A SIMPLE COMPREHENSIVE VALIDATED LIQUID CHROMATOGRAPHIC (HPLC) METHODOLOGY FOR SIMULTANEOUS ESTIMATION OF 5-FLUOROURACIL AND CANNABIDIOL

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Abstract

5-Fluorouracil (5-FU) is a conventional anti-cancer agent. Several studies have shown the synergism effect of Cannabidiol (CBD) along with 5-FU for skin cancers, thus making this combination cogitate for researchers for the management of skin cancer.

A novel, economical, sensitive and robust high-pressure liquid chromatography (HPLC) technique was established for the evaluation of 5-Fluorouracil (5-FU) and Cannabidiol (CBD) simultaneously. Chromatographic elution was done using the stationary phase Hypersil™ C18 reverse phase column 250 mm × 4.6 mm (5 µm) and employing mobile phase as Methanol: Water in ratio 90:10 running at a flow rate of 1.0 mL/min and evaluated at 235 nm. The total run time of the proposed technique was 4 min. The retention time was obtained at 1.43 min (5-FU), and 2.27 min (CBD).

The proposed analytical methodology validation was executed in consistent with International Conference on Harmonization (ICH) regulations and the validation parameter taken into consideration were the limit of detection (LOD), robustness, the limit of quantification (LOQ), and system suitability. A linear standard curve was plotted from the 10 to 100 µg/ml concentration range. The LOD of the proposed method was 4.75 ng/ml for 5-FU and 12.12ng/ml for CBD.

All results are under acceptable limits and the method could be suitable for employed in the synchronous determination of sample drugs in quality control and assay.

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1. INTRODUCTION

A famous fluorinated Pyrimidine analogue antimetabolite 5-Fluorouracil (Chemically known as 5-Fluoro-1H,3H-pyrimidine-2,4-dione) (Figure 1) which was accepted as a medicine in 1962 is still widely used conventional anticancer agent after 60 years of its recognition in the medical field (5-Flourouracil, 2022). It is commonly employed in the prescription of different categories of malignancies and skin problems. Evidences of its curative effect in the therapeutics of Breast carcinoma (Raymond , et al., 1997) , GIT (especially colon) (Raymond , et al., 1997) (Arburk, 1989) , ovarian cancer (Raymond , et al., 1997) and skin cancer ((Khan, et al., 2015) , (Iqubal , et al., 2021)) (Diasio & Harris, 1989) is well established.

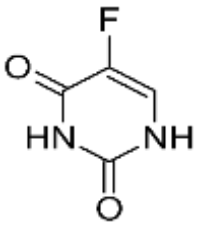


Figure 1: Chemical Structure of 5-Fluorouracil

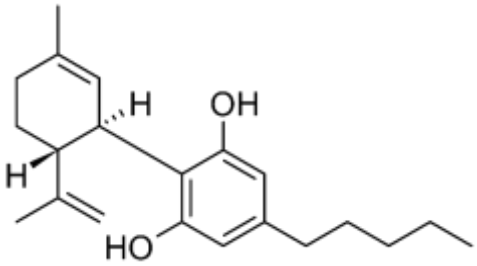


Figure 2: Chemical Structure of Cannabidiol

Cannabidiol (CBD) (chemically named as 2-[(1R, 6R)-6-Isopropenyl-3-methylcyclohex-2-en-1-yl]-5-pentylbenzene-1, 3-diol) (Figure 2) is major non-psychoactive agent derived naturally from plant Cannabis sativa. It has gained fame in the scientific associations due to its pharmacologically activities ranging from antioxidant (Atalay, Jarocka-Karpowicz, & Skrzydlewska, 2020), antipsychotic, antimicrobial (Blaskovich, et al., 2021), anxiolytic (Blessing, Steenkamp , Manzanares, & Marmar, 2015), antiepileptic, antiemetic, anti-inflammatory (Atalay, Jarocka-Karpowicz, & Skrzydlewska, 2020), colorectal cancer (Gustafsson, Lindgren, Jonsson, & Jacobsson, 2009), head and neck squamous cell cancer (Go, Kim, Kim, Chae , & Song, 2020) to skin cancer and lesions ((Scheau , et al., 2020) (Massi, Solinas, Cinquina, & Parolaro, 2012)). (Zhornitsky & Potvin, 2012).

Since both the drug moieties shows well known effect in the area of skin cancer which allows the author to suggest to take this combination ahead for detailed study.

Several Liquid Chromatography (HPLC) methods has been documented for the determination of 5-Fluorouracil (5-FU) individually plus in amalgamation with several different drug moieties in Biological matrices (Muhammad, et al., 2018), (Semail, et al., 2020), along with ion pairing technique (Rustum & Hoffman, 1988), along with Fluorescence derivatisation (Iwamoto, Yoshida, & Hirose, 1984) and in Bulk drug and marketed preparation (Larson, Khazaeli, & Dillon, 2003), (Sinha, Kumar, & Bhinge, 2009) , (Haq, et al., 2013), (Muhammad, et al., 2018), (Tomar, Sharma, Kumar, Jain, & Ahirrao, 2021).

Similarly, several HPLC methods are also developed for Cannabidiol (CBD) in Biological matrices (Zgair , et al., 2015) and in Raw Material, Bulk drug and marketed preparation (Mandrioli , Tura , Scotti, & Toschi, 2019), (Nahar, Onder, & Sarker, 2019), (Vlad, et al., 2019), (Ahmed, Rizwanulla, Usman, Mir, & Amin, 2022), (Piani, Ferfuia, Bortolomeazzi,, Verardo, & Baldini , 2022), (Analakkattillam, Langsi, Hanrahan , & Moore, 2022).

Many HPLC methods are reported for 5-FU and CBD individually in literature, but to the contrary no method has been reported in the literature for the estimation of 5-Fluorouracil (5-FU) and Cannabidiol (CBD) simultaneously. Thus, instigating the authors to develop an innovative synchronous estimation method for 5-Fluorouracil and Cannabidiol which can be intended to be used in assay studies, and routine analysis due to its cost-effectiveness (Tiwari et al 2021).

The intent of the ongoing investigation was to simply develop a rapid, economical and modernistic Reverse Phase HPLC analytical technique for the simultaneous quantification of 5-Fluorouracil (5-FU) and Cannabidiol (CBD) and validate it according to the guidelines of ICH (ICH, 2005).

As per literature, 5-FU has been quantified at various wavelengths, i.e. 205nm, 210nm, 254nm, 275nm, 266nm and 269nm, by HPLC method (Semail, et al., 2020). Furthermore, CBD has been quantified at 221nm by HPLC method (Nahar, Onder, & Sarker, 2019). While developing the method for simultaneous estimation, 5-FU was observed at 267nm and CBD at 221 nm individually. Though, the Isobestic wavelength was found to be 235nm which provides an efficient way for data analysis in the simultaneous quantification of two different drug moieties. Thus, using the optimised wavelength mentioned

earlier, an understandable and methodical analytical way was refined for estimation of 5-FU and CBD simultaneously which was able to beat the issues of the former individual methods. Validation of the current method was in consonance as per ICH guidelines.

2. MATERIAL AND METHODOLOGY

2.1. Material Used

5-Fluorouracil (5-FU) was bought from Sigma-Aldrich and Cannabidiol (CBD) was acquired as a kind from Netherlands, Europe. For mobile phase, Water, Methanol and Acetonitrile (ACN), all were of HPLC grade and were procured from Merck India. HPLC Column used for analysis was Hypersil C-18 which was procured from Thermo fisher India. The syringe filters used were from Merck Millipore. All other reagents used were of analytical (AR) or pharmaceutical grade.

2.2. Identification of Standard Drug:

Bulk drugs' (5-FU & CBD) identification were carried out by determining melting point, Infrared spectroscopy and solubility.

2.3. HPLC Instrument and Chromatographic Conditions

Analytical equipment used for this method development was Waters e-2695 Separation Module, along with Waters e-2998 PDA detector equipped with Software EMPOWER 3 for data acquisition and analysis. Stationary Phase used was Hypersil Reverse Phase column C-18 (250mm* 4.6mm, 5µm). Mobile phase used for the analysis was Methanol: Water (90:10) using isocratic elution mode at temperature 25ºC. Detection was done at 235nm using PDA detector. The flow rate was 1ml/min with ambient column temperature. The volume of injection was 10µl with the total run time of 4 minutes.

2.4. Formulation of Standard Solutions and Working Standards

5-Fluorouracil and Cannabidiol standard solution measuring 1000µg/ml concentration were formulated and were marked as SS1 & SS2 respectively. Stock Solutions of concentration 100µg/ml were formulated from the standard solution (S1& S2) and were marked as A1 and A2 respectively. Working stock solution of different concentration (10, 20, 40, 60, 100 µg/ml) were formulated from the standard solution (A1& A2) for both 5-Fluorouracil and Cannabidiol and the diluent used for working stock solution was Methanol. Furthermore, the samples of FU-CBD combination were formulated in methanol at 10, 20, 40, 80, and 100 µg/ml concentration for both the sample moieties. All the sample solutions were stored in amber-coloured bottles in the freezer at 4ºC before the set analysis. All the samples were filtered via a syringe filter of 0.22µm pore size. (Wrightson, Myers, & Galandiuk, 1995)

3. VALIDATION

Subsequently, the developed optimized method was validated for the synchronous determination of 5-Fluorouracil and Cannabidiol. The validation was attained in accordance with the protocol of ICH guidelines Q2 (R1) (ICH, 2005). The developed HPLC method should be validated on various domains to ensure that the strategy's comprehensive efficacy attributes meet the requirements of its intended purpose only (Zothanpuii & Selvakumar, 2020). The parameter taken into consideration are system suitability testing, linearity, specificity, ruggedness, limit of detection (LOD) and limit of quantification (LOQ).

3.1. System Suitability Test

This system suitability test (SST) is of key importance in liquid chromatography as it aids in the validation of the given testing procedure and ensures reproducibility. The suitability testing was done in six replicates with the sample size of 20µg/ml for both 5-Fluorouracil and Cannabidiol by scrutinizing the R_T and AUC at UV detection value of 235nm. According to the guidelines laid by US-FDA, Relative Standard Deviation (%RSD) should be NMT 2%.

3.2. Specificity

Specificity is described as the capacity of the analytical technique to isolate the sample of analytical nature from the combination (excipients, degraded products, impurities), which is the main distinctive feature of HPLC. Chromatograms of drug samples were compared to blank solution and their amalgamation sample to evaluate specificity. The blank solution did not contain 5-FU or CBD; the rest of the constituents and preparation process were identical to the drug sample.

3.3. Linearity

Linearity of the analytical procedure can be determined by preparing sample mixture of 10, 20, 40, 80, and 100 µg/ml concentration from standard solution. The area under the curve is calculated for each concentration of the sample (individual or mixture). Thus, plotting the calibration graph with concentration (x-axis) and area under the curve (y-axis) helps in derivatisation of regression equation. All standard

curves of 5-Flourouracil, Cannabidiol and their combination were devised for linear equation.

3.4. Ruggedness

Ruggedness means having the capacity to generate an outcome under different conditions, like
Employing varied investigator or device of unsimilar make. We have utilised three different samples of 10, 20, and 30 µg/ml concentration, that were examined by various investigator on the same Liquid chromatographic equipment located in the similar and different laboratory. Ruggedness score was calculated by evaluating the Recovery (%) of 5-Fluoruracil and Cannabidiol in both the situations.

3.5. Sensitivity

Sensitivity of the HPLC analytical method can be determined by calculating the minimum amount of drug which can be isolated and quantified i.e limit of detection (LOD) and limit of quantification (LOQ).

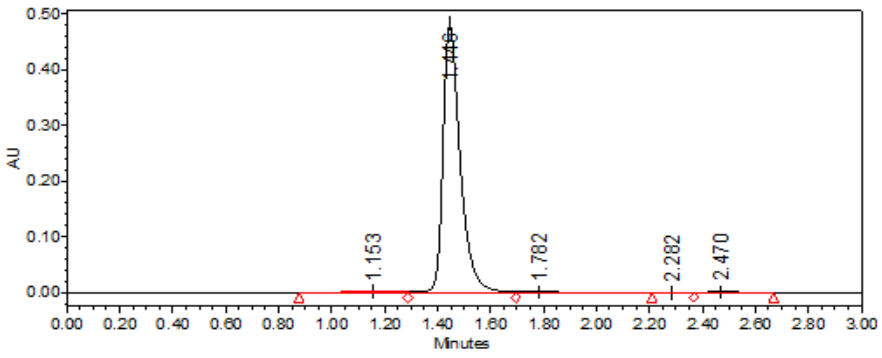
	LOD	**LOQ**
Formula **S= S.D.** **s= Slope**	3.3* S/s	10*S/s
Signal to noise ratio	3:1	10:1

4. RESULT AND DISCUSSIONS

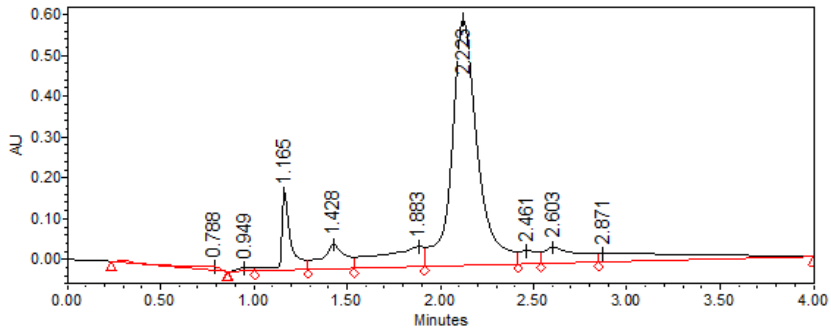
4.1 Development and Optimisation of the Technique

In order to make the prepared approach more financially viable and credible, an isobestic wavelength was screened for the analysis of the both the drug moieties, which is postulated on the basis of the absorption maxima of both drug samples at a particular wavelength. The methanolic solutions of both drug samples (5-FU and CBD) of concentration 10 µg/ml each were analysed by UV spectroscopy in the 400-200 nm spectrum. 5-FU and CBD had the highest absorbance at 267 nm and 221 nm respectively. A single wavelength of 235 nm was chosen as per the Spectral data of both drugs and validated by a PDA detector.
Mobile phase selection for method development was done among ACN/Methanol and Water in varied concentrations in isocratic and gradient flow. The best separation of 5-Florouracil and Cannabidiol was marked in the mobile phase Water: Methanol in a 90:10 ratio and the flow rate of the mobile phase was 1ml/min along with the total run time of 4 mins. The retention time of 1.43 minutes for 5-FU and 2.27 minutes for CBD was obtained. 5-Florouracil and Cannabidiol both analytes were tested independently at λmax 267 and 221 nm respectively (Figure. 1a and 1b). The combination of 5-FU and CBD was also estimated, through which the peaks got separated at 1.4 and 2.2 min respectively for 5-FU and CBD suggesting no change in the retention time of the individual and that combination (Fig. 1c).
The developed method for analysing 5-FU, CBD, and its combination determination indicates supremacy in comparison to the different established techniques because of its accelerated efficacy, and ability to detect both analytes within only 4 minutes of run time.

a) Chromatogram of 5-Fluorouracil at λmax 267nm,



b) Chromatogram of Cannabidiol at λmax 221 nm



c) Chromatogram of 5-Fluorouracil and Cannabidiol at isobestic λmax 235 nm

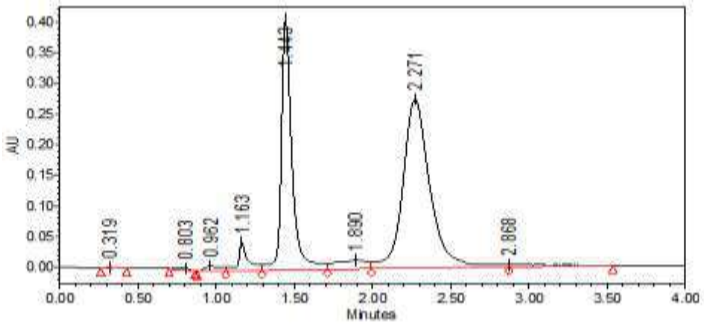
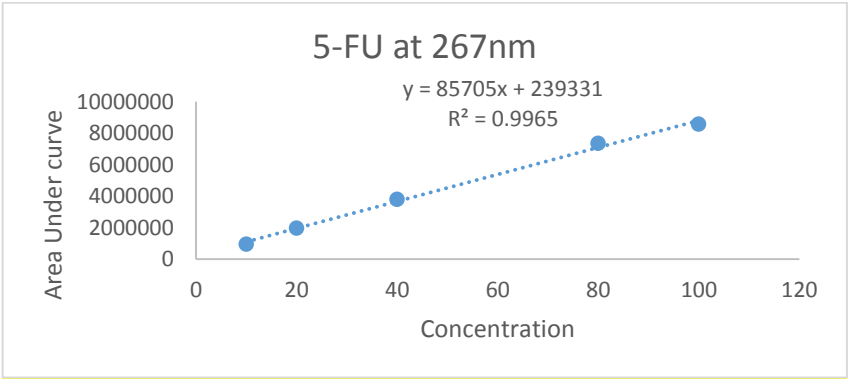


Figure 3: Chromatogram of 5-Flurouracil, Cannabidiol and Combination at their respective wavelength

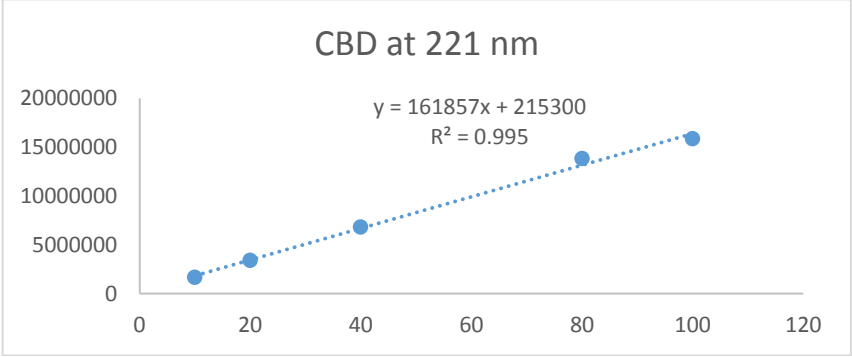
4.2. Method Validation

4.2.1. Linearity

a) Calibration curve of 5-Fluorouracil at 267nm



b) Calibration curve of Cannabidiol at 221nm



c) Calibration curve of 5-Flourouracil & Cannabidiol at Isobestic λmax 235nm

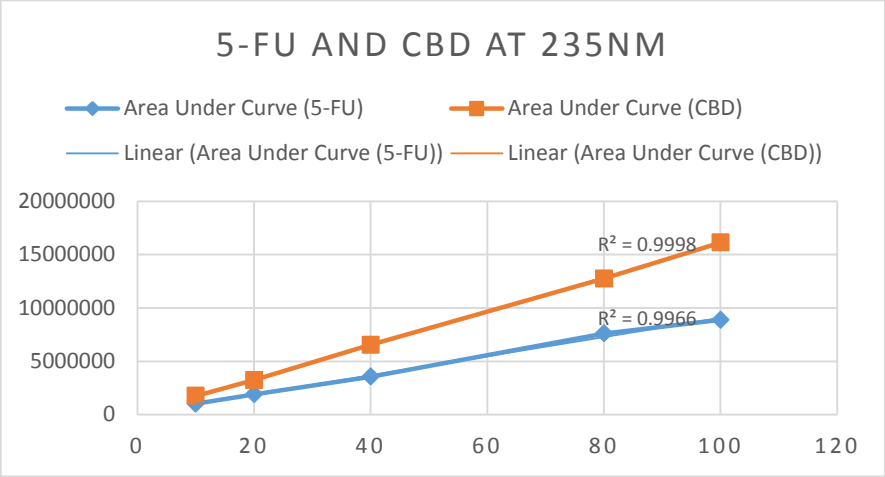


Figure 4: Calibration Curve of 5-Fluorouracil, Cannabidiol and Combination at respective wavelength

Five different samples of 5-FU, CBD and 5FU-CBD of concentration 10, 20, 40, 80 and 100 µg/ml were formulated and analysed independently. To determine linearity of the current HPLC method, 5-Flourouracil, Cannabidiol and their combination were monitored at 267nm, 221nm and 235 nm respectively. A regression graph was devised between the concentration on X-Axis and Area under the curve (AUC) on Y-Axis. The Correlation Coefficient value (R2) for 5-FU at λmax 267nm was 0.9965, for CBD at λmax 221nm was 0.995 and for 5-FU-CBD at λmax 235nm was 0.9966 for 5-FU and 0.9998 for CBD. (Fig 4). The above values showed the current HPLC method's linearity in the stated range of concentration.

4.2.2. System Suitability Test

a) SST of 5-Fluorouracil at 235nm		
SST Criteria	**T_R**	**AUC**
1	1.46	1962575
2	1.45	1996652
3	1.39	1914854
4	1.41	1985485
5	1.44	1952548
6	1.42	1995241
Mean	1.428333333	1967892.5
Standard Deviation	0.026394444	31500.34069
RSD	1.847919057	1.600714505

b) SST of Cannabidiol at 235nm		
SST criteria	**T_R**	**AUC**
1	2.19	3308678
2	2.24	3348182
3	2.17	3395527
4	2.27	3336826
5	2.23	3382535
6	2.18	3324173
Mean	2.213333333	3349320.167
Standard Deviation	0.039327683	35074.94097
RSD	1.776853157	1.047225682

Table 1: a) Retention time & Area under peak data for system suitability test of 5-Flurouracil at 235 nm
b) Retention time & Area under peak data for system suitability test of Cannabidiol at 235 nm

Six samples runs of 5-FU and CBD were conducted to calculate the Relative standard deviation (RSD) of retention time and Area under the curve. The average retention time for 5-FU was 1.4284± 0.026min with RSD of 1.84% and the average retention time for CBD was 2.21± 0.039min with RSD of 1.77%. The average area under the curve of 5-Fluorouracil was observed as 1967892.5± 31500.34 with RSD of 1.60% and the average curve area of CBD was found to be 3349320.167± 35074.94 with RSD of 1.04%. (Table 1). All the obtained Relative Standard deviation (RSD) values for both retention time and area under the curve for 5-Fluorouracil and Cannabidiol are below 2%, thus complying with the parameters for system suitability and reproducibility for the current liquid chromatographic method.

4.2.3. Specificity

By correlating the chromatograms of 5-Fluoruracil, Cannabidiol, and Fluorouracil

Cannabidiol mixture's standard solution along with the blank solution, the assessment of the specificity of the current HPLC procedure was established.

The standard sample which was injected was of 10 µl volume each and all were analysed separately. When the retention time of both drug moieties (5-FU & CBD) were examined individually, they were found to be 1.44 and 2.2 minutes, respectively. 5-Fluorouracil and Cannabidiol had a retention time of 1.44 and 2.2 minutes, respectively, in the 5-Fluorouracil-Cannabidiol mixture (Fig 1). Thus, implies that the retention time is unaltered irrespective the samples are analysed individually or simultaneously, therefore indicating the specific nature of the current analytical method.

4.2.4. Sensitivity

Drug Sample	Slope of the curve	Standard deviation (SD)	LOD	LOQ
5-Flourouracil at λmax 267nm and Cannabidiol at λmax 221nm				
5-FU	85705	53979	2.07841666	6.298232309
CBD	161857	373689	7.61890867	23.08760202
5-Fluorouracil and Cannabidiol at Isobestic λmax 235 nm				
5-FU	89770	129341	4.75465412	14.40804278
CBD	159246	585240	12.1277269	36.75068762

Table 2: Sensitivity Table

The sensitivity of the HPLC analytical method is done by evaluating the calibration curves of the samples and combination to analyse the lowest detection amount of the drug (LOD) and lowest quantified drug value (LOQ). Thus, LOD for 5-Fluorouracil was 2.08 ng/ml at λmax 267nm and LOD for Cannabidiol was found to be 7.6 ng/ml at λmax 221nm. LOD obtained at the common isobestic wavelength 235nm was 4.75ng/ml for 5-Fluorouracil and 12.12ng/ml for Cannabidiol (Table 2). The minimal amount of the analyte that can be measured is known as LOQ, which was calculated to be 6.29 ng/ml for 5-FU at λmax 267nm and LOQ for CBD was estimated to be 23.08 ng/ml at λmax 221nm. The LOQ for the current designed HPLC method at λmax 235nm were calculated to be 14.04 ng/ml for5-Fluorouracil and 36.75 ng/ml for Cannabidiol (Table 2).

Hence, the statistics of LOD and LOQ shows promising evaluation scope for the current HPLC analytical method in assay procedures, and drug development.

4.2.5. Ruggedness

(A)Ruggedness of 5-Fluorouracil and Cannabidiol on varied HPLC unit by same investigator					
Drug Sample	**Sample Concentration (µg/ml)**	**Amount Recovered concentration (in µg/ml)**		**Recovery (In percentage)**	
		HPLC-I	**HPLC-II**	**HPLC-I**	**HPLC-II**
5-FU	10	9.48 ± 0.22	9.61 ± 0.43	94.89	96.14
	20	19.24 ± 0.49	19.08 ± 0.89	96.24	95.42
	30	29.86 ± 0.2	29.54 ± 0.75	99.53	98.49
CBD	10	9.78 ± 0.23	9.50 ± 0.93	97.89	95.02
	20	19.36 ± 0.56	19.73 ± 0.58	96.82	98.66
	30	29.05 ± 0.69	29.03 ± 0.74	96.83	96.78
(B) Ruggedness of 5-Fluorouracil and Cannabidiol by varied investigators on the same HPLC unit.					
Drug Sample	**Sample Concentration (µg/ml)**	**Amount Recovered concentration (in µg/ml)**		**Recovery (In percentage)**	
		Investigator-I	**Investigator-II**	**Investigator-I**	**Investigator-II**
5-FU	10	9.97 ± 0.90	9.64 ± 0.72	99.72	96.48
	20	19.08 ± 0.45	19.68 ± 1.03	95.44	98.43
	30	28.80 ± 0.46	29.79 ± 0.96	96	99.31
CBD	10	9.76 ± 0.41	9.71 ± 0.44	97.67	97.19
	20	19.62 ± 0.89	19.42 ± 0.66	98.11	97.11
	30	28.81 ± 0.59	28.99 ± 1.00	96.04	96.64

Table 3: Ruggedness Score of 5-Fluorouracil and Cannabidiol at λmax 235nm

The robustness test was used to assess the reproducibility of outcome values procured on varied HPLCs with the similar investigator and on single liquid chromatographic setup with various investigators. Then, the test was performed by the same investigator on different HPLC. The amount recovered of 5-FU for sample concentrations 10, 20 and 30 µg/ml at 235nm came out to be 9.48 ±0.22, 19.24 ±0.49 and 29.86 ±0.2 µg/ml along with percentage recovery of 94.89, 96.24 and

99.53 % subsequently on HPLC-1 and the recovered concentration was 9.61 ± 0.43, 19.08 ± 0.89 and 29.54 ± 0.75 µg/ml with percentage recovery 96.14, 95.42 and 98.49 % respectively on HPLC -2. Similarly, the recovered concentration of CBD by the same investigator for sample concentration 10, 20 and 30 µg/ml at 235nm came out to be 9.78 ± 0.23, 19.36 ± 0.56 and 29.05 ± 0.69µg/ml along with percentage recovery of 97.89, 96.82 and 96.83% respectively on HPLC-1 and the recovered concentration was 9.50 ± 0.93, 19.73 ± 0.58and 29.03 ± 0.74µg/ml with percentage recovery 95.02, 98.66 and 96.78% respectively on HPLC -2. (Table 3(A)).

Then, the ruggedness data was collected by different analysts on the same HPLC (HPLC-1). The recovered concentration of 5-FU for sample concentrations 10, 20 and 30 µg/ml at 235nm came out to be 9.97 ± 0.90, 19.08 ± 0.45 and 28.80 ± 0.46µg/ml along with percentage recovery of 99.72, 95.44 and 96% respectively by analyst-1 and the recovered concentration was 9.64 ± 0.72, 19.68 ± 1.03 and 29.79 ± 0.96µg/ml with percentage recovery 96.48, 98.43 and 99.31% respectively by analyst-2. Similarly, the recovered concentration of CBD for sample concentrations 10, 20 and 30 µg/ml at 235nm came out to be 9.76 ± 0.41, 19.62 ± 0.89 and 28.81 ± 0.59µg/ml along with percentage recovery of 97.67, 98.11and 96.04% respectively by analyst-1 and the recovered concentration was 9.71 ± 0.44, 19.42 ± 0.66 and 28.99 ± 1.00µg/ml with percentage recovery 97.19, 97.11and 96.64% respectively by analyst-2. The study findings show an excellent level of ruggedness implying that the current analytical method is capable of producing concurrent results on various HPLC analytical setups and by various researchers. The validation data produced tends to be consistent with the reported work. (Sharma, Goyal, & Chauhan, 2018), (Swartz & Krull, 1997).

CONCLUSION

The current study presents a novel approach for simultaneous determination of 5-FU and C B D in assay procedures and quality control. The developed method is simplified, fast, highly selective, sensitive and meticulous, and the proposed technique was validated in consistent with ICH guidelines.

The run time of the method is less than 4 minutes i.e. faster elution with great resolution, thus saving analyst time and solvents, making it to be cheap for routine analysis. This method could employed as rapid and economical way to quantify both the analytical samples (5-FU and CBD) simultaneously in formulation development and quality control checks.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to acknowledge GD Goenka University, Gurugram for furnishing legitimate infrastructure and material to carry forward investigation work.

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DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE SIMULTANEOUS ESTIMATION OF IMIQUIMOD AND THYMOQUINONE

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ABSTRACT:
A novel and sensitive high-pressure liquid chromatography (HPLC) method was developed for the simultaneous estimation of Imiquimod (IMI) and Thymoquinone (THY). Chromatographic elution was accomplished using Hypersil™ C-18 reverse phase column measuring 250 mm × 4.6 mm (5 µm) and mobile phase as Acetonitrile: Water (0.1% formic acid) in 65:35 ratio. The flow rate used in the method is 1.0 mL/minute and was observed at 249 nm employing PDA Detector. The run time of the analytical procedure was 16 minutes. The retention time was obtained at 4.1 min (IMI), and 14.3 min (THY). International Conference on Harmonization (ICH) guidelines were followed to validate the method and the parameter included a limit of detection (LOD), robustness, limit of quantification (LOQ), and system suitability. The regression graph plotted showed linearity from 10 to 100 µg/ml concentration.
All results are under acceptable limits and the method could be suitably employed for the simultaneous estimation of both drugs in quality control and assay.

Keywords: Imiquimod, Thymoquinone, HPLC, validation, simultaneous estimation

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DOI: - 10.31838/ecb/2023.12.si5.0101

1. INTRODUCTION

An Immunomodulator which is an imidazoquinolone synthetic derivative, Imiquimod is chemically known as 1-(2-methyl propyl)-1H-imidazo [4, 5-c] quinoline-4-amine (Figure 1). Imiquimod has the molecular formula $C_{14}H_{16}N_4$, is a crystalline white powder, and has a molecular weight of 240.30g/mol with a melting point of 292-294 °C. Imiquimod is readily miscible in oleic acid and lactic acid. It is well known for its antiviral and antitumor activity in animal models but not able to exhibit its antiproliferative action in cells. It acts by inducing the cytokines by binding to the toll-like receptor 7. In 1977, Food and Drug Administration (FDA) approved Aldara® 5% imiquimod ointment for the prophylaxis and treatment of genital warts, superficial basal cell carcinomas, and scratchy and scaly patches on the skin due to prolonged sun exposure known as actinic keratosis. (Imiquimod, 2022), (Sharma, Kumar, & Rana, 2020), (Gupta, Browne, & Blu, 2002).Indications of its potential therapeutic effect in the therapies of human papillomavirus warts and infection (HPV), squamous cell carcinoma, and vulvar intraepithelial neoplasia. (Gupta, Browne, & Blu, 2002), Bowen's disease (Stanley, 2002), cervical cancer (Frank, et al., 2020), as an age-defying agent (Metcalf, Crowson, Naylor, Haque, & Cornelison, 2006) is entrenched.

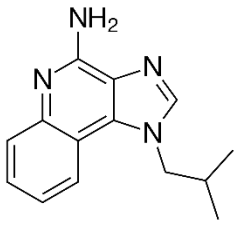


Figure 1: Chemical Structure of Imiquimod

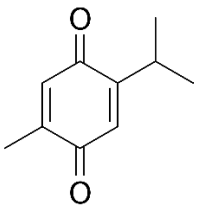


Figure 2: Chemical Structure of Thymoquinone

A monoterpene molecule Thymoquinone (THY) chemically named 2-Methyl-5-(propan-2-yl) cyclohexane-2,5-diene-1,4-dione (Figure 2) was extracted from plant Black cumin i.e., *Nigella sativa* (Ranunculaceae) by El-Dakhakhany in 1963 (Gali-Muhtasib, Roessner, & Schneider-Stock, 2006). Thymoquinone's molecular formula $C_{10}H_{12}O_2$, is a crystalline off-white coloured powder, with a molecular weight of 164.204 g/mol and a melting point of 45-47 °C. It is readily miscible in alcohol and ether. It induces apoptosis and regulates the signalling of both pro-apoptotic and anti-apoptotic genes, acting as a scavenger for free radicals. It has gained fame in the scientific associations due to its pharmacological activities ranging from anti-oxidant, anti-inflammatory, anti-histaminic, anti-microbial, anti-psoriasis, anti-neoplastic and immuno-modulatory. It has remarkable anticancer and cytoprotective properties on different cell lines such as the breast, Cervical, Pancreatic, Colon, Leukaemia, Buccal, Cranial & neck, Lungs, Skin, Ovarian, and Bladder (Muhammad , et al., 2018), (Gupta, Ghosh , & Gupta, 2016), (Gali-Muhtasib, Roessner, & Schneider-Stock, 2006), (Almajali, et al., 2021). It has also been indicated to have gastro-protective, hepatoprotective, nephroprotective, and neuroprotective attributes. It is also believed to have beneficial effects in the therapeutics of epilepsy, diabetes, cardiovascular disease, reproductive complications, and lung diseases, as well as bone complications, arthritis and fibrosis (Goyal, et al., 2017). Furthermore, a significant amount of research implies that Thymoquinone has very few side effects and no significant toxic effects (Darakhshana, Pour, Colagar, & Sisakhtnezhad, 2015).

Although both drug moieties have well-known effects in the area of cancer, thus, the author recommends that this combination to be studied further in depth. As combining naturally derived moiety with the synthetic one is taking us one step closer to the plant-based approach of medicine making it acceptable to accommodate alternative therapy, either showing synergism or antagonism with the conventional medicine because of the drug resistance and adverse effects. (Woo , Kumar , Sethi, & Tan, 2012). Conventional medicine is yet inadequate to offer complete eradication of cancer.

Many High-Pressure Liquid Chromatography (HPLC) Analytical methods are in existence for the estimation of Imiquimod (IMI) individually (Sharma, Sharma, Singh, & Katare, 2019) and in combination with other drug moieties (Tomar, Sharma, Kumar, Jain, & Ahirrao, 2021) has been reported in the literature. The analytical method was also reported in Biological matrices (Mu, et al., 2016), in nanoformulation (Frank, et al., 2020), (Remiro, et al., 2022), in bulk drug (Rao, Patrudu, Rao, & Ganesh, 2020) and in marketed preparation (Bachute & Turwale, 2013).

Additionally, several HPLC techniques have been developed for Thymoquinone (THY) individually

(Aboul-Enein & Abou-Basha, 1995), (Iqbal, Ahmad, & Pandey, 2018), in combination with other drug moieties (Jagtap, Mahajan, Parte, Pananchery, & Jain, 2021), (Soliman, et al., 2020), in biological matrices (Ahmed, Khan, & Alkharfy, Many analytical methods are published in the literature for IMI and THY independently, but no simultaneous estimation analytical method of IMI and THY together has been stated. As a result, the authors were inspired to create a trailblazing simultaneous estimation method for IMI and THY, which is intended to be used in assay studies and routine analysis due to its cost-effectiveness.

The current study aimed to create and validate a novel, smooth, quick, and parsimonious liquid chromatography method for the synchronal evaluation of Imiquimod and Thymoquinone in accordance with guidelines laid by ICH (ICH, 2005).

According to the literature, IMI has been quantified by HPLC at various wavelengths, such as 226 nm (Sharma, Kumar, & Rana, 2020), 242 nm (Frank, et al., 2020), 244 nm (Sharma, Sharma, Singh, & Katare, 2019), 245 nm (Bachute & Turwale, 2013) and 260nm (Rao, Patrudu, Rao, & Ganesh, 2020). Similarly, THY has also been quantified using an HPLC method at 249 nm (Alam, et al., 2022), 254 nm (Ahmed, Khan, & Alkharfy, 2015) (Gilani, et al., 2019), (Soliman, et al., 2020), 255 nm (Ansar, et al., 2020) and 295 nm (Aboul-Enein & Abou-Basha, 1995).

IMI and THY were detected at 244 nm and 255 nm, respectively, while developing the approach for simultaneous estimation. However, the isobestic wavelength was discovered to be 249 nm, allowing for efficient data analyzation in the combined quantification of two distinct drug moieties. Thus, using the previously mentioned optimised wavelength, a simplified precise method for simultaneous estimation of IMI and THY was developed, which was able to overcome the shortcomings of the previous individual methods. The method developed has also been substantiated in congruence with ICH guidelines.

2. MATERIAL AND METHODS

2.1. Materials

Imiquimod (IMI) was gifted sample from Glenmark Pharmaceuticals Ltd, Mumbai. Thymoquinone (THY) was procured from Sigma Aldrich, Mumbai. The supply of Water (HPLC-grade), Methanol and Acetonitrile (ACN) was by Merck Specialties Pvt. Ltd., India. The column

2015), along with Box-Behnken Design (BBD) (Alam, et al., 2022), and in nano formulation (Gilani, et al., 2019), (Ansar, et al., 2020), (Jain, et al., 2017).

used was HPLC Hypersil C-18 Column and was procured by Thermo Fisher, India; and the syringe filters were procured from Axiva Sichem Biotech, New Delhi, India. All other chemicals employed in the method development were of pharmaceutical or analytical grade.

2.2 Identification of Standard Drug:

Identification of the bulk drugs (IMI & THY) was executed by measuring the melting point, infrared spectroscopy, and solubility.

2.3 HPLC Instrument and Chromatographic Conditions

Analytical equipment used for this method development was Waters- e2695 Separation Module, along with Waters-e2998 PDA detector equipped with Software EMPOWER 3 for data acquisition and analysis. The stationary phase used was Hypersil C-18 Reverse Phase column measuring 250mm * 4.6 mm, 5 µm. ACN: Water (0.1% formic Acid) in a 65:35 ratio was used as mobile phase using isocratic elution mode at a temperature of 25ºC. Detection was done at 249nm using a PDA detector. The flow rate was maintained at 1ml/min with ambient column temperature. The volume of injection was 10 µl with a run time of 16 minutes.

2.3. Preparation of Standard and Working Solutions

IMI and THY standard solutions were formulated separately at 1000µg/ml concentration and labelled S1 and S2, respectively. Stock solutions of concentration 100µg/ml were made from the standard solutions (S1& S2) and labelled as A1 and A2. Using the mobile phase as a diluent, a working stock sample of varied concentrations (10, 20, 40, 60, and 100 µg/ml) was made from the stock solution (A1& A2). Furthermore, IMI-THY combination samples were prepared in the mobile phase at concentrations 10, 20, 40, 80, and 100 µg/ml for both drugs. All samples were kept in amber-coloured bottles in a freezer at 4ºC prior to HPLC analysis. Filtration of all the samples was done through a syringe filter (pore size: 0.22 µm). (Wrightson, Myers, & Galandiuk, 1995)

3. VALIDATION

Soon after the creation of an optimization technique, the method of simultaneous estimation

of IMI and THY was validated in accordance with ICH guidelines Q2 (R1) (ICH, 2005). HPLC methods should be validated using various parameters to ensure that the overall performance attributes of the strategy correspond to the demands of its desired uses only (Zothanpuii & Selvakumar, 2020). The parameters considered are system suitability testing, linearity, ruggedness, specificity, detection limit, and quantification limit (Tiwary et al. 2021).

3.1. System Suitability Test

This Suitability test is pivotal in liquid chromatography because it helps validate the testing method and ensures reproducibility. Suitability testing was performed in six replicates with a sample size of 20g/ml for both IMI and THY, with the RT and Peak area examined at a UV detection value of 249nm. As per US-FDA guidelines, the acceptable limit of relative standard deviation, %RSD is not more than 2%.

3.2. Specificity

While developing an analytical process, Specificity is the capacity to isolate standard drug from its combination such as other drugs, excipients or impurities. To assess specificity, individual solution chromatograms were compared to a blank solution and their combination. The blank solution lacked IMI and THY, but the remaining constituents and preparation method were exactly equivalent to the sample drug solution.

3.3. Linearity

Solutions of 10, 20, 40, 80, and 100 µg/ml concentration were prepared from standard solution for linearity determination. Standard curve was prepared from the values of peak area (Y-axis) and corresponding concentration (X-axis).
The linear equation was established from IMI, THY, and IMI-THY calibration curves that were plotted individually by arranging concentration and peak area.

3.4. Ruggedness

Ruggedness refers to the capability of producing an outcome under varying conditions, like by using variant investigator or same instrument of unsimilar make. We have used three distinct samples with concentration levels of 10, 20, and 30 µ g/ml which were analysed by two varied investigator using the same HPLC analytical equipment and on different HPLC by the same researcher in separate laboratory. Percentage amount concentration recovery for both analytes was calculated, and ruggedness findings from two separate investigators were compared.

3.5. Sensitivity

The sensitivity of the LC analytical method is determined by its ability to detect and quantify the minimal amount of drug sample i.e. LOD (limit of detection) and LOQ (limit of quantification).

	LOD	**LOQ**
Signal to noise (s/n) Ratio	3:1	10:1
Calculation Formula **S= S.D.** **s=Slope**	3.3 S/s	10S/s

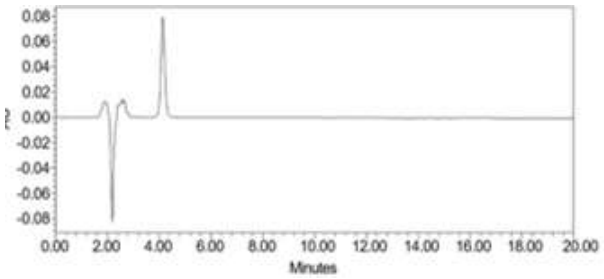
4. RESULT AND DISCUSSION

4.1. Development and Optimisation of the Method

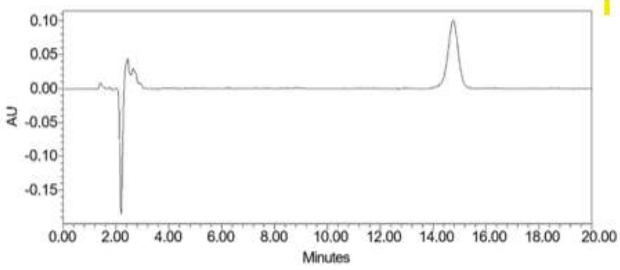
To make the prepared methodology more economically sustainable and reliable, an isosbestic wavelength was chosen for an analytical study that is predicted by observing the absorption maxima of IMI and THY at a particular wavelength. IMI and THY solutions having 10 µg/ml concentration each were analysed by UV Spectroscopy in the range of 400-200 nm.

IMI has the highest absorbance at 244 nm, followed by THY at 255 nm. A single wavelength of 249 nm was chosen based on spectral data and it was validated by a PDA detector. Mobile phase selection for method development was done among ACN/Methanol and Water in varied concentrations in isocratic and gradient flow.
The best isolation of IMI and THY was observed in an ACN: Water (0.1% Formic Acid) in a 65:35 ratio as mobile phase with a flow rate of the mobile phase in stationary column is 1ml/minute and the total run time of the analytical method was 16 minutes. IMI had a retention time of 4.1 minutes and THY had a retention time of 14.3 minutes. IMI and THY both were tested at their respective maxima, 244 and 255 nm, individually (Figs. 1a and b). The combination of IMI and THY was also analysed, and the peaks were separated at 4.1 and 14.3 min for IMI and THY, respectively, implying no change in the retention time of the individual and that combination (Fig. 1c).

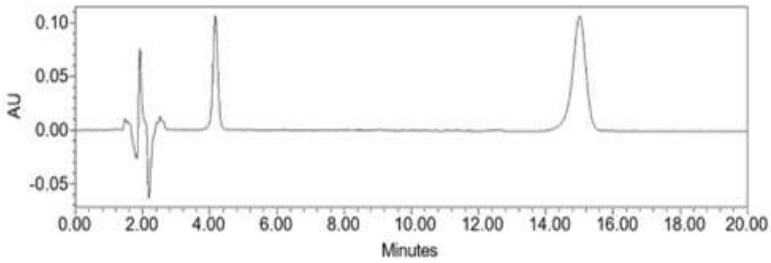
The developed method for evaluating IMI, THY, and their combinations suggests it to be economical, efficient, and can detect both analytes in only 16 minutes of run time. Thus, it proves its efficiency over other stated methods of estimation.



a) Chromatogram of Imiquimod at λmax 244nm



b) Chromatogram of Thymoquinone at λmax 255 nm

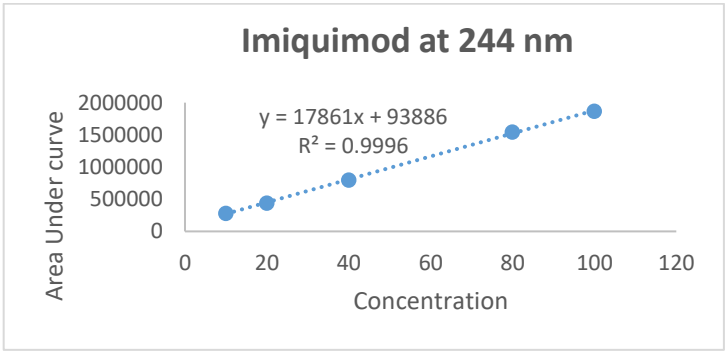


c) Chromatogram of Simultaneous determination of Thymoquinone and Imiquimod at isosbestic wavelength 249 nm.

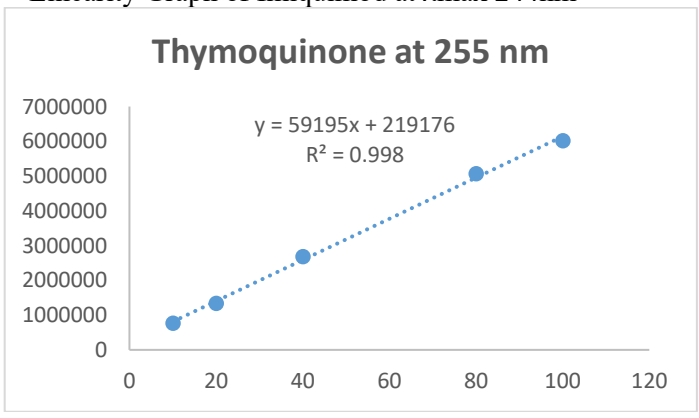
Figure 3: Chromatogram of Imiquimod, Thymoquinone and its combination at their respective wavelength.

4.2. Method Validation

4.2.1. Linearity

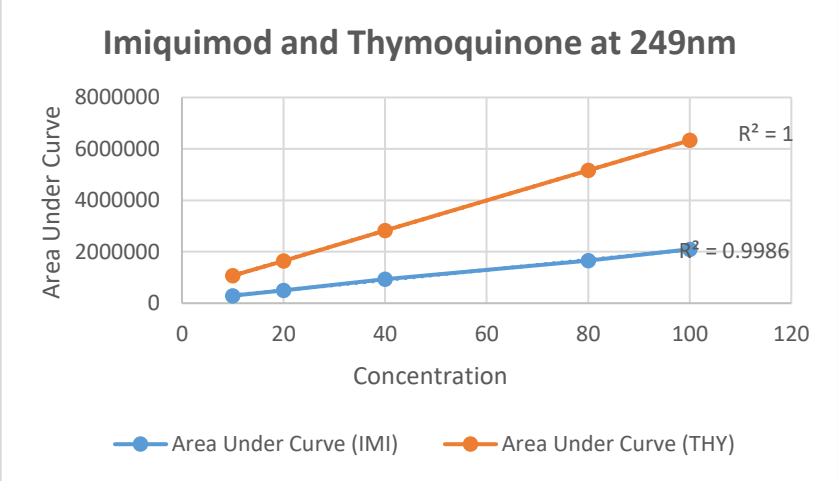


a) Linearity Graph of Imiquimod at λmax 244nm



b) Linearity Graph of Thymoquinone at λmax 255nm

c)



Linearity Graph of Simultaneous estimation of Thymoquinone and Imiquimod at isosbestic wavelength 249 nm

Figure 4: Linearity Graph of Imiquimod, Thymoquinone and its Combination at respective wavelengths**.**

Five distinct concentration levels of 10, 20, 40, 80, and 100 µg/ml were prepared as well as analysed for IMI, THY, and IMI -THY independently. IMI, THY, and IMI-THY were analysed at 244nm, 255 nm, and 249 nm, respectively, to determine the linearity of the Analytical method. A graph was created by plotting the concentration of the drug moiety on the X-axis with its comparative AUC on the Y-axis. The R_2 value for IMI at λmax 244 nm was 0.9996, for THY at λmax 255 nm was 0.998, and for IMI-THY at max 249 nm was 0.9986 for IMI and 1 for THY. (Fig 4). The above values demonstrated that the current analytical Methodology is linear in the defined concentration range.

4.2.2. System Suitability Test

a) System Suitability of Imiquimod at 249 nm		
System suitability criteria	**Retention Time**	**Peak Area Count**
1	4.12	413209
2	4.18	421378
3	4.06	408971
4	4.15	418107
5	4.11	407816
6	4.08	414271
Average	4.116666667	413958.6667
Standard deviation	0.044121046	5206.225146
Relative standard deviation (RSD)	1.07176629	1.25766787
b) System Suitability of Thymoquinone at 249nm		
System suitability criteria	**Retention Time**	**Peak Area Count**
1	14.34	1335501
2	14.26	1312634
3	14.38	1298632
4	14.36	1341783
5	14.39	1351352
6	14.42	1347929
Average	14.35833333	1331305.167
Standard deviation	0.055287129	21737.89004
Relative standard deviation (RSD)	0.385052555	1.632825485

Table 1: a) System suitability (R_t & Peak area Count) for Imiquimod at 249 nm;
b) System suitability (R_t & Peak area Count) for Thymoquinone at 249 nm.

Six IMI and THY sample runs were performed to determine the RSD of peak area count and retention time. The mean retention time for IMI was 4.11± 0.044 min with an RSD of 1.07% and 14.35 ± 0.055 min with an RSD of 0.38%. The average peak area of IMI was 413958.67± 5206.2 with an RSD of 1.25%, while the average peak area of THY was 1331305.167± 21737.89 with an RSD of 1.64%. (Table 1). All of the obtained RSDs for Peak Area count and retention time for both IMI and THY are less than 2 %, indicating that the current Analytical technique meets the criteria for suitability and reproducibility.

4.2.3. Specificity

The specificity of the current analytical method was evaluated by correlating chromatograms of an IMI, THY, and IMI -THY standard solution with a blank solution.

Each standard sample was injected in a volume of 10 µl and was analysed separately. IMI and THY retention times were observed to be 4.1 and 14.3 minutes, respectively, when examined separately. In the IMI-THY mixture, IMI and THY had retention times of 4.1 and 14.3 minutes, respectively (Fig 1). Thus, indicating the specific nature of the analytical method as the retention time remains constant whether the samples are evaluated individually or simultaneously.

4.2.4. Sensitivity

Sample	Slope of linear curve	Standard deviation (SD)	LOD	LOQ
Imiquimod at λmax 244 and Thymoquinone at λmax 255				
Imiquimod	17861	39961.14	7.383223896	22.37341
Thymoquinone	59195	126538.2	7.05424546	21.3765
Imiquimod & Thymoquinone at Isobestic λmax 249 nm				
Imiquimod	19748	59556.3	9.952187057	30.15814
Thymoquinone	58458	291580.7834	16.45996417	49.87868

Table 2: Sensitivity Table of Imiquimod and Thymoquinone individually and in combination at their respective wavelength

The sensitivity of the HPLC analytical method is assessed by examining the standard curve of the samples and combinations to ascertain the LOD i.e. lowest detection amount of the drug and LOQ i.e. lowest quantified drug value. Thus, the LOD for IMI at λmax 244 nm was calculated to be 7.38 ng/ml, and the LOD for THY at λmax 255 nm was 7.05 ng/ml. LOD at the isobestic wavelength of 249 nm was 9.95 ng/ml for IMI and 16.45 ng/ml for THY (Table 2).

LOQ is the minimum iota of drug sample that is assessed by the proposed method. It was estimated to be 22.37 ng/ml for IMI at λmax 244nm and 21.37 ng/ml for THY at λmax 255nm. The LOQ for said current anticipated analytical method at λmax 249 nm was 30.15 ng/ml for IMI and 49.87 ng/ml for THY (Table 2).

As a result, the LOD and LOQ statistics demonstrate an intriguing evaluation possibility for the current analytical method in assay procedures and formulation development.

4.2.5. Ruggedness

(A) Ruggedness Score of Imiquimod and Thymoquinone on different HPLC unit by same investigator					
Drug Sample	**Sample Concentration (µg/mL)**	**Amount Recovered**		**Recovery (%)**	
		HPLC-I	**HPLC-II**	**HPLC-I**	**HPLC-II**
IMI	**10**	9.61 ±0.68	9.89 ± 0.76	96.10	98.929
	20	19.55 ± 0.35	19.75 ± 0.37	97.77	98.7725
	30	29.68 ± 0.69	28.91 ± 0.87	98.95	96.37
THY	**10**	9.68 ± 0.42	9.64 ± 0.35	96.80	96.49
	20	19.41 ± 0.18	19.44 ± 0.08	97.06	97.24
	30	29.76 ± 0.65	29.33 ± 0.26	99.23	97.79
(B) Ruggedness Score of Imiquimod and Thymoquinone by the different investigator on same HPLC system.					
Drug Sample	**Sample Concentration (µg/mL)**	**Amount Recovered**	**Recovery (%)**		
		Investigator-I	**Investigator-II**	**Investigator-I**	**Investigator-II**
IMI	**10**	9.74 ± 1.55	9.85 ± 1.77	97.42	98.57
	20	19.43 ± 1.21	19.89 ± 0.77	97.19	99.48
	30	29.73 ± 1.46	29.41 ± 0.94	99.12	98.03
THY	**10**	9.85 ± 1.24	9.65 ± 0.41	98.51	96.53
	20	19.77 ± 0.25	19.55 ± 0.09	98.87	97.79

Table 3: Ruggedness Score of Imiquimod and Thymoquinone at λmax 249 nm

The robustness of the test was used to evaluate the reproducibility of the outcomes obtained on different HPLCs by the same investigator and on the same HPLC by different investigators. The test was then repeated on different HPLCs by the same analyst. The recovered concentration of IMI for sample concentration 10, 20 and 30 µg/ml at 249 nm came out to be 9.61 ± 0.68, 19.55 ± 0.35 and 29.68 ± 0.69 µg/ml along with percentage recovery of 96.10, 97.77 and 98.95 % respectively on HPLC-1 and the recovered concentration was 9.89 ± 0.76, 19.75 ± 0.37 and 28.91 ± 0.87 µg/ml with percentage recovery 98.92, 98.77 and 96.37 % respectively on HPLC -2. Similarly, the recovered concentration of THY by the same analyst for sample concentration 10, 20 and 30 µg/ml at 249 nm came out to be 9.68 ± 0.42, 19.41 ± 0.18 and 29.76 ± 0.65 µg/ml along with percentage recovery of 96.80, 97.07 and 99.23 % respectively on HPLC-1 and the recovered concentration was 9.64 ± 0.35, 19.44 ± 0.08 and 29.33 ± 0.26 µg/ml with percentage recovery 96.49, 97.24 and 97.79% respectively on HPLC -2. (Table 3(A)).

The ruggedness data was then collected by different analysts using the same HPLC (HPLC-1). The recovered concentration of IMI for sample concentration 10, 20 and 30 µg/ml at 249 nm came out to be 9.74 ± 1.55, 19.43 ± 1.21 and 29.73 ± 1.46 µg /ml along with percentage recovery of 97.42, 97.19 and 99.12 % respectively by analyst-1 and the recovered concentration was 9.85 ± 1.77, 19.89 ± 0.77 and 29.41 ± 0.94 µg/ml with percentage recovery 98.57, 99.48 and 98.03 % respectively by analyst-2. Similarly, the recovered concentration of THY for-sample concentration 10, 20 and 30 µg/ml at 249 nm came out to be 9.85 ± 1.24, 19.77 ± 0.25 and 29.62 ± 1.05 µg/ml along with percentage recovery of 98.51, 98.87 and 98.76 % respectively by analyst-1 and the recovered concentration was 9.65 ± 0.41, 19.55 ± 0.09 and 9.71 ± 0.70 µg/ml with percentage recovery 96.53, 97.79 and 99.05 % respectively by analyst-2.

The study results show a significant level of ruggedness, implying that the HPLC analytical method is experimentally verified on separate systems and by varied researchers. The results obtained from validation are congenial with ICH Guidelines. (Sharma, Goyal, & Chauhan, 2018), (Swartz & Krull, 1997)

CONCLUSION

The current research presents an innovative method for estimating IMI and THY simultaneously in assay procedures and process monitoring. The methodology is simple, rapid, highly selective, sensitive and precise, and it has been validated per ICH guidelines.

The method takes only around 16 minutes to complete. Thus, faster elution of the combination with high resolution saves researcher time and solvents, making the proposed method economical and quick for routine analysis. This method could be used to simultaneously estimate both analytes (IMI and THY) in formulation development, assay procedures and quality control checks.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

The authors would like to acknowledge GD Goenka University, Gurugram for furnishing all paramount machinery and material to carry out the experimentation work.

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Journal of Chemical&Engineering Data

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Understanding Interactions between Amino Acids and Food Additives via Physicochemical and Spectroscopic Approaches

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Cite This: *J. Chem. Eng. Data* 2023, 68, 4−13 Read Online

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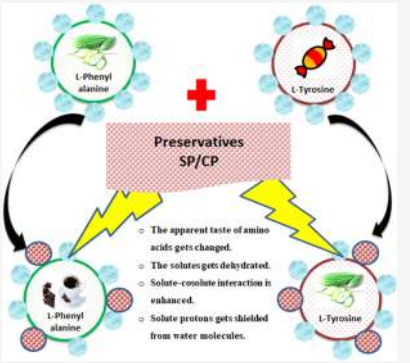
ABSTRACT: The effects of preservatives (E281 and E282) on the physicochemical and structural properties of amino acids in processed foods and other commodities have been studied using thermophysical (density and viscosity) and spectroscopic techniques in aqueous solutions. The explication of the derived properties shows that the addition of a cosolute intensifies the densities and viscosities of L-phenylalanine and L-tyrosine aqueous solutions and changes in the taste and hydration numbers of solute molecules. Preservatives have been designed to be physically and spectroscopically dehydrating; they thereby enhance solute−cosolute interactions over the hydration effect.



1. INTRODUCTION

The pre-eminence of amino acids in biological processes[1−3] is evaluated in a significantly important research class. Undoubtedly, as an essential constituent of nutrition, amino acids are widely used to produce fertilizers, drugs, medicines, biodegradable plastics, and chiral catalysts.[4−6] The hydrophobic aromatic rings of essential and nonessential amino acids[7,8] such as L-phenylalanine and L-tyrosine have applications in the food, pharmaceutical, and agriculture industries. L-Phenylalanine, primarily utilized in producing food items and traded as dietary supplements, has the breast milk of mammals as its primary natural source. Apart from its consumption in manufacturing food items and nutritional supplements, pharmaceutical practices involve it as analgesics and antidepressants to treat depression, Parkinson's disease, rheumatoid arthritis, chronic pain, osteoarthritis, and alcohol withdrawal symptoms. The process of protein synthesis in cells involves L-tyrosine or 4-hydroxyphenylalanine (substituted with a polar side group) and their derivatives (L-DOPA, melanin, phenylpropanoids, and others) employed in pharmaceuticals as precursors to neurotransmitters, dietary supplements, and food additives. However, amino acids are always accompanied by other additives such as fortifying agents, colors, antioxidants, preservatives, and flavor enhancers in processed foods, medicines, fertilizers, and drugs, as a pervasive component. These additives in the above-mentioned products have been considered physiologically inert, as suggested by the Food and Drug Administration (FDA). Chemical preservatives are the top category of chemicals used in the current international market, finding applications as flavor enhancement agents, improving nutritive values, and helping to diminish microbial growth and spoilage. Sodium propionate, also known as E281/SP, and calcium propionate, also known as E282/CP, are propionate-based preservatives that are listed as E numbers in the Codex Alimentarius.[9]

These preservatives help prevent mold development in bakery and dairy preparations and act as an antifungal agent employed in the treatment of mycoses.[10−12] In agronomy, prevention of milk fever and feeding supplementation in cows are the major chores accomplished by these propionate salts. As prescribed by the research evidence procured by the Ministry of Health of Turkey, CP is nontoxic[13] and harmless if utilized to the maximum limit of 3000 mg kg^{-1}. However, the presence of this salt may impact the physicochemical properties, such as enthalpy, hydration, molecular structure, taste behavior, and water structuring characteristics. Therefore, aqueous solutions of these propionate-based preservatives need to be explored in terms of properties and structures[14−16] to understand their significant impact on the biologically important molecules (amino acids). As a result, the effect of CP and SP on the volumetric transport behavior, structural properties, and taste quality of L-phenylalanine and L-tyrosine at various concentrations and temperatures is reported in this paper. Moreover, the changes in the hydration properties and

Received: June 20, 2022
Accepted: November 23, 2022
Published: December 7, 2022

Table 1. Specification of the Chemicals Used

compound [molecular formula]	molar mass (g·mol^{-1})	mass fraction purity[a]	source	CAS number
calcium propionate [$C_6H_{10}CaO_4$]	186.22	0.97	Sisco Research Lab.	4075-81-4
sodium propionate [$C_3H_5NaO_2$]	96.06	0.99	Sisco Research Lab.	137-40-6
L-tyrosine [$C_9H_{11}NO_3$]	181.19	≥0.99	Sigma Chemical Co.	60-18-4
L-phenylalanine [$C_9H_{11}NO_2$]	165.19	≥0.99	Sigma Chemical Co.	63-91-2
deuterium oxide [D_2O]	20.02	≥0.999 D atom[b]	Sigma Chemical Co.	7789-20-0
sodium chloride [NaCl]	58.44	≥0.99	Sisco Research Lab.	7647-14-5

[a]Declared by the supplier. [b]Isotopic purity of D_2O as declared by the supplier.

taste behavior of amino acids in various concentrations of these salts have been explored using spectroscopic methods.

2. EXPERIMENTAL SECTION

2.1. Materials. Table 1 lists the chemical specifications that were used. Anhydrous $CaCl_2$ has been used in a vacuum desiccator to absorb the chemicals' moisture before experimentation. A Mettler balance accurate to a value of ±0.01 mg has been utilized to prepare the solutions. To prepare the solutions, the water is procured from an Ultra UV/UF Rions lab water system. The specific conductance was less than 1.29 × 10^{-4} S m^{-1}. The molality of the prepared aqueous solutions in this report has a relative uncertainty of $u_r(m_A) = u(m_A)/m_A = 0.03000$ and $u_r(m_B) = u(m_B)/m_B = 0.01$.

2.2. Methods. To determine the densities of the solutions, a digital densimeter (Model: DMA 60/602, Anton Paar, Austria) equipped with a Julabo F-25, thermostatic water bath with a stability of ±0.01 K, was used. Before using the densimeter, calibration was carried out employing dry air and pure water. Furthermore, the working of the instrument was checked by measuring the densities of $NaCl_{(aq)}$ solutions at 298.15 and 308.15 K (Table 2), which agreed well with the literature values.[17] The standard uncertainty in density measurements, $u(\rho)$, which is a function of temperature, time periods, constant K, and effect of sample purity, is 0.000011 g·m^{-3}.

The viscosities of solutions have been evaluated using a Ubelohde-type capillary viscometer further employed with a Julabo F-25. The flow times of various aqueous solutions were measured with the help of a digital stopwatch (resolution of ±0.01 s). The viscometer is calibrated by measuring the efflux time of water at 298.15 and 318.15 K. The values are in good agreement with the literature values.[18] The relative uncertainty in viscosities is $u(\eta)/\eta = 0.0101$, which also includes 1% relative uncertainty in viscosity of the calibrated solvent, i.e., water.

The ultraviolet absorption spectra of pure L-phenylalanine and L-tyrosine and CP and SP aqueous solutions were enumerated using a SHIMADZU (UV-1800) spectrophotometer furnished with a quartz cuvette of 0.01 m path length. The wavelength range for the study of absorption spectra has been chosen to be 200−400 nm, and each spectrum was corrected for the baseline. Aliquots (2 mL) of 10^{-3} mol·kg^{-1} solutes have been titrated by consecutive additions (20 μL) of 1.0 mol·kg^{-1} SP and CP solution, maintaining the solute molality constant in the cell. The consistency for λ_{max} of the recorded spectra was found to be within ±0.1 nm.

^{1}H and ^{13}C NMR spectra have been obtained by employing a NMR spectrometer (Bruker Ascend 500 MHz) at 300.15 K. As a lock solvent, D_2O has been utilized, and the center of the HDO signal has been used to establish the chemical shifts, δ, for other nuclei. The NMR spectra of sheer phenylalanine and tyrosine and mixtures with CP and SP have been recorded. The observations in terms of change in the chemical shifts (Δδ) and different concentrations of cosolutes were characterized and discussed.

3. RESULTS AND DISCUSSION

3.1. Volumetric Properties. The apparent molar volumes, $V_{2,\phi}$, of L-phenylalanine and L-tyrosine have been determined in water and in m_B = (0.1, 0.25, 0.50, and 0.75) mol·kg^{-1} SP and CP aqueous solutions from the density measurements at varied temperatures (298.15 and 308.15 K) by means of relation

$$V_{2,\phi} = \{M/\rho\} - \{(\rho - \rho_o)/(m_A\rho\rho_o)\} \quad (1)$$

where molar mass and molality terms of amino acids are denoted by M and m_A, respectively, and ρ_o and ρ are the symbols used to denote densities of solvent and ternary solutions, respectively. The density values of solutes in water and in SP and CP aqueous solutions are given in Table 2. The density data for both L-phenylalanine and L-tyrosine in water are compared with the literature values.[19−27] The experimental densities for L-phenylalanine in water show good agreement with the literature data[19−21,23,24,27] at 298.15 K (Figure S1A) and show some deviations from the literature values[22,25,26] for L-phenylalanine at 308.15 K and for L-tyrosine at 298.15 and 308.15 K (Figure S1B−D). Das and Dash[22] have reported that the densities values for (0.0100−0.0811) mol·kg^{-1} L-phenylalanine in water are lower than the present values at 308.15 K (Figure S1B). The density values for L-tyrosine reported by Carto[25] have been compared with the present data, which are higher than those reported by Carto[25] at 298.15 and 308.15 K (Figure S1C,D). Savaroglu and Ildaser[26] have reported the densities for (0.000125−0.002000) mol·kg^{-1} L-tyrosine in water, and their values of densities are slightly higher than the present values at 298.15 K, whereas at 308.15 K, the present density values are higher (Figure S1C,D). The small deviation in the values may be due to differences in the concentration ranges studied, and other factors including purity of materials, solution preparation, experimental methods, etc. may also be responsible for the deviations.

The apparent molar volumes of solutes in water and SP and CP aqueous solutions are given in Table S1. The trend in the data depicts the increase in densities and $V_{2,\phi}$ values of amino acids with ascending concentrations of SP and CP. Solute−solvent interactions experience an intensification as the molar mass of the solutes used in the present study increases. The higher $V_{2,\phi}$ values in aqueous solutions of CP compared to those of SP indicate that Ca^{2+} ions have a greater dehydration effect on these amino acids than that of Na^+ ions.[28] Furthermore, standard partial molar volumes at infinite dilution, $V_{2,\phi}^o$, were evaluated using the following relation

https://doi.org/10.1021/acs.jced.2c00394
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Table 2. Densities, ρ, and Viscosities, η, of Sodium Chloride in Water and L-Phenylalanine and L-Tyrosine in Water and in Aqueous Solutions of SP and CP over T/K = 298.15 and 308.15 and at the Pressure p = 0.1 MPa[ab]

m (mol·kg^{-1})	ρ (g·cm^{-3})	η (mPa·s)	ρ (g·cm^{-3})	η (mPa·s)
	298.15 K		308.15 K	
Sodium Chloride in Water				
0.10215	1.000936		0.997782	
0.15234	1.002818		0.999591	
0.22547	1.005532		1.002201	
0.27125	1.007211		1.003813	
0.31264	1.008711		1.005254	
0.36417	1.010572		1.007040	
0.41201	1.012280		1.008679	
L-Phenylalanine in Water				
0.00495	0.997265	0.8969	0.994275	0.7221
0.00660	0.997336	0.8976	0.994346	0.7249
0.00922	0.997451	0.8986	0.994457	0.7269
0.02970	0.998342	0.9052	0.995329	0.7321
0.04998	0.999214	0.9144	0.996186	0.7400
0.06943	1.000042	0.9266	0.997000	0.7477
L-Phenylalanine in Aqueous Sodium Propionate Solutions at m_B = 0.10 mol·kg^{-1}				
0.00496	1.001371	0.9430	0.998314	0.7664
0.00678	1.001447	0.9446	0.998389	0.7672
0.00932	1.001553	0.9455	0.998492	0.7684
0.01029	1.001593	0.9463	0.998525	0.7685
0.03000	1.002413	0.9538	0.999330	0.7749
0.05113	1.003281	0.9652	1.000172	0.7833
L-Phenylalanine in Aqueous Sodium Propionate Solutions at m_B = 0.25 mol·kg^{-1}				
0.00517	1.007527	0.9984	1.004416	0.8109
0.00696	1.007600	0.9992	1.004479	0.8116
0.00897	1.007682	1.0001	1.004580	0.8133
0.01019	1.007732	1.0009	1.004611	0.8149
0.02909	1.008498	1.0099	1.005361	0.8238
L-Phenylalanine in Aqueous Sodium Propionate Solutions at m_B = 0.50 mol·kg^{-1}				
0.00538	1.017731	1.1129	1.013314	0.9064
0.00927	1.017883	1.1145	1.013515	0.9073
0.01045	1.017928	1.1158	1.013611	0.9079
0.03015	1.018689	1.1216	1.014640	0.9102
0.05000	1.019451	1.1292	1.015393	0.9152
L-Phenylalanine in Aqueous Sodium Propionate Solutions at m_B = 0.75 mol·kg^{-1}				
0.00551	1.026451	1.1935	1.022842	0.9590
0.00725	1.026515	1.1949	1.022905	0.9604
0.00944	1.026596	1.1960	1.022984	0.9627
0.01079	1.026646	1.1981	1.023033	0.9636
0.03113	1.027390	1.2119	1.023760	0.9713
L-Phenylalanine in Aqueous Calcium Propionate Solutions at m_B = 0.1 mol·kg^{-1}				
0.00516	1.005812	0.9866	1.002773	0.8037
0.00723	1.005897	0.9878	1.002857	0.8048
0.00900	1.005969	0.9896	1.002929	0.8052
0.01000	1.006011	0.9914	1.002970	0.8059
0.03258	1.006929	1.0009	1.003882	0.8126
L-Phenylalanine in Aqueous Calcium Propionate Solutions at m_B = 0.25 mol·kg^{-1}				
0.00586	1.019163	1.1435	1.015865	0.9192
0.00700	1.019208	1.1460	1.015909	0.9224
0.00900	1.019286	1.1478	1.015986	0.9236
0.01032	1.019338	1.1493	1.016037	0.9254
0.03149	1.020160	1.1607	1.016851	0.9325
L-Phenylalanine in Aqueous Calcium Propionate Solutions at m_B = 0.50 mol·kg^{-1}				
0.00586	1.035504	1.3732	1.032051	1.0890
0.00597	1.035508	1.3758	1.032062	1.0926
0.00819	1.035590	1.3797	1.032148	1.0936
0.01032	1.035669	1.3824	1.032215	1.0956
0.03116	1.036438	1.3937	1.033008	1.1055
L-Phenylalanine in Aqueous Calcium Propionate Solutions at m_B = 0.75 mol·kg^{-1}				
0.00519	1.052744	1.7296	1.049585	1.3671
0.00678	1.052798	1.7307	1.049638	1.3684
0.00831	1.052850	1.7339	1.049690	1.3711
0.01115	1.052946	1.7369	1.049785	1.3732
0.03125	1.053622	1.7519	1.050453	1.3863
L-Tyrosine in Water				
0.00011	0.997053	0.8905	0.994069	0.7195
0.00034	0.997066	0.8906	0.994082	0.7196
0.00059	0.997080	0.8908	0.994096	0.7197
0.00084	0.997094	0.8908	0.994110	0.7198
0.00106	0.997107	0.8909	0.994122	0.7198
L-Tyrosine in Aqueous Sodium Propionate Solutions at m_B = 0.1 mol·kg^{-1}				
0.00019	1.001166	0.9370	0.998124	0.7622
0.00035	1.001174	0.9371	0.998139	0.7623
0.00049	1.001182	0.9373	0.998146	0.7624
0.00072	1.001195	0.9374	0.998159	0.7624
0.00100	1.001211	0.9374	0.998174	0.7625
L-Tyrosine in Aqueous Sodium Propionate Solutions at m_B = 0.25 mol·kg^{-1}				
0.00039	1.007178	0.9847	1.004117	0.7991
0.00053	1.007186	0.9848	1.004124	0.7993
0.00077	1.007199	0.9848	1.004137	0.7993
0.00098	1.007210	0.9850	1.004148	0.7994
L-Tyrosine in Aqueous Sodium Propionate Solutions at m_B = 0.5 mol·kg^{-1}				
0.00025	1.017132	1.1034	1.013817	0.9091
0.00040	1.017140	1.1035	1.013825	0.9109
0.00066	1.017154	1.1036	1.013838	0.9118
0.00084	1.017163	1.1038	1.013847	0.9132
0.00107	1.017175	1.1039	1.013859	0.9145
L-Tyrosine in Aqueous Sodium Propionate Solutions at m_B = 0.75 mol·kg^{-1}				
0.00035	1.025974	1.1847	1.022463	0.9561
0.00063	1.025988	1.1848	1.022477	0.9563
0.00075	1.025994	1.1850	1.022483	0.9563
0.00096	1.026005	1.1851	1.022494	0.9564
L-Tyrosine in Aqueous Calcium Propionate Solutions at m_B = 0.1 mol·kg^{-1}				
0.00021	1.005998	0.9875	1.00294	0.8003
0.00039	1.006008	0.9876	1.00295	0.8004
0.00060	1.006019	0.9878	1.00297	0.8005
0.00089	1.006035	0.9879	1.00298	0.8006
0.00107	1.006045	0.9880	1.00299	0.8008
L-Tyrosine in Aqueous Calcium Propionate Solutions at m_B = 0.25 mol·kg^{-1}				
0.00019	1.017433	1.1242	1.01418	0.9052
0.00039	1.017443	1.1243	1.01419	0.9053
0.00058	1.017453	1.1244	1.01420	0.9054
0.00085	1.017467	1.1246	1.01422	0.9056
0.00103	1.017476	1.1248	1.01422	0.9052
L-Tyrosine in Aqueous Calcium Propionate Solutions at m_B = 0.5 mol·kg^{-1}				
0.00024	1.036691	1.3559	1.033120	1.0919
0.00040	1.036699	1.3560	1.033128	1.0920
0.00056	1.036707	1.3563	1.033136	1.0921
0.00074	1.036716	1.3564	1.033144	1.0923
0.00096	1.036727	1.3567	1.033155	1.0925
L-Tyrosine in Aqueous Calcium Propionate Solutions at m_B = 0.75 mol·kg^{-1}				

https://doi.org/10.1021/acs.jced.2c00394
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Table 2. continued

m (mol·kg^{-1})	ρ (g·cm^{-3})	η (mPa·s)	ρ (g·cm^{-3})	η (mPa·s)
	298.15 K		308.15 K	
0.00039	1.053693	1.7260	1.049962	1.1498
0.00048	1.053697	1.7261	1.049966	1.1499
0.00059	1.053702	1.7262	1.049971	1.1501
0.00078	1.053711	1.7264	1.049980	1.1504
0.00099	1.053721	1.7267	1.049990	1.1508

[a]m_B is the molality of CP/SP in water. [b]m_A is the molality of the solute in water or water + SP/CP. Standard uncertainties are $u(T)$ = 0.01 K, $u_r(m_A) = u(m_A)/m_A$ = 0.03000 and $u_r(m_B) = u(m_B)/m_B$ = 0.01, $u(\rho)$ = 0.000011 g·m^{-3}, $u(\eta)/\eta$ = 0.0101, and $u(p)$ = 0.0005 MPa.

Table 3. Partial Molar Volumes at Infinite Dilution ($V_{2,\phi}^{\circ}$) and Hydration Numbers, N_h, of L-Phenylalanine and L-Tyrosine in $SP_{(aq)}$ and $CP_{(aq)}$ Solutions at Temperature T = (298.15 and 308.15)K

m_B (mol·kg^{-1})	$V_{2,\phi}^{\circ}$ (cm^3·mol^{-1})	N_h	$V_{2,\phi}^{\circ}$ (cm^3·mol^{-1})	N_h
	298.15 K		308.15 K	
	L-Phenylalanine in Sodium Propionate$_{(aq)}$ Solutions			
0.00	121.474 ± 0.038 (6.60)[a] (121.80)[b]	4.96	122.782 ± 0.026 (3.18) (122.77)	4.82
0.10	122.901 ± 0.054 (9.85)	4.48	123.952 ± 0.064 (16.06)	4.43
0.25	123.714 ± 0.027 (3.96)	4.21	124.521 ± 0.032 (15.85)	4.24
0.50	124.611 ± 0.039 (4.71)	3.91	125.660 ± 0.034 (7.87)	3.86
0.75	125.784 ± 0.008 (6.13)	3.52	126.461 ± 0.053 (17.57)	3.60
	L-Phenylalanine in Calcium Propionate$_{(aq)}$ Solutions			
0.10	123.431 ± 0.009 (10.04)	4.30	123.990 ± 0.035 (9.38)	4.41
0.25	123.951 ± 0.028 (3.55)	4.13	124.752 ± 0.036 (10.51)	4.16
0.50	124.881 ± 0.009 (2.02)	3.82	125.801 ± 0.027 (3.32)	3.81
0.75	125.972 ± 0.035 (12.06)	3.46	126.571 ± 0.007 (17.41)	3.55
	L-Tyrosine in Sodium Propionate$_{(aq)}$ Solutions			
0.00	124.431 ± 0.003 (−75.10) (124.58)	5.23	125.275 ± 0.001 (−83.29) (126.63)	5.24
0.10	125.251 ± 0.002 (−60.31)	4.95	126.272 ± 0.006 (−63.02)	4.90
0.25	126.313 ± 0.002 (−69.58)	4.60	127.234 ± 0.001 (−49.24)	4.58
0.50	127.321 ± 0.003 (−62.49)	4.26	128.552 ± 0.005 (−56.88)	4.14
0.75	128.351 ± 0.002 (−50.13)	3.92	129.312 ± 0.002 (−50.14)	3.89
	L-Tyrosine in Calcium Propionate$_{(aq)}$ Solutions			
0.10	126.491 ± 0.004 (−67.61)	4.54	127.442 ± 0.004 (−66.96)	4.51
0.25	127.672 ± 0.004 (−60.39)	4.15	128.653 ± 0.004 (−55.64)	4.11
0.50	128.582 ± 0.002 (−67.83)	3.84	129.681 ± 0.002 (−67.84)	3.77
0.75	129.502 ± 0.003 (−64.51)	3.54	130.523 ± 0.003 (−64.52)	3.49

[a]Parenthesis contains S_v values. [b]Parenthesis contains literature values of L-phenylalanine/L-tyrosine in water. "±" values are the standard deviation.

$$V_{2,\phi} = V_{2,\phi}^{\circ} + S_v m \quad (2)$$

where the symbol S_v stands for the slope.

The $V_{2,\phi}^{\circ}$ values calculated for amino acids prove good concurrence with the literature values.[29] The solitary presence of amino acids in water contributed to lower $V_{2,\phi}^{\circ}$ values (Table 3). In contrast, the mutual presence of preservatives enhanced the values, which further showed incremental behavior with ascending cosolute concentration and ascending temperature. The high charge density of Ca^{2+} ions may result in higher $V_{2,\phi}^{\circ}$ values of L-phenylalanine and L-tyrosine in CP aqueous solutions compared to those in SP aqueous solutions. Moreover, the strong hydration effect of CP affects the exclusion of water from the amino acid hydration shell. Here, it can be pointed out that a 2:1 electrolyte ($(CH_3COO^-)_2$ Ca^{2+}) persuades the $V_{2,\phi}^{\circ}$ values to change to a more considerable extent as compared to what a 1:1 electrolyte (CH_3COO^- Na^+) does. Here, it is pertinent to mention that the ratios 2:1 and 1:1 signify the charge ratio in electrolytes. Additionally, S_v represents the volumetric virial parameter and gives evidence for the solute−solute interactions. The S_v values have very small magnitudes in comparison to V_2°, which shows the predominance of solute−solvent interactions over the solute−solute interactions. Furthermore, partial molar volumes of transfer, $\Delta_{tr}V_{2,\phi}^{\circ}$, for the studied amino acids at infinite dilution from water to SP and CP aqueous solutions were estimated as

$$\Delta_{tr}V_{2,\phi}^{o} = V_{2,\phi}^{o}\ (\text{in aqueous SP/CP}) - V_{2,\phi}^{o}\ (\text{in } H_2O) \quad (3)$$

The data of $\Delta_{tr}V_{2,\phi}^{\circ}$ are given in Table 4. Constructive $\Delta_{tr}V_{2,\phi}^{\circ}$ values of L-phenylalanine and L-tyrosine were observed in both SP and CP (Figure 1) and increased with a increase in the concentration of the cosolute, and the magnitude of transfer values are higher for CP compared to those for SP. In L-phenylalanine, $\Delta_{tr}V_{2,\phi}^{\circ}$ values show a descent with ascending temperature, whereas the $\Delta_{tr}V_{2,\phi}^{\circ}$ values follow the reverse trend with temperature for L-tyrosine. Moreover, the difference in the magnitude of transfer values at higher molalities is more extensive than in L-phenylalanine. The $\Delta_{tr}V_{2,\phi}^{\circ}$ values are more significant for L-tyrosine than for L-phenylalanine, which could be due to its more polar character, induced by the presence of the −OH group on the aromatic ring, making it hydrophilic.

Following the co-sphere overlap model,[30] the most probable form of interactions between the molecules of the solute and cosolute is due to the displacement of the co-sphere material when there is an overlap between their hydration spheres,

https://doi.org/10.1021/acs.jced.2c00394
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Table 4. Volume of Transfer, $\Delta_{tr}V_{2,\phi}^{o}$, and Viscosity *B*-Coefficients of Transfer, $\Delta_{tr}B$, for L-Phenylalanine and L-Tyrosine in CP and SP Aqueous Solutions at T = (298.15 and 308.1)K

m_B (mol·kg^{-1})	$\Delta_{tr}V_{2,\phi}^{o}$ (cm^3·mol^{-1})	$\Delta_{tr}B$ (cm^3·mol^{-1})	$\Delta_{tr}V_{2,\phi}^{o}$ (cm^3·mol^{-1})	$\Delta_{tr}B$ (cm^3·mol^{-1})
	298.15 K		308.15 K	
L-phenylalanine in SP aqueous solutions				
0.10	1.43	0.014	1.17	0.011
0.25	2.24	0.015	1.74	0.012
0.50	3.14	0.016	2.88	0.013
0.75	4.31	0.019	3.68	0.017
L-phenylalanine in CP aqueous solutions				
0.10	1.96	0.014	1.21	0.011
0.25	2.48	0.017	1.97	0.014
0.50	3.41	0.018	3.02	0.015
0.75	4.5	0.02	3.79	0.017
L-tyrosine in SP aqueous solutions				
0.10	0.82	0.003	1	0
0.25	1.88	0.003	1.96	0.001
0.50	2.89	0.004	3.24	0.001
0.75	3.92	0.005	4.04	0.002
L-tyrosine in CP aqueous solutions				
0.10	2.06	0.012	2.17	0.01
0.25	3.24	0.013	3.38	0.011
0.50	4.15	0.013	4.41	0.011
0.75	5.07	0.014	5.25	0.012

which further causes the variations in the thermodynamic parameters of solutions. Positive $\Delta_{tr}V_{2,\phi}^{o}$ values (Figure 1) result from hydrophilic−ionic interactions among hydrophilic−ionic (−OH, $-NH_3^+$, $-COO^-$) sites of solutes and ions (Na^+, Ca^{2+}, $C_3H_5O_2^-$) of cosolutes. In contrast, negative $\Delta_{tr}V_{2,\phi}^{o}$ values result from hydrophobic−hydrophobic/ionic interactions amid ions of cosolute molecules and solute molecules. Mutual interactions among L-phenylalanine and L-tyrosine with cosolute molecules cause dehydration of both the solutes and cosolute, liberating more water as bulk water. The positive $\Delta_{tr}V_{2,\phi}^{o}$ values possibly result from the more significant volume contribution via bulk water, with CP having a more substantial dehydration effect. Preservatives had a more significant dehydration impact on L-phenylalanine and L-tyrosine than on L-proline and L-serine[31] and on L-leucine, *β*-alanine, and L-glycine.[32]

3.2. Apparent Massic Volume (v_ϕ) and Taste Behavior. Apparent massic volumes, v_ϕ, define the accessibility and efficiency of a solute to stimulate the taste receptor. The wide range for human taste observation is probably restricted to molecules having v_ϕ between 0.1 and 0.95 cm^3 g^{-1}, which can be determined as

$$v_\phi = V_{2,\phi}\ /\mathrm{M} \tag{4}$$

The v_ϕ values are related to the taste behavior in the order bitter > sweet > sour >salty[33] and indicate the depth of receivers in the taste bud. The v_ϕ values for L-tyrosine in water (Table S1) fall in the sweet taste quality range (0.52−0.71) cm^3 g^{-1}, which descends in the presence of preservatives, whereas values for L-phenylalanine lie in the bitter taste range (0.71−0.93) cm^3 g^{-1}, which becomes more bitter with the addition of SP and CP.

3.3. Hydration Number. The hydration number, N_h, of the amino acid was evaluated by using the relation[34]

$$\Delta_{tr}V_{2,\phi}^{o} = [N_h\ (\text{in water}) - N_h\ (\text{in SP and CP})]\ {}^{*}3.0 \tag{5}$$

Further N_h values of L-phenylalanine and L-tyrosine in water were calculated employing the equation earlier used by[35] as follows

$$N_h\ (\text{in water}) = V_{elect}/(V_e^{o} - V_b^{o}) \tag{6}$$

where the symbols V_e^{o} and V_b^{o} represent the molar volume of electrostricted and bulk water, respectively. The $(V_e^{o}-V_b^{o})$ values are about −3.47 and −3.3 cm^3·mol^{-1} at 298.15[36] and 308.15 K,[37] respectively. The calculation of V_{elect} values has been done using the relation

$$V_{elect} = V_{2,\phi}^{o} - V_{int}^{o} \tag{7}$$

where intrinsic molar volume, V_{int}^{o}, was obtained by the crystallographic volume[38] V_{cryst}.

$$V_{int}^{o} = (0.7/0.634)/V_{cryst} \tag{8}$$

Table 3 represents the hydration number, N_h, stipulating the dehydration of amino acids, i.e., L-phenylalanine and L-tyrosine, in association with the preservative molecules, contributing further to concentration increases of the cosolute. The N_h values for L-tyrosine are more significant in magnitude than those of L-phenylalanine. However, a further decrease in N_h values with concentration is prominent in L-phenylalanine compared to those of L-tyrosine, suggesting higher solute−cosolute interactions and less electrostatic influence. The N_h values show that calcium propionate offers itself as a superior preservative intended for the dehydration process. As a result,

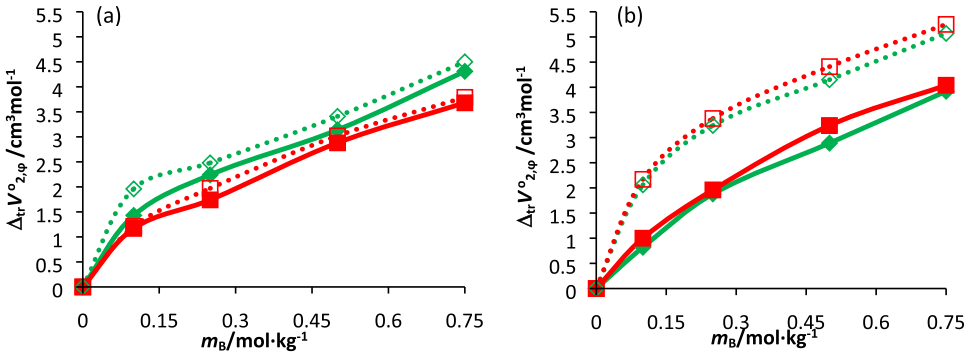

Figure 1. Partial molar volume of transfer, $\Delta_{tr}V_{2,\phi}^{o}$, versus molality, m_B, of SP and CP for (a) L-phenylalanine (b) L-tyrosine at ◆ 298.15 K and ■ 308.1 K K (dotted line: CP, solid line: SP).

https://doi.org/10.1021/acs.jced.2c00394
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Table 5. Viscosity B Coefficients, $B/V_{2,\phi}^{\circ}$, and dB/dT for L-Phenylalanine and L-Tyrosine in CP and SP Aqueous Solutions at T = (298.15 and 308.15)K

m_B (mol·kg^{-1})	B (cm^3·mol^{-1})	$B/V_{2,\phi}^{\circ}$ (cm^3·mol^{-1})	B (cm^3·mol^{-1})	$B/V^{\circ}_{2,\phi}$ (cm^3·mol^{-1})	10^{-4} dB/dT (cm^3·mol^1·K^1)
	298.15 K		308.15 K		
		L-phenylalanine in SP aqueous solutions			
0.00	0.585	4.81	0.589	4.80	5
0.10	0.599	4.87	0.600	4.85	1
0.25	0.600	4.85	0.601	4.85	1
0.50	0.601	4.82	0.602	4.82	1
0.75	0.604	4.80	0.606	4.80	2
		L-phenylalanine in CP aqueous solutions			
0.10	0.599	4.83	0.600	4.83	1
0.25	0.602	4.82	0.603	4.83	1
0.50	0.603	4.79	0.604	4.80	2
0.75	0.605	4.78	0.606	4.78	1
		L-tyrosine in SP aqueous solutions			
0.00	0.625	5.02	0.628	5.01	3
0.10	0.628	5.01	0.628	5.03	1
0.25	0.628	4.97	0.629	4.99	1
0.50	0.629	4.93	0.629	4.96	1
0.75	0.629	4.90	0.630	5.13	1
		L-tyrosine in CP aqueous solutions			
0.10	0.637	4.97	0.638	5.00	1
0.25	0.638	4.94	0.639	4.96	1
0.50	0.638	4.89	0.639	4.92	1
0.75	0.639	4.87	0.640	5.10	1

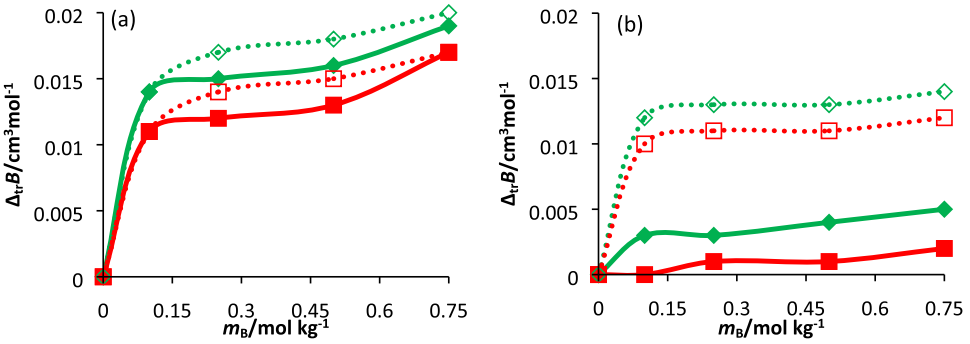

Figure 2. Viscosity B coefficients of transfer $\Delta_{tr}B$ versus molality m_B of SP and CP for (a) L-phenylalanine (b) L-tyrosine at ◆ 298.15 K and ■ 308.15 K (dotted line: CP, solid line: SP).

such properties are utilized to preserve dairy products by eliminating water in curds and cheese via salting. It reduces the chances of microbial spoilage and pathogen growth, enhancing the shelf life of foodstuffs.[39−41]

3.4. Viscosity. The flow time measurements have been used to calculate the viscosities η of solutions using the following relation

$$\eta/\rho = at - (b/t) \tag{9}$$

where t is used to represent the efflux time of solutions and a and b represent the viscometric constants. The η data for both L-phenylalanine in water are compared with the literature values.[20−23,42] The experimental viscosities for both solutes studied in water show some deviation from the literature data[20,22] (Figure S2A,B). Das and Dash[22] have reported the viscosities for (0.0100−0.0811) mol·kg^{-1} L-phenylalanine in water, and their values of viscosities are slightly higher than the present values at (298.15 and 308.15) K (Figure S2A,B). The η values reported by Kumar et al.[20] for (0.02−0.12) mol·kg^{-1} L-phenylalanine in water are lower than the present values at (298.15 and 308.15) K (Figure S2A,B). The small deviation in the values may be due to differences in the concentration ranges studied, and other factors including purity of materials, solution preparation, experimental methods, etc. may also be responsible for the deviations.

The viscosity B-coefficients were evaluated from the relative viscosities (Table 2), which were fitted in the following (Jones−Dole) relation[43]

$$\eta_r = 1 + Bc \tag{10}$$

where c is the molarity of solutions and the viscosity B coefficient has repercussions on the solute−solvent interactions and the structure of the solvent when present in the nearest contact of the solute molecules. Temperature induces a increase in the B values of amino acids (Table 5), as does the molality of the cosolute, reflecting it more in CP than in SP. The variation of the B coefficient is closely associated with the size of solute molecules; hence, the B value for L-tyrosine is

https://doi.org/10.1021/acs.jced.2c00394
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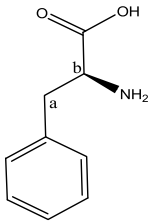
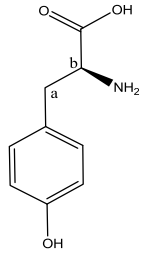
Table 6. ^{1}H NMR Data for L-Phenylalanine and L-Tyrosine in the Presence and in the Absence of CP and SP

Solutes	$\delta_{aqueous}$(ppm)	$\delta_{with\ NP}$(ppm)	$\Delta\delta$ (ppm)	$\delta_{with\ CP}$(ppm)	$\Delta\delta$(ppm)
	L-phenylalanine				
	1D ^{1}H NMR 9:1(w/w) H_2O-D_2O				
 l-phenylalanine	δ_{H1}= 3.9332	$\delta_{H1=}$3.9167	-0.0106	$\delta_{H1=}$3.8249	-0.1083
	$\delta_{H2=}$3.9225	δ_{H2}= 3.9061	-0.0100	$\delta_{H2=}$3.8133	-0.1092
	δ_{H3}=3.9169	δ_{H3}= 3.9006	-0.0015	$\delta_{H3=}$3.8091	-0.1078
	δ_{H4}=3.9063	δ_{H4}= 3.8900	-0.0497	$\delta_{H4=}$3.7976	-0.1087
	δ_{H5}=3.2333	δ_{H5}=3.2174	-0.0165	δ_{H5}=3.1347	-0.0986
	δ_{H6}=3.2226	δ_{H6}=3.2067	-0.0164	δ_{H6}=3.1270	-0.0956
	δ_{H7}=3.0381	δ_{H7}=3.0257	-0.0109	δ_{H7}=2.9311	-0.1070
	δ_{H8}=3.0216	δ_{H8}=3.0093	-0.0163	δ_{H8}=2.9141	-0.1075
	δ_{H9}=3.2042	δ_{H9}=3.1883	-0.0159	δ_{H9}=3.1059	-0.0983
	δ_{H10}=3.1934	δ_{H10}=3.1775	-0.0159	δ_{H10}=3.0955	-0.0979
	δ_{H11}=3.0673	δ_{H11}=3.0549	-0.0124	δ_{H11}=2.9605	-0.1068
	δ_{H12}=3.0508	δ_{H12}=3.0386	-0.0122	δ_{H12}=2.9435	-0.1073
	L-Tyrosine				
	1D ^{1}H NMR 9:1(w/w) H_2O-D_2O				
 l-tyrosine	δ_{H1}= 3.8749	$\delta_{H1=}$3.8598	-0.0151	$\delta_{H1=}$3.7677	-0.1072
	$\delta_{H2=}$3.8638	δ_{H2}= 3.8488	-0.0150	$\delta_{H2=}$3.7529	-0.1109
	δ_{H3}=3.8593	δ_{H3}= 3.8437	-0.0156	$\delta_{H3=}$3.7407	-0.1186
	δ_{H4}=3.8482	δ_{H4}= 3.8331	-0.0151	$\delta_{H4=}$ 3.7315	-0.1165
	δ_{H5}=3.1421	δ_{H5}=3.1288	-0.0133	δ_{H5}=3.0488	-0.0933
	δ_{H6}=3.1325	δ_{H6}=3.1200	-0.0125	δ_{H6}=3.0388	-0.0937
	δ_{H7}=2.9635	δ_{H7}=3.9493	-0.0142	δ_{H7}=2.8558	-0.1077
	δ_{H8}=2.9467	δ_{H8}=3.9334	-0.0133	δ_{H8}=2.8396	-0.1071
	δ_{H9}=3.1131	δ_{H9}=3.1009	-0.0122	δ_{H9}=3.0195	-0.0936
	δ_{H10}=3.1026	δ_{H10}=3.0904	-0.0122	δ_{H10}=3.0100	-0.0926
	δ_{H11}=2.9932	δ_{H11}=2.9791	-0.0141	δ_{H11}=2.8862	-0.1312
	δ_{H12}=2.9766	δ_{H12}=2.9628	-0.0138	δ_{H12}=2.8699	-0.1067

slightly higher than that of L-phenylalanine. Furthermore, higher values of viscosity B coefficients in CP could be attributed to the more significant dehydration impact of the Ca^{2+} ion and CP ionic strength than in SP, suggesting an increasingly more structured environment.

In the case of ions, it has been discovered that hydrated ions have larger ionic radii than those of anhydrous metal ions.[44] Moreover, the ion having smaller radii in an anhydrous state possesses greater radii when hydrated. A comparison of the B coefficients for L-phenylalanine and L-tyrosine in the incidence of SP/CP aqueous solutions has been characterized. An increase in the size of hydrated metal ions induces an increase in the B coefficient values. The opposite tendency is perceived with the anhydrous metal ion size. Improved knowledge about the structure making or structure breaking behavior of the solute can be sought from the temperature dependence of the B coefficient (dB/dT) compared to B values.[45] Both L-phenylalanine and L-tyrosine display positive dB/dT values in water and SP and CP aqueous solutions (Table 5), attributing to the chaotropic behavior of the solutes owing to their more extraordinary hydrophilic character. In addition, viscosity B coefficients of transfer, $\Delta_{tr}B$, were evaluated utilizing the equation analogous to eq 5, and values are given in Table 4.

An incremental rise in $\Delta_{tr}B$ values (Figure 2) with ascending concentration of SP/CP has been observed, which is again larger for CP than for SP, which further suggests the strong dehydration outcome of CP. In both cases of L-phenylalanine and L-tyrosine, the $\Delta_{tr}B$ values decrease with temperature.

3.5. Solvation. The ratio of $B/V^{\circ}_{2,\phi}$ provides information on the solvation behavior of solutes in water and in aqueous solutions of cosolutes. The dissolution of amino acids in water indicates $B/V^{\circ}_{2,\phi}$ values greater than 2.5 (Supporting Information, Table S2), which propose them as solvated spherical species.[29] The values are further increased in the presence of propionate-based preservatives. However, the magnitude decreases with the concentration increases of cosolutes, which could be due to the hydrophobic nature of interactions. Furthermore, the greater $B/V^{\circ}_{2,\phi}$ values compared to those of L-phenylalanine specify the more significant solvation behavior of tyrosine molecules owing to their extra polar nature.

3.6. NMR Spectroscopy. Proton (^{1}H) NMR spectra for pure phenylalanine and tyrosine (m_A = 0.3 mol·kg^{-1}) and in SP and CP (m_B = 0.75 mol·kg^{-1}) were obtained in a 9:1 (w/w) H_2O-D_2O solvent, and chemical shift changes ($\Delta\delta$) for nuclear magnetic resonance spectra are reported in Table 6. The protons of amino acids in ^{1}H NMR spectra go upfield in the presence of the molecules of CP and SP, as compared to the individual solute molecules in the D_2O: H_2O system, which could be due to the reduced hydrogen bonding of the solute molecules with water molecules due to the dehydration effect of cosolute molecules. Increased hydrophilic−ionic interactions induce a downfield shift. On the other hand, an upfield shift could be obtained due to the domination of hydrophobic−ionic/hydrophobic interactions. The higher upfield shift in the presence of CP in the present study can be attributed to its higher ionic strength and the tiny size of Ca^{2+} ions. However, the present report's decrease in the water content of solutes and cosolutes may be considered the reason for the experimental upfield shift. Also, these observations are consistent with volumetric and viscometric results.

https://doi.org/10.1021/acs.jced.2c00394
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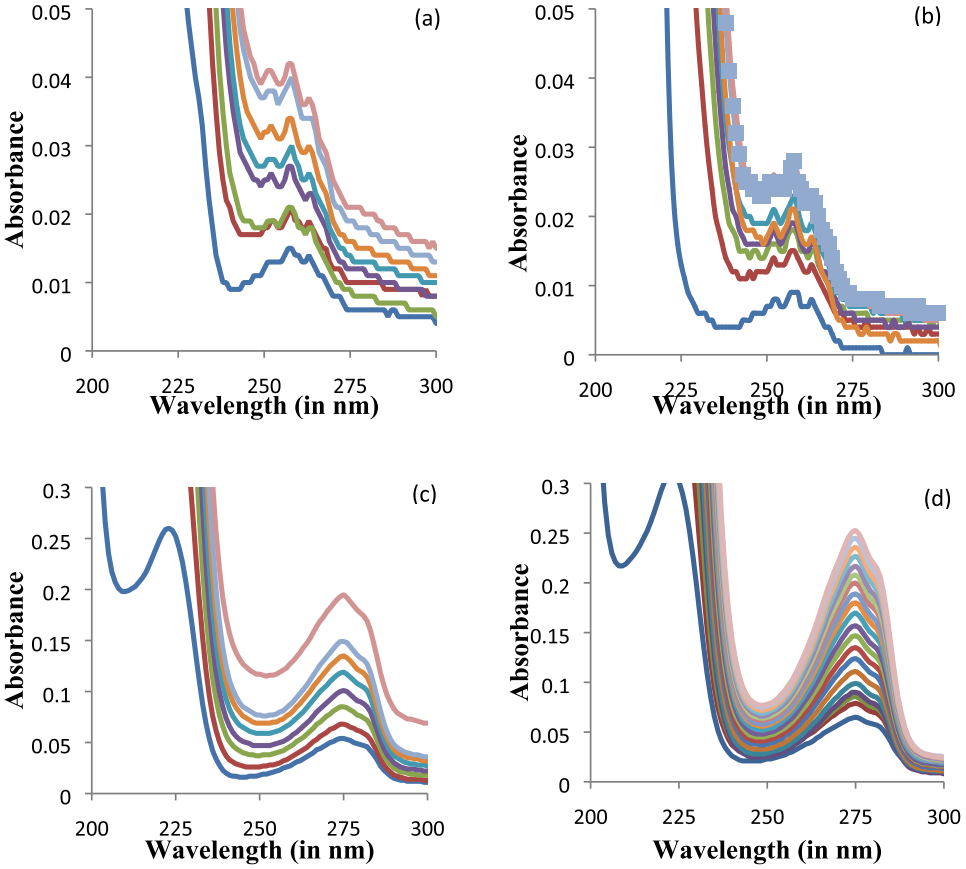

Figure 3. Absorption spectra for (a) L-phenylalanine in SP, (b) L-phenylalanine in CP, (c) L-tyrosine in SP, and (d) L-tyrosine in CP.

3.7. UV−Visible Spectroscopy. The absorption spectra were recorded for L-phenylalanine and L-tyrosine in water and in association with SP and CP. For pure L-phenylalanine and pure L-tyrosine, the absorption maxima occur at, λ_{max}, 257.60 and 274.80 nm, respectively, and are in accordance to the literature.[46] On addition of the aqueous solution of propionate salts to the aqueous amino acids, the absorbance intensified (hyperchromic shift) in both the cases (Figure 3). The value of E_{max} increases with addition of CP/SP without change in λ_{max}, evidencing hydrophilic interactions between solute (amino acids) and cosolute (SP/CP) molecules.[47] Undoubtedly, it is larger in CP compared to that in SP, indicating larger solute−cosolute interactions in the former.[48]

4. CONCLUSIONS

The results of physicochemical examinations of the interactions of L-phenylalanine and L-tyrosine aqueous solutions with propionate-based preservatives manifest increases in the density and viscosity of the solute molecules. Apparent molar volumes of transfer and viscosity B coefficients of transfer also increase with the concentration of preservatives, elucidating the increasing solute−cosolute interactions. Moreover, the change in $v\phi$ values specifies the sweet taste of L-tyrosine, the increase in the bitter taste of L-phenylalanine, and the decrease in the hydration number for the solutes, suggesting the dehydration effect of the preservatives. The dB/dT coefficient is positive for L-phenylalanine and L-tyrosine, indicating their chaotropic behavior. The ascending absorbance and the upward shift in the proton NMR spectra of solutes also contribute to the solute−cosolute interactions and the dehydration effect. Hence, variation in various obtained parameters is significant for studying the interaction of solutes with preservatives, which further monitors their hydration and taste behavior.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jced.2c00394.

Apparent molar volumes, $V_{2,\phi}$, of L-phenylalanine and L-tyrosine in water and in aqueous solutions of SP and CP at T/K = 298.15 and 308.15; $B/V_{2,\phi}^{\circ}$ values for L-phenylalanine and L-tyrosine in aqueous SP and CP solutions at T/K = 298.15 and 308.15; comparison of the densities (ρ) of the amino acids in water; and comparison of the viscosities (η) of L-phenylalanine in water at temperatures of 298.15 and 308.15 K (PDF)

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https://doi.org/10.1021/acs.jced.2c00394
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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are thankful to Dr. Tarlok Singh Banipal and Emerging Life Sciences, GNDU, for providing the necessary laboratory facilities.

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https://doi.org/10.1021/acs.jced.2c00394
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Individual floral phenology of *Barringtonia racemosa* observed from bud stage (A – D) to breakage (E,) full opening (F – I), until dehiscence (J – K).

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Environmental and **E**xperimental **B**iology

Environ Exp Biol (2022) 20: 55–60

Orginal Paper

Indirect organogenesis in *Ephedra foliata*

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Abstract

Ephedra foliata Boiss. ex C.A. Mey (Ephedraceae) is a well-known source of the alkaloid ephedrine used for the treatment of chronic asthma and associated respiratory ailments. This investigation was conducted to standardize an efficient regeneration protocol for *E. foliata* via indirect organogenesis through intermodal explants. Maximum callus induction (80.3%) was achieved on Murashige and Skoog (MS) medium supplemented with 0.5 mg L-1 2,4-dichlorophenoxyacetic acid + 0.5 mg L-1 kinetin and additives (50 mg L-1 ascorbic acid and 25 mg L-1 citric acid). The maximum shoot regeneration (n = 5.27) was achieved on MS medium containing 6.0 mg L-1 kinetin, followed by 5.0 mg L-1 6-benzylamino purine (n = 4.27). MS half strength medium with 3.0 mg L-1 α-naphthalene acetic acid resulted in the highest rooting percentage (32%). Sixty percent of the plantlets survived during acclimatization and were successfully transferred under field conditions. These plants and callus will be a suitable source of plant material for mass multiplication, genetic modification for enhanced bioactive constituents, and germplasm conservation.

Key words: callus induction, *Ephedra foliata*, *ex vitro* acclimatization, growth regulators, organogenesis.

Environ Exp Biol (2022) 20: 55–60
DOI: http://doi.org/10.22364/eeb.20.06

Print ISSN 1691-8088 – Online ISSN 2255-9582

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http://doi.org/10.22364/eeb.20.06

Original Paper

Indirect organogenesis in *Ephedra foliata*

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ISSN 2255-9582

Abstract

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Key words: callus induction, *Ephedra foliata, ex vitro* acclimatization, growth regulators, organogenesis.
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine; IBA, indole-3-butyric acid; KIN, kinetin; MS, Murashige and Skoog; NAA, α-naphthalene acetic acid.

Introduction

Ephedra foliata Boiss. ex C.A. Mey (Ephedraceae), commonly known as 'unthphog' and 'shrubby horsetail', is widely distributed in deserts of Africa, Arabian Peninsula and India. In India, it is found in arid and semi-arid regions of the north-western (Bhandari 1990; Lodha et al. 2014). It is the only gymnosperm that grows sporadically on sand hills in arid and semiarid areas in the Thar Desert of India (Shekhawat et al. 2012). At present, *E. foliata* is considered to be a threatened species in India (IUCN 2017; Meena et al. 2019). The plants are woody climbers with highly reduced scale-like foliage leaves arranged in a decussate pattern on nodes. The branches and seeds of this plant are an important component of the diet of camel, sheep, goat and insect species surviving in the nutrient poor arid desert of Rajasthan. It also acts as a major soil binder (Singh et al. 2007).

The species is known to contain the alkaloids ephedrine and pseudoephedrine, which are of great importance for their biological and pharmacological potential (Ghiasvand et al. 2019). It is used to treat bronchial asthma, hypersensitivity, fever, influenza, chills, colds, hack, cerebral pains, nasal blockage and other respiratory problems (Elhadef et al. 2020). It possesses antimicrobial, antioxidant, antidiabetic, hepatoprotective and cardiovascular activity (Al-Snafi 2017).

The conventional propagation of this plant is through seeds. The percentage seed germination and establishment is low due to abiotic stressors like high ambient temperature and soil alkalinity, which are common severe environmental conditions of the region. Pre-dispersal seed predation and post-dispersal seed predation by insects groups, rodents and other burrowing animals is a serious issue in restricting recruitment of new *E. foliata* population (Singh et al. 2007). Also, anthropogenic activities also have impact on the dwindling population of *E. foliata* (Singh 2004; Lodha et al. 2014).

Micropropagation offers an efficient method for mass propagation of threatened medicinal plants via direct and indirect organogenesis under *in vitro* conditions for *ex situ* conservation, genetic improvement and commercial applications, without any seasonal limitations (Yadav, Singh 2012; Yadav et al. 2012; Groach et al. 2014). Although there are many reports on *in vitro* propagation of *E. foliata* (Lodha et al. 2014a; Lodha et al. 2014b), considerable efforts are still required to make it more economical and practical. Therefore, the development of an efficient micropropagation protocol for *E. foliata* is urgently needed for both germplasm conservation and to expand pharmaceutical prospects. The present investigation deals with indirect organogenesis through callus-mediated induction of internodal explants of *E. foliata* as an alternative to naturally grown plants.

Materials and methods

A mature healthy plant of *E. foliata* growing in the Herbal Garden of the Department of Botany, Kurukshetra University, Haryana (India) was used as an explant source. Healthy internodal explants (1.0 to 1.5 cm) were surface sterilized by washing with 5% liquid detergent (Tween 20) followed by washing under running tap water in a plastic sieve for ten minutes to remove the adhering dust particles. Thereafter, the explants were disinfected using 0.1 % (w/v) of mercuric chloride (Hi-Media, India) for 3 to 5 min followed by a brief rinse with 70% ethanol and five times washing with sterilized double distilled water to remove the traces of mercuric chloride under aseptic conditions.

Explants after trimming the ends were inoculated on Murashige and Skoog (MS; 1962) medium containing 3% (w/v) sucrose, 0.8% (w/v) agar, additives (50 mg L^{-1} ascorbic acid and 25 mg L^{-1} citric acid) supplemented with various concentrations of auxin-type growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA) and cytokinin-type growth regulators 6-benzylaminopurine (BAP), kinetin (KIN) individually or in different combinations (0.5 to 2.0 mg L^{-1}) for callus induction. After explanting, the culture tubes, flasks capped with non-absorbent cotton plugs, were incubated at 25 ± 2 °C and 60 to 70 % relative humidity under a 16 h photoperiod at 40 µmol m^{-2} s^{-1} photon flux density of photosynthetically active radiation provided by Philip's cool white fluorescent tube lights. The pH of the medium was adjusted to 5.8 and autoclaved at 1.5 kg cm^{-2} and 121 °C for 20 min.

After 4 weeks of incubation, the calli formed from intermodal explants were periodically sub-culturing for multiplication and maintenance on various callus proliferation media (Table 1 and 2) and finally, a mass of calli was harvested. Visual observations like number of days required for callus induction, frequency of the callus induction (%) and nature of callus (colour, texture) were also recorded. For shoot regeneration, the best *in vitro* regenerated calluses from the 3rd successive sub-culture were excised aseptically and implanted on the different shoot induction medium (Table 3).

For root induction, the regenerated shoots (2.5 to 3.0 cm) were excised and cultured on half-strength MS media supplemented with various concentrations (1.0 to 4.0 mg L^{-1}) of IAA, NAA and indole-3-butyric acid (IBA) alone. The well-rooted plantlets were gradually pulled out from the culture tubes and gently washed with a soft brush under running tap water to remove the adhering agar with minimum injury.

After washing these plantlets, they were then transferred to small plastic cups containing autoclaved vermiculite/sand (3:1) potting mixture. In order to maintain the elevated humidity around the plants, the plantlets were covered with a glass jar. They were supplied with half-strength MS salt solution on alternate days. In the third week, the glass jars were removed for 3 to 4 h daily to expose the plants to the natural field conditions. After 4 weeks, these plants were transferred to bigger pots and were maintained in a greenhouse for acclimatization. Finally, the plants were transferred to field conditions.

All the experiments were repeated thrice with a

Table 1. Effect of plant growth regulators on callus induction of *E. foliata* recorded after four weeks on MS medium. (–) no response, (+) poor growth, (++) moderate growth, (+++) good growth. Data shown are mean ± SE of 45 replicates .Mean values followed by different letters within a column do not differ significantly at $P = 0.05$ according to Duncan's Multiple Range Test

Growth regulator (concentration mg L^{-1})	Time required for callus induction (days)	Callus induction (%)	Nature of callus	Visual growth of callus
Control	–	–	–	–
2,4 D (0.25)	–	–	–	–
2,4 D (0.5)	28	20.0 cd	Creamish yellow	+
2,4 D (1.0)	27	40.3 cb	Creamish yellow	+
2,4 D (2.0)	21	65.1 a	Fluorescent green	+++
NAA (0.25)	–	–	–	–
NAA (0.5)	–	–	–	–
NAA (1.0)	25	45.2 b	Yellowish brown	++
NAA (2.0)	26	47.1 ab	Yellowish brown	++
BAP (0.25)	–	–	–	–
BAP (0.5)	27	22.1 c	Light green	++
BAP (1.0)	26	15.0 cd	Light green	+
BAP (2.0)	–	–	–	–
KIN (0.25)	21	5.1 d	Green	+
KIN (0.5)	17	40.4 cb	Dark green	+
KIN (1.0)	16	45.1 b	Dark green	++
KIN (2.0)	16	42.2 cb	Dark green	++

Table 2. Effect of growth regulators in combinations on callus induction recorded after four weeks on MS medium. (–) no response, (+) poor growth, (++) moderate growth, (+++) good growth. Data shown are mean ± SE of 45 replicates. Mean values followed by different letters within a column do not differ significantly at $P = 0.05$ according to Duncan's Multiple Range Test

Growth regulator (concentration mg L^{-1})	Time required for callus induction (days)	Callus induction (%)	Nature of callus	Visual growth of callus
Control	–	–	–	–
KIN (0.25) + 2,4D (0.5)	32.1	10.0 e	Brownish	+
KIN (0.25) + 2,4D (1.0)	27.2	13.2 de	Brownish	++
KIN (0.25) + 2,4D (2.0)	35.7	21.1 d	Brownish	+
KIN (0.5) + 2,4D (0.5)	21.4	80.3 a	Dark green	+++
KIN (0.5) + 2,4D (1.0)	25.2	45.2 c	Dark green	+
KIN (0.5) + 2,4D (2.0)	25.9	42.1 c	Dark green	+
KIN (1.0) + 2,4D (0.5)	35.2	38.1 cd	Brownish green	++
KIN (1.0) + 2,4D (1.0)	36.3	34.1 cd	Brownish green	+
KIN (1.0) + 2,4D (2.0)	38.0	28.4 cd	Brownish green	+
KIN (0.25) + NAA (0.5)	32.0	62.0 b	Brown friable	++
KIN (0.25) + NAA (1.0)	31.5	62.7 b	Brown friable	++
KIN (0.25) + NAA (2.0)	31.0	63.1 b	Brown friable	++
KIN (0.5) + NAA (0.5)	32.1	55.1 bc	Brown friable	++
KIN (0.5) + NAA (1.0)	32.3	55.6 bc	Brown friable	++
KIN (0.5) + NAA (2.0)	30.0	56.0 bc	Brown friable	++
KIN (1.0) + NAA (0.5)	33.0	47.0 c	Brown friable	+
KIN (1.0) + NAA (1.0)	32.8	47.7 c	Brown friable	++
KIN (1.0) + NAA (2.0)	33.1	48.5 c	Brown friable	++
BAP (0.25) + NAA (0.5)	30.0	46.0 c	Light green	++
BAP (0.25) + NAA (1.0)	30.6	46.3 c	Light green	+
BAP (0.25) + NAA (2.0)	30.8	45.1 c	Light green	+
BAP (0.5) + NAA (0.5)	29.8	46.2 c	Light green	++
BAP (0.5) + NAA (1.0)	29.0	46.0 c	Light green	+
BAP (0.5) + NAA (2.0)	28.0	45.4 c	Light green	+
BAP (0.5) + NAA (0.5)	30.1	46.0 c	Light green	+
BAP (0.5) + NAA (1.0)	30.0	45.1 c	Light green	+
BAP (0.5) + NAA (2.0)	30.0	44.4 c	Light green	+

minimum of fifteen replicates per treatment and one explant per replicate. The statistical calculations were all carried out using SPSS (V. 16.0) statistical software. The difference between means was analyzed by one-way analysis of variance (ANOVA) using Duncan's multiple range test at a significance level $p = 0.05$.

Results

All of the tested auxins showed a better callogenic response over cytokinins (Table 1). MS medium supplemented with 2,4-D (2.0 mg L^{-1}) resulted in the highest per cent callus induction (65%) within 21 days of inoculation (Fig. 1A). The callus obtained was fluorescent green in colour. Among auxins, 2,4-D was found to be superior over NAA regarding the percent callus induction in a lower number of days.

Since MS medium supplemented with auxins resulted in better results, the effect of 2,4-D and NAA in combination with KIN and BAP was studied on callogenesis in intermodal explants (Table 2). The best callus induction percentage (80%) with higher growth was noticed in media supplemented with KIN (0.5 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}) (Fig. 1B). All of the different concentrations of KIN + NAA supplemented media resulted in the production of brownish friable callus while light green was observed with all of the concentrations of BAP + NAA. The different concentrations of KIN + 2,4-D showed different nature of calli ranging from brown, brownish green to dark green.

For differentiation of shoots from intermodal derived callus, various concentrations (1.0 to 6.0 mg L^{-1}) of BAP and KIN were tested (Table 3). Shoots bud formation was occurred in both BAP and KIN fortified medium. Vigorous growth of callus was visible in the form of green patches after two weeks of transfer on various shoot induction media. After three weeks, these green patches further developed into multiple shoots.

Both full and half-strength MS medium devoid of growth regulator failed to produce roots (Table 4). Among NAA and IBA as root inducers, both NAA and IBA resulted in significantly better results. However, the best root

Table 3. Effect of plant growth regulators on shoot regeneration of *E. foliata* from callus recorded after four weeks on MS medium. (–) no response. Data shown are mean ± SE of 45 replicates. Mean values followed by different letters within a column do not differ significantly at $P = 0.05$ according to Duncan's Multiple Range Test

Growth regulator (concentration mg L^{-1})	Time required for formation of green patches (days)	Number of shoots per explant
Control	–	–
BAP (0.5)	–	–
BAP (1.0)	16.0	1.13 de
BAP (2.0)	16.2	1.19 de
BAP (3.0)	15.8	1.82 d
BAP (4.0)	15.9	3.45 c
BAP (5.0)	15.5	4.27 b
KIN (1.0)	–	–
KIN (2.0)	–	–
KIN (3.0)	17.3	1.86 d
KIN (5.0)	17.1	3.21 c
KIN (6.0)	16.9	5.37 a
2,4D (1.0) + BAP (1.0)	–	–
2,4D (1.0) + BAP (3.0)	20.0	0.98 e
2,4D (1.0) + BAP (5.0)	16.8	3.75 bc

Table 4. Effect of plant growth regulators on root development of *E. foliata* recorded after four weeks on 0.5 MS medium. (–) no response. Data shown are mean ± SE of 45 replicates. Mean values followed by different letters within a column do not differ significantly at $P = 0.05$ according to Duncan's Multiple Range Test

Growth regulator (concentration mg L^{-1})	Root-producing shoots (%)	Number of roots per shoot
Control	–	–
NAA (1.0)	–	–
NAA (2.0)	23.00 ± 0.66 b	1.20 ± 0.01 c
NAA (3.0)	32.00 ± 0.78 a	2.10 ± 0.04 a
NAA (4.0)	25.00 ± 0.05 ab	1.30 ± 0.02 bc
IBA (1.0)	–	–
IBA (2.0)	–	–
IBA (3.0)	16.00 ±0.07 bc	1.50 ± 0.02 b
IBA (4.0)	13.00 ± 0.39 c	1.30 ± 0.00 bc

formation was observed with 3.0 mg L^{-1} NAA (Table 4; Fig. 1D).

The period of acclimatization is one of the most important stages, where the plant self-fixes the abnormalities to ensure survival under *ex vitro* conditions. The in vitro raised plantlets were then transferred to small plastic cups containing autoclaved vermiculite: sand (3:1) potting mixture (Fig. 1E). Sixty per cent of the plantlets survived during acclimatization and were successfully transferred to field conditions.

Discussion

Indirect organogenesis involves the formation of callus from cultured explants followed by shoot bud differentiation. Callus is an undifferentiated proliferative mass of cells, obtained by culturing explants aseptically on nutrient medium under controlled experimental conditions (Hussain et al. 2012).

The MS medium devoid of any plant growth regulators did not show any callogenic response in the tested explants, which may be due to the inadequate level of endogenous growth hormones in explants to induce callusing and requirement of an external contribution of growth regulators to trigger cell division (Huang et al. 2012). 2,4-D is the most commonly used plant growth regulator in callus induction (Zang et al. 2016; Mostafiz, Wagiran 2018; Carsono et al. 2021). The resulting colour variation in callus might be due to the type and concentration of the growth regulator. Compared to only auxin, a combination of auxin and cytokinin resulted in a greener callus, caused by cytokinin, which tends to promote chlorophyll formation (George et al. 2008). Other factors like pigment, nutrients and exposure to light also account for changes in callus colour (Evans et al. 2003).

Explants of *E. foliata* on MS medium fortified with KIN

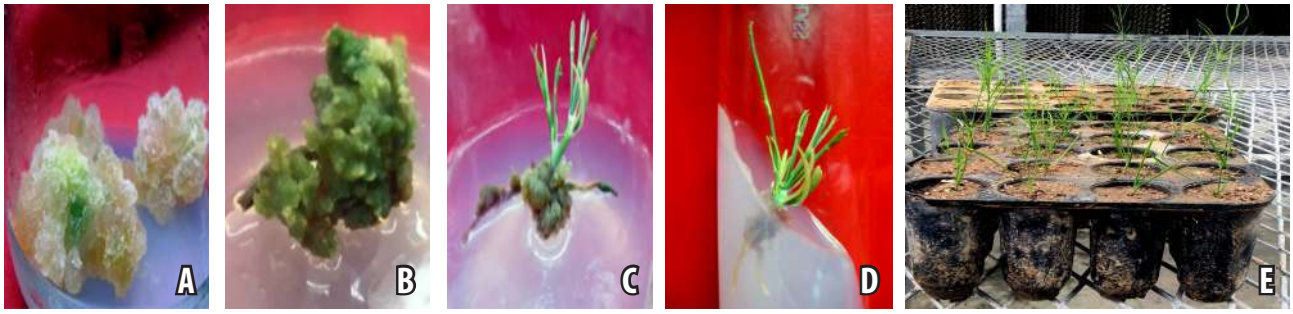

Fig. 1. *In vitro* plant regeneration of *E. foliata*. A, callus from internodal explants on MS medium supplemented with 2,4-D (2.0 mg L^{-1}). B, callus formation on MS + KIN (0.5 mg L^{-1}) + 2,4-D (0.5 mg L^{-1}). C, shoot differentiation on MS + KIN (6.0 mg L^{-1}). D, root induction on ½ MS medium + NAA (3.0 mg L^{-1}). E, *in vitro* raised plantlets before transfer to field conditions.

(6.0 mg L^{-1}) produced 5.37 shoots per callus culture within 16.9 days (Fig. 1C). Kinetin was found to be more potent over other cytokinins with or without the combination of auxin for the differentiation of multiple shoots from callus in *Ananas comosus* (Akbar et al. 2003), *Oryza sativa* (Libin et al. 2012), *Plectranthus rotundifolius* (Asha et al. 2013) and *Plectranthus bourneae* (Elangomathavan et al. 2017). The addition of 2,4-D in lower concentration also facilitated better morphogenesis and enhanced the rate of shoot bud differentiation in different plant species (Parveen et al. 2012; Mehaboob et al. 2019; Putri et al. 2020). In contrast, BAP was found to be more effective than kinetin for shoot multiplication in *Albizia lebbeck* (Yadav, Singh 2011), which may be due to the ability of BAP to induce and produce natural hormones, such as zeatin, within the tissue through the natural hormone system (Sharma, Wakhlu 2003).

Rooting is necessary to generate the whole plantlet from *in vitro* regenerated shoots. The plantlets have difficulty in survival under *ex vitro* conditions without an appropriate root system (De Klerk 2002). MS medium without any growth regulators proved ineffective for root induction in *Prosopis cineraria* (Kumar, Singh 2009) and *Stevia rebaudiana* (Verma et al. 2011). The effectiveness of half-strength medium over full strength medium in inducing *in vitro* rooting has also been reported by many researchers (Yadav et al. 2012; Kumar et al. 2013; Groach et al. 2014). The rooting of elongated shoots on MS medium supplemented with NAA or IBA has been well documented in *Stevia rebaudiana* (Verma et al. 2011), *Simmondsia chinensis* (Kumar et al. 2013) and *Vitex negundo* (Groach et al. 2014). In the culture laboratory, *in vitro* raised plants are constantly maintained under a controlled environment of high humidity, low light intensity, photoperiod, optimum temperature, supplementary sugar supply and growth regulators (Hazarika, Bora 2006; Yadav et al. 2013).

The efficient protocol developed for *Ephedra foliata* in the present study could help in using this plant material for mass multiplication, genetic modification for enhanced bioactive constituents and germplasm conservation.

Acknowledgements

The authors are grateful to Kurukshetra University, Kurukshetra, for providing laboratory facilities to carry out this investigation. One of the author (MS) is thankful to UGC, New Delhi for providing financial assistance.

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Received 29 January 2022; received in revised form 21 February 2022; accepted 10 March 2022

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Quarterly journal edited by the Institute of Natural Fibres and Medicinal Plants

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From Botanical to Medical Research

ISSN 0018-0599

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Ministry of Science and Higher Education (2021): **70** points

Index Copernicus ICV(2020): **120.75** points

CiteScore 2021: **1.5**

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Evaluation of antimicrobial activity and phytochemical qualitative analysis of Ephedra foliata Boiss. ex C.A. Mey.

MAHABIR SINGH, MINAKSHI RAJPUT, KULDEEP YADAV, NARENDER SINGH

Abstract

Introduction: Ephedra foliata Boiss. ex C.A. Mey of the Ephedraceae family is an evergreen shrub distributed throughout North Africa and Southwest Asia. It has been a characteristic source of alkaloids like ephedrine, pseudoephedrine and other related mixes, which are of great importance for their biological and pharmacological potential. **Objective:** This study is aimed to evaluate the antimicrobial potential and phytochemical constituents studies on stem, leaves and flowers extracts of E. foliata. **Method:** The air-dried plant sample was powdered with an electric grinder, then extracted successively with solvents, namely petroleum ether, petroleum benzene, ethyl acetate, methanol, and aqueous using Soxhlet apparatus for 72 hours. The solid matter was separated by filtration and then solvents were evaporated with a vacuum rotary evaporator to obtain the crude extracts. Freshly prepared crude extracts were subjected to the standard procedures of preliminary phytochemical screening for the investigation of the presence or absence of different phytoconstituents. The result showed the presence of reducing sugars, flavonoids, and cardiac glycosides. Antimicrobial activity of the crude extract was determined by agar well diffusion method. **Results:** Ethyl acetate extract showed the highest antimicrobial activity against all the tested pathogens (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Yersinia enterocolitica, Streptococcus pneumonia, Aspergillus terreus, Cladosporium herbarum and Candida tropicalis). All five extracts inhibited the growth of Y. enterocolitica. **Conclusion:** The antimicrobial properties of E. foliata extracts are of great interest in light of the ongoing threat of microbial resistance to conventional antibiotics. Phytoconstituents present in the E. foliata extracts might be a good alternative to modern antimicrobials as a natural compound.

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International journal edited by the Institute of Natural Fibres and Medicinal Plants
National Research Institute
Vol. 68 No. 2 2022

Received: 2021-10-10
Accepted: 2022-01-22
Available online: 2022-05-30

DOI: 10.2478/hepo-2022-0007

EXPERIMENTAL PAPER

Evaluation of antimicrobial activity and phytochemical qualitative analysis of *Ephedra foliata* Boiss. ex C.A. Mey.

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Summary

Introduction: *Ephedra foliata* Boiss. ex C.A. Mey of the *Ephedraceae* family is an evergreen shrub distributed throughout North Africa and Southwest Asia. It has been a characteristic source of alkaloids like ephedrine, pseudoephedrine and other related mixes, which are of great importance for their biological and pharmacological potential.

Objective: This study is aimed to evaluate the antimicrobial potential and phytochemical constituents studies on stem, leaves and flowers extracts of *E. foliata*.

Method: The air-dried plant sample was powdered with an electric grinder, then extracted successively with solvents, namely petroleum ether, petroleum benzene, ethyl acetate, methanol, and aqueous using Soxhlet apparatus for 72 hours. The solid matter was separated by filtration and then solvents were evaporated with a vacuum rotary evaporator to obtain the crude extracts. Freshly prepared crude extracts were subjected to the standard procedures of preliminary phytochemical screening for the investigation of the presence or absence of different phytoconstituents. The result showed the presence of reducing sugars, flavonoids, and cardiac glycosides. Antimicrobial activity of the crude extract was determined by agar well diffusion method.

Results: Ethyl acetate extract showed the highest antimicrobial activity against all the tested pathogens (*Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Streptococcus pneumonia*, *Aspergillus terreus, Cladosporium herbarum* and *Candida tropicalis*). All five extracts inhibited the growth of *Y. enterocolitica.*

Conclusion: The antimicrobial properties of *E. foliata* extracts are of great interest in light of the ongoing threat of microbial resistance to conventional antibiotics. Phytoconstituents present in the *E. foliata* extracts might be a good alternative to modern antimicrobials as a natural compound.

Key words: ***Ephedra foliata, crude extract, phytochemical screening, antimicrobial activity***

Słowa kluczowe: ***Ephedra foliata, surowy ekstrakt, badania fitochemiczne, aktywność antybakteryjna***

INTRODUCTION

Medicinal plants are edible or non-edible plants which at least accumulate substances in one of their organs that reflect health benefits in the treatment or prevention of mental illnesses [1]. It has been estimated that 20 to 85% of the world's population in the developed and developing countries use medicinal plants [2]. *Ephedra foliata*, ordinarily known as Somalata, is a gymnosperm plant belonging to family *Ephedraceae*, is an evergreen shrub developing from 0.15 to 1.0 m in height. It is distributed in North Africa and Southwest Asia. It is one of the most established medicinal plants in the world, notable in conventional Chinese drug, used to treat hypersensitivities, bronchial asthma, chills, colds, hack, fever, influenza, cerebral pains and nasal blockage. It is a source of alkaloids like ephedrine, pseudoephedrine and their mixes [3]. The stems of most individuals from this family contain ephedrine and are used in the treatment of asthma and numerous different contradictions of the respiratory framework. The activity of ephedrine is more delayed than that of adrenaline, being a functioning vasoconstrictor; alkaloids from *Ephedra* can be utilized to hoist circulatory strain and respiratory rate [4]. It possesses antimicrobial, antioxidant, antidiabetic, hepatoprotective and cardiovascular activities [5].

Secondary metabolite has a pharmacological or bioactivity used in drug discovery process. Based on the diverse uses of *E. foliata* in folk medicine, it is interesting to perform the antimicrobial potential and phytochemical qualitative analysis of different plant extracts (stem, leaves and flower) of *E. foliata*.

MATERIALS AND METHODS

Plant materials

E. foliata chosen for the present study has proficient medicinal significance. The plant material was collected from the Herbal Garden, Botany Department, Kurukshetra University, Kurukshetra.

Preparation of extracts

The aerial portions, stem, leaves and flowers of *E. foliata* were collected and washed with tap water pursued by distilled water to take out the dust and dirt on the surface of the plant. The plant material was dried at room temperature for 15 days. The dried plant material was powdered with an electric grinder. The different extracts were prepared by taking 50 g of plant powder extracted by immersing with 200 ml of five solvents namely petroleum ether, petroleum benzene, ethyl acetate, methanol, and aqueous using Soxhlet apparatus for 72 hours. The extracts were filtered with filter paper (Whatman No. 1) and solvents were evaporated with a vacuum rotary evaporator to obtain the crude extracts, residue stored at 4°C until further use. The final concentration of 200 mg/ml was made to test antimicrobial activity by dissolving extracts into DMSO (dimethyl sulfoxide) with the concentration of 1 mg/ml being evaluated for biological potential.

Test microorganisms

The microbial strains used in the research experiment namely, *Escherichia coli* MTCC 1570, *Staphylococcus aureus* (NCIM 5345), *Pseudomonas aeruginosa* MTCC 1034, *Yersinia enterocolitica* (MTCC 3235), *Streptococcus pneumoniae* (MTCC 2672), *Aspergillus terreus* (NCIM 1325), *Cladosporium herbarum* (NCIM 1112) and *Candida tropicalis* (MTCC 184) were collected from the IMTECH Chandigarh and NCIM, Pune.

Preparation of inoculums

Stock cultures were sustained at 4°C on slants of nutrient agar test tubes. Active cultures were readied by moving cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacterial strains, incubated for 24 h at 37°C and Sabouraud Dextrose broth (SDB) for fungal strains that were incubated for 7 days at 30°C.

Antimicrobial assay

The extracts were assessed against pathogens by agar well diffusion method. The turbidity of the inoculum was adjusted to 1.5 × 108 CFU/ml (corresponding to 0.5 McFarland standards). The antibacterial viability of plant extracts were compared by standard antibiotics streptomycin and antifungal activity by fluconazole. The final concentrations for antimicrobial drugs were streptomycin (15 μg/ml) and fluconazole (130 μg/ml) according to CLSI AST norms. Streptomycin was dissolved in distilled water and fluconazole in DMSO to make the final concentration for antimicrobial testing. Distilled water and DMSO act as a negative control. A 6 mm diameter cork borer was used to make well in the medium and filled with 50 μl of final concentrations of control and plant extracts. The zone of inhibition was measured in millimetre (mm) as clear zones seen around the wells.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of crude extracts was decided by broth microdilution method. Stock solutions were set up with the concentration of 200 mg of crude plant extracts in 1 ml of DMSO and further diluted using two-fold serial dilution in sterile broth. A series of test concentrations of 200, 100, 50, 25, 12.5, 6.25 and 3.13 were given by serial dilution. Afterward, 100 μl volumes of samples were poured into the well of 96-well microtiter plate followed by 100 μl volume of test strain broths. After incubation, the minimum concentration of the test sample which inhibited the growth of the test organism was considered as MIC of the plant extract.

Preliminary phytochemical screening

Freshly prepared crude extracts of *E. foliata* were subjected to the standard methodologies (Table 1) of preliminary phytochemical screening for the investigation of the presence or absence of different phytoconstituents [6, 7]. The qualitative results of the analysis were expressed as + for the presence and - for the absence of phytochemical.

Ethical approval: The conducted research is not related to either human or animal use.

Table 1.

Preliminary phytochemical screening methods

Phytochemical	Test/ Reagent	Method	Observation	Inference
Alkaloids	Wagner's reagent	Extract + 3–5 drops of Wagner's reagent	reddish brown precipitate or coloration	alkaloids present
Reducing sugars	Fehling's Test	1 ml of extract + few drops of Fehling's reagent and stirring	rusty red precipitate	reducing sugars present
Tannins	Braymer's test	2 ml of extract + 10% alcoholic ferric chloride	blue or greenish coloration	tannins present
Phenols	Ferric chloride test	Extracts + aqueous 5% ferric chloride	deep blue or black coloration	phenols present
Flavonoids	Alkaline reagent test	2 ml of extract + few drops of 20% sodium hydroxide + dil. HCl	intense yellow color, turns colorless on addition of acid	flavonoids present
Saponins	Foam test	2 ml of extract + 6 ml of water and shaken well	formation of persistent foam	saponins present
Amino acids	Ninhydrin test	2 ml of extract + 2–5 drops of 1% ninhydrin solution in acetone and boiled for 1–2 min.	purple coloration	amino acids and protein present
Cardiac Glycosides	Keller Kelliani's test	5 ml extract + 2 ml glacial acetic acid + 1 drop of ferric chloride + 1 ml conc. H_2SO_4	brown to blue color ring at the interface	cardiac glycosides present
Steroids	Salkowki's test	2 ml of extract + 1 ml chloroform + few drops of conc. H_2SO_4	red color on the upper layer and yellow color on lower layer	steroids present

RESULTS AND DISCUSSION

Antimicrobial Activity

In vitro antimicrobial activity of different solvent extracts of *E. foliata* was assessed by investigating the presence or absence of zones of inhibition. The data of the antimicrobial activity of the extracts of *E. foliata* and reference standard antimicrobials are shown in Table 2. The stem extracts of *E. foliata* in different solvents have potent antimicrobial efficacy against many bacterial strains and fungal strains carried out by agar well diffusion method. The antimicrobial activity of all the crude extracts could be explained by the presence of several phytochemicals such as reducing sugars, alkaloids, phenols, flavonoids, and cardiac glycosides etc., which could be responsible for the observed biological activities [8]. Results showed some variation among different extracts (petroleum ether, petroleum benzene, ethyl acetate, methanol and aqueous) of *E. foliata* against most of the tested strains (Gram-positive and Gram-negative) in a dose-dependent manner. Indeed, the bacterial strain *Y. enterocolitica* was observed to be the most sensitive pathogen among all the test microorganisms. All the five extracts inhibited the growth of *Y. enterocolitica* and showed the zone of inhibitions ranging from 28±0.66 to 34±0.15 mm and the ethyl acetate extract was observed to be the most effective extract followed by aqueous, petroleum benzene, methanol and petroleum ether. Ethyl acetate extract gave the highest antimicrobial activity against all the tested pathogens with the zone of inhibition 34±0.15 mm against *Y. enterocolitica* followed by *S. pneumoniae* (32±0.03 mm), *S. aureus* (31±0.34 mm), *E. coli* (30±0.38 mm), *P. aeruginosa* (29±0.26 mm), *C. herbarum* (20±0.54 mm), *A. terreus* (16±0.20 mm), and *C. tropicalis* (10±0.07 mm). Streptomycin was used to compare the sensitivity of the bacterial strains and *S. pneumoniae* as well as *Y. enterocolitica* were found the most sensitive with inhibition zone 22±0.63 mm and 22±0.58 mm respectively. Fluconazole was used as a reference antifungal drug and was found to be most effective against *A. terreus* and *C. tropicalis* with the zone of inhibition 21±0.90 mm and 20±0.25 mm respectively. Among the different solvent tested, hydrophobic fractions were petroleum ether and petroleum benzene while hydrophilic fractions were ethyl acetate, methanol and aqueous. Among all these the hydrophilic fraction, ethyl acetate dissolve more compounds and gave best inhibition activity. Besides this, methanol extracts of *E. foliata* have been well observed for their antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa,* and *E. coli*. [3]. Mathur *et al.* [9] reported the antimicrobial efficacy of *E. foliata* against human pathogenic bacteria. Bissa [10], evaluated the antimicrobial potential of different plant part (stem and leaves) extracts (aqueous, alcoholic, chloroform and petroleum ether) of *E. foliata* against human pathogenic (*E. coli, S. typhi, K. pneumoniae,* and *Enterobacter aerogenes*) as well as plant pathogenic (*Agrobacterium tumefaciens*) bacteria and reported that all the plant parts exhibited antimicrobial activity that is comparable to the present research work.

Minimum inhibitory concentration (MIC)

The MIC of the extracts of *E. foliata* is shown in Figure 1. The MIC of the four different extracts

Table 2.

Zone of inhibition of different solvent extracts of *E. foliata* against MTCC microbial strains

Microbial strains	Zone of inhibition [mm]							
	Petroleum ether	Petroleum benzene	Ethyl acetate	Methanol	Aqueous	DMSO	Streptomycin	Fluconazole
E. coli	17±0.62	29±0.54	30±0.38	25±0.86	22±0.32	–	20±0.16	NT
P. aeruginosa	30±0.59	27±0.19	29±0.26	31±0.98	31±1.01	–	18±0.47	NT
S. aureus	28±0.98	28±0.25	31±0.34	27±0.15	30±1.11	–	08±0.07	NT
*Y. enterocolitic*a	28±1.06	30±0.68	34±0.15	29±0.66	33±1.34	–	22±0.58	NT
S. pneumoniae	26±0.82	27±0.63	32±0.03	26±0.63	33±1.10	–	22±0.63	NT
A. terreus	02±0.01	03±0.07	16±0.20	–	–	–	NT	21±0.90
C. tropicalis	02±0.04	-	10±0.07	–	–	–	NT	20±0.25
C. herbarum	04±0.00	06±0.06	20±0.54	–	–	–	NT	10±0.04

Values are the mean of three replicates; significant at $p \leq 0.05$ level of analysis of variance; diameter of well: 6 mm; MTCC – Microbial Type Culture Collection; DMSO – dimethyl sulfoxide; NT – not tested

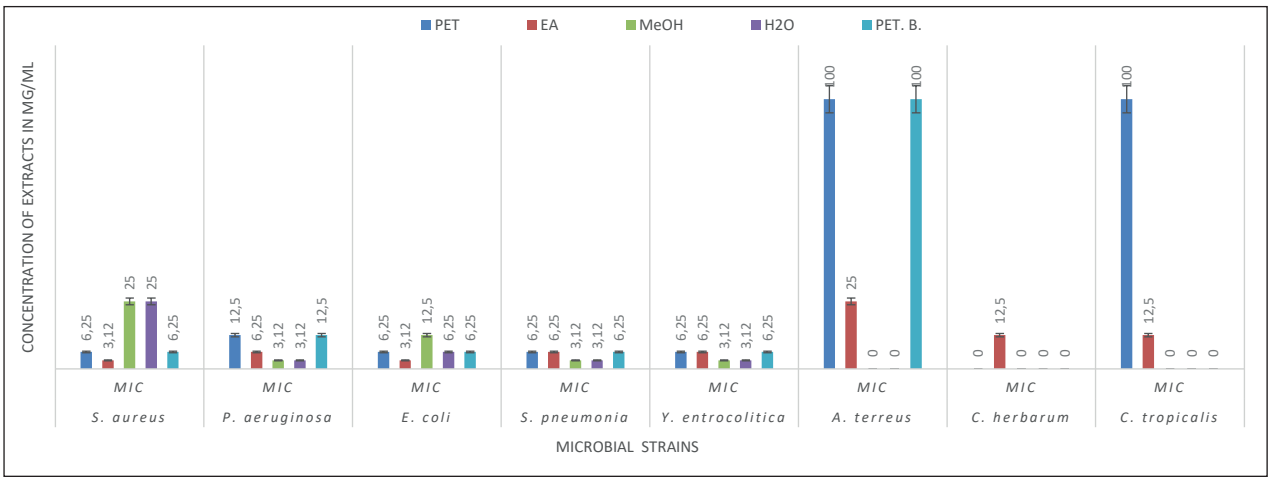

Figure 1.

MIC [mg/ml] of different solvent extracts of *E. foliata* against MTCC microbial strains

ranged from 3.12-25 mg ml^{-1} for *S. aureus,* 3.12-12.5 mg ml^{-1} for *P. aeruginosa* and *E. coli,* 3.12-6.25 mg ml^{-1} for *S. pneumoniae* and *Y. enterocolitica,* 25-100 mg ml^{-1} for *A. terreus,* 12.5 mg ml^{-1} for *C. herbarum* and 12.5-100 mg ml^{-1} for *C. tropicalis.* The lowest range of MIC (3.12-12.5 mg ml^{-1} and 3.12-25 mg ml^{-1}) were observed in the case of ethyl acetate and methanol extracts respectively of *E. foliata* against all the tested pathogens.

Phytochemical Screening

The qualitative phytochemical screening of the fresh and dried shoot powder extracts of *E. foliata* in five different solvents showed the presence of several phytochemicals. Reducing sugars, flavonoids, and cardiac glycosides were found present in all the five extracts however, saponins and amino acids were found absent in all the extracts (Table 3). The existence of alkaloids, reducing sugars, flavonoids, phenols and cardiac glycosides has been reported in methol, petroleum ether and petroleum benzene extract. In an aqueous extract, alkaloids, phenols, tannins, saponins, amino acids and steroids were not found. While high yields of phytochemicals with the presence of alkaloids, reducing sugars, flavonoids, phenols, tannins, cardiac glycosides and steroids were documented from ethyl acetate extract. According to previously conducted studies, phenolic compounds are the active ingredients of *Ephedra* plant [11, 12]. The antimicrobial activity of many species of *Ephedra* species like *E. major*, *E. monosperma*, *E. fragilis*, *E. distachya, E. foeminea*, *E. alata*, *E. sinica* and *E. vulgaris* showed variations in their phytochemical constituents levels using different types of extracts [11, 12, 13, 14, 15, 16, 17].

Ephedra contains alkaloids (pseudoephedrine, norephedrine and methylephedrine), flavonoids (leucodelphinidin, leucoanthocyanidin, leucopelargonine, lucenine, vicenin-1, and vicenin-2), phenols (kaemferol 3-rhamnoside, quercetin 3-rhamnoside, herbacetin 8-methyl ether 3-O-glucoside-7-O-rutinoside and furanofuran) and tannins (proanthocyanidines) [18].

In the present research work, the stem extract of *E. foliata* exhibited the presence of several phytochemicals with comparatively more concentration of alkaloids that is comparable with Bissa [10]. This variation in concentration may be due to several factors such as soil characteristics, harvesting, storage conditions and analytical quantification techniques [18]. These different phytoconstituents interact with the membrane proteins of the bacteria through inter-helical hydrogen bonding, causing changes in membrane permeability and cell destruction, penetrating bacterial cells and coagulating the cell contents [19]. The cytoplasmic membrane of the bacteria also gets damaged through the perforation action of the flavonoid [20]. Flavonoid causes retardation in the growth of microorganisms by inhibiting their nucleic acid synthesis and energy metabolism [21]. Therefore, *E. foliata* species depicts the presence of various phytochemicals which may be acting independently or synergistically with other compounds to show medicinal as well as therapeutic efficacies. They showed potentials antimicrobial activity that can be exploited as the alternatives of several antibiotics in the treatment of microbial infections [22].

Table 3.

Preliminary phytochemical screening of different solvent crude extracts of *E. foliata*

Phytochemical compounds	Petroleum ether	Petroleum benzene	Ethyl acetate	Methanol	Aqueous
Alkaloids	+	+	+	+	–
Reducing sugars	+	+	+	+	+
Tannins	–	-	+	–	–
Phenols	+	+	+	+	–
Flavonoids	+	+	+	+	+
Saponins	–	–	–	–	–
Amino acids	–	–	–	–	–
Cardiac glycosides	+	+	+	+	+
Steroids	–	–	+	–	–

+: present; –: absent

CONCLUSION

It has been well known the rate of microbial resistance towards antibiotics increasing day by day. Therefore, the medicinal plants and their phytocompounds are creating a new interest as an antimicrobial agent. The background of the manuscript indicates that *E. foliata* phyto-complex extracts have an efficacious antimicrobial potential, as evidenced by the inhibitory effect on bacterial growth of different human pathogens. The antimicrobial properties of *E. foliata* extracts are of great interest in light of the ongoing threat of microbial resistance to conventional antibiotics. Phytoconstituents present in the *E. foliata* extracts might be a good alternative to modern antimicrobials as a natural compound.

ACKNOWLEDGMENTS

The authors are grateful to Kurukshetra University, Kurukshetra, for providing laboratory facilities to carry out this investigation. One of the author (MS) is thankful to UGC, New Delhi for providing financial assistance.

Conflict of interest: Authors declare no conflict of interest.

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त्रैमासिक साहित्यिक पत्रिका
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एक ओंकार की ध्वनि से समस्त वाङ्मय, भूमंडल, अखिल ब्रह्माण्ड क्षिति, सम्पूर्ण दिशाओं को धन्य करने वाले परम विवेकी, अत्याधिक सौम्य संत गुरु नानक देव जी ने अपनी दिव्य एवं पीयूष वर्षी वाणी के द्वारा मनुष्य के मनुष्यत्व, उसके अंतःकरण के बुद्धत्व को जागृत करने का प्रयास किया। गुरु नानक वाणी के एक - एक शब्द अपने अर्थ में समस्त चेतना को समाहित किए हुए है। युगों-युगों से गुरु वाणी शुष्क एवं क्लांत चित्त को रस-विभोर एवं आह्लादित करती आ रही है। गुरु नानक जी ने केवल उपदेशों के दस्तावेज हम मरणधर्माप्राणियों को नहीं दिए, प्रत्युत अपने व्यवहार द्वारा कर्म द्वारा करनी के द्वारा उसे प्रमाणित भी किया। गुरु वाणी एक ऐसा प्रकाश पुंज है, जिसके अध्ययन मात्र से जीव इस जागतिक प्रपंचों से परिचित होता है,सचेत होता है, सजग होता है, जिस प्रकार क्लांत एवं विश्रांत पथिक तरुओं की छाया के नीचे शांति पाता है, सुख का अनुभव करता है, उसी प्रकार गुरु वाणी से इस जगत के त्रय-ताप से उद्वेलित व्यक्ति मन परम आनंद एवं सुख का अनुभव करता है। मन पर चढ़ा ईर्ष्या, असूया, अमर्ष जैसे भयावह जेर तिरोहित होने लगता है, पार्थिव विवक्षाओं एवं ऐषणाओं का दोहन होनेलगता है, व्यक्ति अपने स्वरूप में विराजता है, ममता के उद्घोषक, परदुःखकातरता के गायकप्रेम के पोषक धर्म के मर्मज्ञ, विश्वबंधुत्व के संवाहक, सार्थकयायावर, सौहार्द के उन्नायक,मिथ्याडम्बरों के भंजक, युग पुरुष युग - नियंता युग प्रवर्त्तक गुरु नानक बीजों के बीजत्व में, कण-कण के कणत्व में, जड़ सम पदार्थों में, समस्त दृश्यमान जगत् में एक चिन्मय सत्ता,एक जोत, एक तत्त्व, एक बिंदु का दर्शन करने वाले गुरुनानक देव जी की वाणी आत्मनिरीक्षण, आत्म शोधन, आत्म मंथन करने की सार्थक औषधि है। जापक जब गुरु मंत्र को शुद्ध एवं केन्द्रित एवं अक्षत मन से उच्चरित करने बैठते हैं, तो एकअलौकिक एवं अनिर्वचनीय अनहद नाद, एक अनुपम गूंज एक अद्वितीय संसृ, उस के भीतर घटित होता है। वह राग एवं विराग की रेखाओं को तोड़ता हुआ अनुराग की अवस्था पर स्वयं को पहुंचा हुआ पाता है, गुरुनानक देव का आविर्भाव निश्चित रूप से इस त्रस्त वसुंधरा, दलित लोगों के लिए मंगलकारी था। लोक चेतना से अनुप्राणित इनका साहित्य मात्र उत्तर भारत में ही नहीं, अपितु विदेशों में भी अपनी छटा प्रकीर्ण कर रहा है। गुरु नानक नेअपनी वाणी में सामाजिक विचारों एवं जनमानसकी आवाज को निर्भीक किन्तु विनम्र भाव सेमुखरित किया। मूर्धन्य साधक महान् दार्शनिक,समाज सुधारक, विचक एवं चिंतक गुरुनानक देव जी जीव को अपनी ओजस्वी एवं तेजस्वीवाणी के द्वारा निर्द्वन्द्व, निर्बोझ, निर्विकार,निर्भार एवं निर्मल कर दिया। आज यपि व्यक्तिमानसिक कष्ट भोग रहा है, जिन दुर्बलताओं के समाधान में असमर्थ है, उसका कारण एक ही हैकि व्यक्ति गुरुमुखी होने की अपेक्षा अंतर्मुखी हो गया। तुलसीदास ने भी मानस में बालकांड में सर्वप्रथम गुरु का नाम किया,यह प्रमाणित सत्य है कि बिना गुरु के व्यक्ति, बिना मार्गदर्शनके व्यक्ति जर्जर पत्ते के समान दिग्भ्रमित हो, यहाँ-वहाँ भटकता है। गुरु पथ भी देते दृष्टि भी और प्रकाश भी।गुरु नानक देव जी ने भी अपनी वाणी में गुरु को सर्वशक्तियों, सभी पदार्थों,सभी ऐश्वर्यों से उच्च बताया। गुरु के सान्निध्य बिना शास्त्रप्रदत्त सिद्धांतों पर चले बिना उस तत्त्व के दर्शन नहीं हो सकते, गुरु व्यक्ति के चित्त में विवेक जागृत करते हैं, उस ज्ञात सत्ता के प्रति प्यास पैदा करते गुरु जगत् की मृगमरीचिका से जीव को अवगत कराते हैं, वह अपने सत्य को यथार्थ का दर्शन करवा माया के विभिन्न रूपों, माया की विकरात,माया का प्रभाव,माया का परिणाम आदि दुष्प्रवृत्तियों से परिचित करवा मनुष्य को उस दिव्यसत्ता के साथजोड़ते हैं। अपने महनीय ग्रन्थ 'आसा की वार' में गुरु नानक गुरु महिमा का प्रस्तुतीकरण करते हैं कि गुरु के नैकट्य बिनाजीव शून्य है, उसका वजूद नगण्य है, उसकी अस्मिता निरर्थक है-

"बिनु सतीगुरु किनै न पाओ बिनु सतिगुरु किनै न पाइआ।
सतिगुरु विचे आपु रखिनु करि परगटु आखि सुणाइआ।
सतिगुरु मिलिऐ सदा मुकतु है जिनि विचहु मोहु चुकाइया।
उत्तम एहू बीचारू है जिनी सचे सिउ चितु लाइया।/जगजीवनु दाता पाइया"1

आज स्वार्थों के लिए, अपने हितों को साधने के लिए प्रकृति का दोहन हो रहा है। बड़ी-बड़ी अट्टालिकाओं, भवनों, स्मारकों, महामहालयों सदनों का निर्माण त्वरित गति से हो रहा है ।नदियों, जलाशयों, पोखरों को दूषित कर दुस्साध्य रोगों का बीजारोपण किया जा रहा है। स्वयं को साधन सम्पन्न बनाने के लिए, मनुष्य ने प्रकृति की उपादेयता को पायदान पर मान प्रकृति की सौम्यता को गिद्ध हाथों से नोचा। आज पूरा विश्व प्रकृति की प्रतिकूलता से त्रस्त है, इसका कारण हम स्वयं हैं। इस संकट का आह्वान स्वयं हमने किया प्रकृति के नैसर्गिक रूप को छेड़ कर हमने विकराल बाढ़ों, भयावह अतिवृष्टि, अनावृष्टि, सूखा, शुष्कता,भू-प्रकम्पन को निमंत्रण दिया। गुरुनानक वाणी में यह सत्य उद्घाटित किया गया है किप्रकृति के साथ मानवीय संबंधों को स्थापित किया जाना चाहिए। जैसे एक परिवार के सदस्य आपस में स्वार्थ के धरातल पर नहीं प्रेम के धरातल पर सम्बंधित होते हैं वैसे ही प्रकृति के साथ व्यक्तिको संबंधों को निर्मित करउसके प्रति कृतज्ञ होना चाहिए। 'जपुजी' साहिब में गुरुनानक देव जीने इन उदात्त संबंधों की परिकल्पना की है। वायु को गुरु, पानी को पिता एवं धरा को माँ की संज्ञा दी ताकि हम प्रकृतिके प्रत्येक घटक के प्रति आदरणीय भाव रखें,उसकी रक्षा हेतु आबद्ध हों, प्रकृति की शाश्वता को बनाये रखने के लिए प्रतिबद्ध हो, प्रकृति के सौष्ठव एवं चारुत्व को बचाए रखने के लिए सम्बद्ध हो जाए। गुरु नानक देव जीकी वाणी इस तथ्य की पुष्टि करती है-

"पवणु गुरु पाणी पिता माता धरति महत।/दिवसु राति दुइ दाई दाइआ खेलै सगल जगतु।/चंगिआईआ बुरिआईआ वाचै धरमु हदूरि
करमी आपो आपणी के नेड़े के दूरि।"2

गुरु नानक वाणी में हउमै(अहंकार) पर विस्तृत चर्चा है। गुरु वाणी साधक को सहजता के मार्गकी ओर प्रवृत करती है। आज अहंकारों के पोषण के लिए कर्मकांड, दान, यज्ञ,बड़ी-बड़ी कथाओं का आयोजन किया जा रहा है। मेरे ऐश्वर्य, मेरे वैभव, सम्पन्नता, मेरे भवनों से जगत परिचित हो इस कुटिल भावना से बड़े-बड़े धार्मिक समारोहों का आयोजन किया जारहा है। मैं समाज में प्रतिष्ठित हो जाऊं, मेरी संपत्ति के चर्चे हर व्यक्ति के मुख पर हो, आजव्यक्ति इसी ग्रंथि से पीड़ितहै। गुरु नानक वाणी हमें इस घृणित वृत्ति से ऊपर उठाती है।गुरु नानक देव जी ने उदबाहु घोषकिया है कि यदि हमारा कोई भी संकल्प, कर्म, समर्पण,अहंकार से प्रेरित है तो उन संकल्पों गरिमा शून्य है। व्यक्ति को सहज हो, निर्व्याज रूप से,निस्वार्थ रूप से कर्म की साधना में रत होना चाहिए। गुरु नानक देव जी ने इस जगत को स्वाभाविक एवं विवेकी होने की दृष्टि प्रदान की-

"लख नेकीआ चंगिआईआ लख पुंना परवाणु
लखतब उपरि तीरथा सहज जोग वेवाणु।
लख सूरतण सगराम रण महि छुटहि पराण
लख सूरती, लख गिआन विआन पड़ीअहि पाठ पुराण।
नानक मति मिथिआ करमु सचा नीसाणु"3

अपरिमित सम्पत्ति, वैभव, साधनों, ज्ञान के स्वामी होने पर भी व्यक्ति को अपनी स्वाभाविकतानहीं छोड़नी चाहिए। भले ही आप कितने भी सुन्दर हो, भले ही समाज, जाति अथवा देश मेंआपका कितना भी सम्मान हो, कितने भी आप लोकप्रियता के शिखरों पर हों। किन्तु आपसहज बने रहें, गुरु वाणी इस शुद्ध बुद्ध भाव का समर्थन एवं वकालत करती है। गुरु नानकदेवजी ने उन तमाम अंधविश्वासों एवं मिथ्याडम्बरों की आलोचना की, जो व्यक्ति को गुमराह करतेहैं। समाज में प्रचलित सारहीन एवं जर्जर प्रथाओं को अपनी वाणी के द्वारा निरस्तकिया।गुरु जी ने समाज में प्रचलित 'सूतक' आदि परम्परा की नयी एवं सार्थक व्याख्या प्रस्तुत की।गुरुवाणी धार्मिक प्रक्रियाओं के द्वंद्वों में जकड़े फंसे जीव को सहजता की ओरप्रवृत करती है।गुरु नानक देव जी कहते हैं कि सूतक केवल भ्रम है, व्यक्ति केवल भयभीत होकर इन कर्मकांडों का अनुसरण करता है। अपनी वाणी में गुरु जी ने इन तथ्यों का समर्थनकिया कि व्यक्ति की अपरामेय ऐषणाएँ उसके मन का सूतक है। मिथ्या भाषण जिह्वा का सूतक है।

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ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:2(1), February: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd February 2023
Publication Date:10th March 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/12.02.05
www.ijmer.in

CONFLICT RESOLUTION IN SOUTH ASIA WITH REFERENCE TO GANGA WATER CONFLICT

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Abstract

South Asia is one of the most conflict prone regions of the world. Inter-state relations in South Asia are characterized by the existence of a number of bilateral disputes which proved to be too difficult to resolve. While some of them are rooted in the historical past, others are in the current dynamics of bilateral as well as intra-state relations. The lists of these disputes are too long. But here the major bilateral disputes have been discussed. In fact, inter-state relations involve territorial disputes inherited from the colonial past like Indo-Pak dispute, the dispute over the sharing of water resources of common rivers including that of the Ganga like between India and Bangladesh, intra-state conflict involving ethno-linguistic and religious groups with cross-border affiliation, and conflicting economic interests and other issues etc. Thus, such issues have actually been the source of conflicts in South Asia. However, these issues have the potential to escalate into violent conflicts at the same time they are also proving to be a source of bilateral or multilateral cooperation among the states. In fact, the nature of such conflicts is such that their resolution needs continued collective efforts of the sovereign state actors across borders.

Keywords: Conflict, Bilateral Disputes, Cross-Border.

Introduction

South Asia is one of the important regions of the world. Most of the countries of this region have traditionally been the subject of colonial rule. They got independence in the last decade of the first half of the 20th century. Almost all the countries of this region are characterized by similar history, culture, socio-economic development etc. The factors like religion, poverty, unemployment, illiteracy, population growth etc. influence the politics of almost all the countries of this region. The presence of such common problems among the South Asian countries provides a good ground for cooperation to resolve them peacefully. However, in the past there have been very few instances of such cooperation in this region. The factors that reflect the similarity in this region also contribute toward making it as a region of mutual mistrust, endemic tension and occasional hostilities.

The inter-state relations in South Asia in recent years have shown little signs of improvement and cooperation. The areas and issues of conflicts have been on the rise while those of cooperation remain dormant. The emergences of various conflicts in this region hinder the process of cooperation. The disputed boundaries, cross-border dispersion of ethno linguistic and religious groups, conflicting economic interests, problems in distribution and management of shared resources and above all the problem in the processes of nation building and economic development are amongst the factors that constitute roots of the conflicts in South Asia.

South Asia profile

South Asia occupies an important strategic position in the world. Its population amounts to one fifth of the total world population. There is a big disparity among the countries of this region. In the world, it is most impoverished region where about half of the region's total population live below the poverty line. The ratio of resources to population is very low. The increasing pressure of population growth on the limited amount of natural resources leads to intensive exploitation of such resources. The growing subsistence needs ask for larger demands on water, arable land and forest. Almost all the countries of this region suffer from a series of environmental problems in the form of deforestation, soil erosion and fresh water scarcity. Moreover, the presence of these factors also precludes the possibilities of mutual cooperation among these states for the peaceful resolution of conflicts. These problems lead to the situation of conflict among these countries.

South Asia is also a region of great disparity in the terms of the size, population as well as military capability of its units. The region has been known by many unresolved conflicts for a long time. There are a lot of disputes between India and its neighboring countries. South Asian countries are also facing numerous internal cleavages and conflicts. These conflicts involve cross-section of classes, social strata, ethnic and linguistic groups, religions, communities and geographical regions. Religion plays a dominant role in the intra and inter-states relations of this region. Pakistan and Bangladesh are created on the basis of religion. Religion based confrontations have been a major source of long standing tension and periodic hostilities between India, Pakistan and Bangladesh.


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www.ijmer.in
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

South Asia is one of the most conflict prone regions of the world. Inter-state relations in South Asia are characterized by the existence of a number of bilateral disputes which proved to be too difficult to resolve. While some of them are rooted in the historical past, others are in the current dynamics of bilateral as well as intra-state relations. The lists of these disputes are too long. But here the major bilateral disputes have been discussed. In fact, inter-state relations involve territorial disputes inherited from the colonial past like Indo-Pak dispute, the dispute over the sharing of water resources of common rivers including that of the Ganga like between India and Bangladesh, intra-state conflict involving ethno-linguistic and religious groups with cross-border affiliation, and conflicting economic interests and other issues etc. Thus, such issues have actually been the source of conflicts in South Asia. However, these issues have the potential to escalate into violent conflicts at the same time they are also proving to be a source of bilateral or multilateral cooperation among the states. In fact, the nature of such conflicts is such that their resolution needs continued collective efforts of the sovereign state actors across borders.

The conceptual and theoretical analysis of conflict and conflict resolution

Conflict is an all-pervasive social phenomenon. It is a situation where different actors are pursuing incompatible goals. Conflict exists where at least two parties try to pursue the same scarce resources at the same time. Conflicts are not static. They are dynamic. Conflict occurs at different levels of social life: interpersonal, inter-group, international and inter-organizational. Broadly speaking, the concept of conflict encompasses overt and latent interactions amongst contending groups and interests which may be non-violent or otherwise. The diverse urges, aspirations, and expectations of these groups constitute the propelling force behind perennial nature of the conflict. But actual conflict situations are precipitated when these components of propelling force are translated by the leadership of those groups into specific demands for prestige, status, share in power and concrete material gains. Conflicts can be constructive as well as destructive. They lead to cooperation among parties for peaceful resolution.

Conflict Resolution deals with the affairs of humans. It is a situation where the possibility of the use of force is reduced. Mediation, negotiation, good offices etc. are the technique of Conflict Resolution which are used for the peaceful resolution of the conflict. In the process of Conflict Resolution focus is shifted from incompatibility to compatibility between the related parties. Among the various paradigms of Conflict Resolution, power politics paradigm and analytical problem-solving paradigm are the most important. Analytical problem solving approach is an advancement on the power politics paradigm. The power politics model is premised on the unrestricted behaviour of states as sovereign territorial entities and on the prolific use of force or the threat to use such force to settle dispute among them. Whereas, analytical problem solving approach advocates the resolution of conflict with the help of a mediator who facilitates the parties to resolve the conflict. Analytical problem solving approach aims at minimizing the role of power in any form to the maximum extent for the peaceful resolution of conflict. It lays emphasis on the mutual and constructive efforts among the parties in the conflict for its resolution. The resolution of the conflict, it further reiterates, has to be equally beneficial for all the parties to the conflict.

Conflict over the sharing of the Ganga water between India and Bangladesh.

This conflict has been the main conflict since the construction of Farakka Barrage. The work on Farakka Barrage started in 1961 and completed in 1971. It became operational in 1975. The Ganga River system is equally important for both India and Bangladesh. For India it is like a lifeline because it serves in several ways like hydropower generation, irrigation, navigation etc. For Bangladesh its uses are several such as irrigation, power generation, fisheries, navigation etc. Thus, it has been the most serious conflict between both the countries because different governments of the respective countries, at different times, took this issue from varying point of views. In fact, India's contention was that as an upper riparian country it has every right to utilize waters flowing in its territory. The diversion at Farakka does not create any shortage of waters into Bangladesh. Bangladesh's contention was that the withdrawal of waters by India at Farakka which has caused scarcity of waters into its territory is illegal, unjustified and inhumane. This problem remained unsolved during the Pakistan period. But the problem took a serious turn after the emergence of Bangladesh. Both these countries tried to resolve the problem of sharing of Ganga waters. For this purpose, both the countries concluded many agreements and accords to solve the problem. But they could not reach to any permanent solution for a long time. Because, the problem was not only the scarcity of water during lean season but was that of difference of perceptions, approaches, attitudes of the both parties towards each other. Due to these reasons over the last forty years the nature of the conflict continued to be fluid. Therefore, the nature of the agreements which were concluded from time to time to resolve this conflict has also been different. The most important agreement among these is December 1996 agreement. Earlier to this agreement many techniques of Conflict Resolution has been used like bargaining, consultations etc. but there was a lack of mutual understanding.

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Online Copy of Article Publication Available (2023 Issues)
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Article Received: 2nd February 2023
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Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/12.02.05
www.ijmer.in

Ganga water conflict: Conflict Resolution Perspective

How Ganga water conflict is a conflict constitute the moot question of this chapter. For a conflict to exist, in the Conflict Resolution framework, there need to be a minimum number of two parties and at least one issue of contention among them based on the scarcity of its resources. All these elements for a conflict to exist were present in the case of Ganga water conflict between India and Bangladesh. Bangladesh and India comprised of the parties; the sharing of lean season water flow fulfill the condition of the issue of contention which further fulfill the condition of scarcity in the form of availability of water during the lean seasons. Another important theme of this chapter highlighted the model of Conflict Resolution being applied on Ganga water conflict. Various efforts, agreements, accords and visits of the Prime Ministers of both the countries helped in shifting the focus from incompatibility to compatibility which is the necessary element of conflict resolution.

Another equally important element in the process of conflict resolution is the notion of scarcity. The December 1996 agreement is considered to be the most important for the resolution of the problem of scarcity during the lean season. Though, this agreement has been successful in resolving the conflict, but the problem of scarcity still remains there. The nature of scarcity in the present case revolves around the limited flow of water during the lean season, which fails to fulfill the respective requirements of India and Bangladesh. The resolution of the scarcity in this regard confined mainly to the equal sharing of the limited amount of water. Whereas, the actual amount of water remains scarce for both of the parties. In such condition the Conflict Resolution help only in the form of reaching to an agreement for sharing of the limited resources in a peaceful way. It is here that a delicate situation emerges where the scarcity of the resources comes to stay permanently. In case any of the party tries to jump the agreement, the ugly face of scarcity would engulf immediately the concerned parties into conflict. Conflict Resolution aims at managing scarce resources in such a manner that all the parties to the conflict express their willingness to the consensus and fell satisfy by the outcomes. In the case of Ganga water conflict this was done in 1996 agreement because this agreement was made by the mutual understanding and to the satisfaction of the parties concerned. However, the scarcity of the resources has not been resolved materially.

The parties have mutually agreed through the process of constructive interactions made possible by various visits of head of the state of both countries to each other and through their various consultative meetings. Through these efforts India and Bangladesh have unanimously agreed to shift the focus from incompatibility to compatibility over the issue of contention. Through various agreements which eventually culminated into a peaceful December 1996 agreement. This agreement made the provisions to successfully manage the scarce water flow during the lean seasons to the satisfaction of both the parties. The way of approaching the conflict was different in this agreement and this is the best example of Conflict Resolution in South Asia.

This approach can be extended to resolve other conflicts between India and Bangladesh and to other conflicts in South Asian countries. India and Pakistan can adopt this approach to resolve their long-standing bilateral conflicts. The dispute over boundaries, distribution of river water, Kashmir and nuclear issue etc. can successfully be resolved if both the parties willingly adopt this approach of mutual understanding towards each other. The problem of runn of Kutch has been resolved by India and Pakistan by adopting this approach. Besides Pakistan and Bangladesh, India has no serious conflict with other South Asian countries. So far as Bangladesh is concerned the serious problem of sharing of Ganga waters has been resolved through the conflict resolution process. There are still other minor problems between both these countries like Chakma refugee problem, New Moore Island, and border dispute. But these problems can easily be resolved by resorting to the same technique of mutual understanding.

In the end we can say that conflicts are inevitable to emerge in the international politics. But until and unless parties to a conflict are motivated to seek the underlying causes of their contention through self-introspectory and mutually interactive way involving dialogue, any attempt to settle or resolve conflict will ultimately lead to failure. If conflict results out of people's or parties struggle for incompatible goals or their efforts to fulfill their basic interests the resolution of the conflict then too lies in the very sincere efforts of the people or parties involved in the conflict. Thus, Conflict Resolution through mutual understanding is the most practical and ideal means to resolve them.

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ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:2(1), February: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd February 2023
Publication Date:10th March 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/12.02.05
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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
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Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

GOOD GOVERNANCE WITH SPECIAL REFERENCE TO KAUTILYA'S ARTHASHASTRA

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Abstract

Good governance is a term which has become an exercise and agenda of almost all institutions. Good governance has different implications to different organizations and institutions. But Kautilya's Arthashastra is a crossover treatise which deals with the art of government, its functions, the duties and responsibilities of the leader/ king and his ministers and above all the concerns of his citizens. As a good number of works has been completed on the issues related to good governance. The idea of good governance has been undergoing expansion and acquiring new features and elements over centuries in human history. Great thinkers, scholars and philosopher have raised the issue of good governance from time immemorial in political discourses, treatise and produced many writings over the centuries. They have conceptualized many aspects, dimensions and characteristics of good governance in the process. In the background of all these facts this paper aims at exploring the various aspects of good governance with special reference to Kautilya's Arthashastra.

Keywords: Good Governance, Arthashastra, Exploring, Functions.

Introduction

Governance is a dire need of many countries in the world today. The intellectuals, bureaucrats and civil society members have been accepting this need day by day. The concept of governance is not new. In fact, the concept of governance is as old as human civilization. It is widely accepted that good governance First and for most presupposes justice. The well-established hallmarks of good governance include

- Justice for All citizens
- Justice between state/government officials
- Justice between men, women and individuals
- Justice between minority groups and majority groups

Now the question arises is what justice is and how it can be secured or administered. These specific questions constitute the subject matter of Plato's "The Republic". Plato's conception of good governance is evident in his theory of ideal state in which philosophers i.e. after undergoing years of training and learning to distinguish well from evil would be kings. The Development and promotion of the mental and physical health of the citizens would be the responsibility of the state and the citizens would perform well-defined public duties.

If good governance is another name of democratic government then Aristotle should be considered as the strongest defender of it. As per Aristotle's theory the Citizenship signifies participatory Citizenship by which citizens actively participate in governmental /administrative decision making processes. The citizen shares in the administration of justice and in the holding of public office. Aristotle highlighted the need for observance of ethics in public life i.e. Moral code of conduct for politicians and administrators for the First time. Good governance calls for Corruption free management of public affairs and promotion of public good. Adherence to High Moral standard by members of political class and the bureaucracy and finally peace+ prosperity for the public. So, the credit for stressing the importance of public service ethics in his self-titled works goes to Aristotle. As this master philosopher reminded the humanity that good governance can be possible only if the government shows Great concern for equality of all the citizens because the existence of inequality in society is bound to trigger revolution as per this theory of revolution.

After the Second World War and decolonisation, the emergency of new nations i.e. third world acted as a big impetus for further evolution of the term good governance for developing nations. The issue of Development became a question of life and death. Good government meant removal of ill health, poverty, unemployment, illiteracy, ignorance and good governance became synonyms. In India, since 1991 the government has effected a paradigm shift in its development policy. The state led modal i.e. command model has been substituted by people participative, democratic and decentralized Model of Development which is based on the policy of privatization, liberalization and globalization.


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DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

The need for sustainable Development and sustainable human rights including child development women empowerment rehabilitation of the displaced and disabled and below poverty line people have been brought with in the ambit of good governance since 1975. Good governance means dedicated participation and total commitment of the state civil society, NGO's citizens and individuals everywhere working from the grass roots to the national and international level to oppose, supress and root out the terrorism and other heinous crimes against humanity.

THE CONCEPT OF GOOD GOVERNANCE: KAUTILYA'S ARTHASHASTRA

Kautilya's Arthashastra is a "treasure- trove filled with precious gems of wisdom". Irrespective of the forms of government the concepts and principles of Kautilya have relevance to administrators of society. It is necessary to relate his principles of good governance to modern times and concepts and today's problems in each political system. Rather than focusing on the theories of origin and functions of the state Kautilya's Arthashastra deals with the subject of statecraft i.e. policy of the state administration. It may be said that Arthashastra is the first comprehensive treatise on politics in ancient India. As Artha means material wellbeing of the people living on the earth in this reflection Arthashastra may be defined as the Shasta which shows how acquisition and protection of earth and its inhabitants may be carried out. Generally, it is the responsibility of the state.

According to Kautilya's Arthashastra, good governance has three basic objectives:

1. The provision for national security and infrastructure- such as roads to facilitate and promote commerce should be ensured by the king.
2. The king should ensure the formulation of efficient policies and their effective implementation, he should make arrangements for removal of all obstructions to economic growth which encourage capital formation.
3. The king should ensure a fair and clean administration.

The concept of good governance refers to a political system in which the king exercises his power in utilizing the economic and social resources for the welfare of his citizens. Arthashastra states: "In the happiness of his subject lies his happiness; in their welfare his welfare; whatever pleases himself he shall not consider as good, but whatever makes his subjects happy, he shall consider good". The work of government is threefold- that what the king sees with his own eyes, that what he knows indirectly through reports submitted to him and that what he infers about work not done by knowing about work that has been done. A King should be bound to guarantee that the common good (dharma) is preserved. So, good governance works as foundation for the sustainability of human development.

Collaboration of different factors in society/community

According to the Kautilya's Arthashastra, good governance comes with peace and order in the system and it can be achieved through the collaboration of various different factors in a community. The first of among these factors is the leader. The leader is the one who takes responsibility for everything what is happening in a community. The leader or the king who leads the nation plays a very significant role in Indian society. Therefore, a leader must be manifested with a lot of virtues. Because the Kautilya's Arthashastra's main goal is to nurture the welfare of the people, which is called Yoga-kshema, the King or the leader of the society must work for achieving this goal. Kautilya says, in the most categorical pronouncement of the concept of good governance, in the 19th chapter of book1:

In the happiness of his people lies his happiness,

In their welfare his welfare,

Whatever pleases himself he shall not consider as good, but

Whatever pleases his people he shall consider as good.

Thus, Kautilya is for a welfare state where the welfare and prosperity of the people are the supreme concern of the ruler. This welfare goal must be the most important and prime concern even against the King/ leader's own needs. This is the true service of a leader.

The king's deference to the law

Apart from rendering his welfare services to the people, the king's deference to the law is the second most important factor. The king of the state must be righteous and must become an example for his citizens. As what is written in the first chapter of the

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DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

Arthashastra, "as when the king is active the servants become active following his example. If he is too remiss, they too become remiss along with him." So, if the king himself humbly follows rules and regulations, so will the people. Therefore, the king must be morally upright in every way. The Arthashastra talks about various qualities of a good leader and stresses on honesty and trustworthiness of the King. Kautilya gave much emphasis on the issue of corruption as it was ravaged in the Indian society during that time. Even though with the passage of time corruption still remains in the present society.

Political stability and sagacity of the king

Kautilya's views on politics were largely based on secular politics. He believed that politics must be considered as an independence science. As per Kautilya's Arthashastra, intelligence and knowledge were the most important factors in maintaining the system of the kingdom. Kautilya occupies pre-eminence position among the famous ancient Indian political thinkers because of his ideas on politics and ethics. His analysis of the relationship of politics and ethics reflects the most enlightened view-point of one of the most renowned political thinkers of ancient India. Artha is an imposing element in this world and politics is also related to it. This idea is in complete similarity to materialistic philosophy of Karl Marx, according to which political power is based on economic factors. His Arthashastra is "truly an anthology of political wisdom and art of statecraft. Thus, Kautilya declared that politics was the supreme art and supreme science. The prosperity and utility of all sciences hinged on the wellbeing of the politics.

The main duty of the king

The first and form of duty of the king is to ensure the protection of the people. Dakshina aur Palna mint primary the protection of the people and property of the state. The ruler most maintain order of the society disorder and offences against the law must be repressed without and you severity or leniency. Discipline lays at the route of the success. Kautilya's whole concept of the duties of the rulers is positive and explicitly suggested improvement of every aspect of social life. According to Kautilya justice is the Bedrock of the society. The king must follow the views and wishes of the friends and leaders of the people. If the ruler works against the wishes of the people or if he fails to keep his promises to the people, he shall become unreliable and untrustworthy. The ruler must also find his own prosperity in the prosperity of the people and he must give priority to provide them security and wellbeing. He should help the miserable helpless and deceased persons. According to Kautilya's Arthashastra a good ruler is expected to attend the problems himself faced by women, the aged, and the afflicted and helpless person. The ruler should be bound by his vow to be always in readiness for action in the discharge of his duties.

Alertness of ruler

According to Kautilya's Arthashastra if the ruler is strong and full of energy the people will be equally so. It is necessary that the ruler is always wide awake and alert. With the help of the prime Minister, he is expected to keep an examining watch over the character and conduct of his ministers. He says that a ruler must devote 18th his time each day for attending the problems and concerns of the common people. Kautilya says that a ruler or King must never cause his petitioners to weight at the door.

Kautilya on Economics

According to Arthashastra, there is a strong relation between good governance and the economy of a nation. As stated in the Summary on Kautilya's Arthashastra: It's Contemporary Relevance, "the end is economic governance while political governance is the means" (Chapter 2). It means that good management and good governance has an effect on the economic aspect of a nation. The Arthashastra then serves as basic guidelines for the proper management of the economy of the country. Having the knowledge about how to manage resources, such as monetary funds, can contribute to the development of the economy.

And since, the political governance is the means for economic governance, there is a need for good political governance. Kautilya noted, "Hence the king shall be ever active in the management of the economy. The root of wealth is economic activity and lack of it brings material distress. In the absence of fruitful economic activity, both current prosperity and future growth are in danger of destruction. A king can achieve the desired objectives and abundance of riches by undertaking productive economic activity"

Views regarding need of Education

Kautilya also recognise the need of education of the king and administrator for developing their skills in state apart from giving guidelines on good governance. He focused on the overall development of the personality of each person so that he may be able to make reasonable judgments. His focus was not only limited to the education of politics. He also imposed upon the ruler strict moral discipline as well as control of the senses. It was for the first time that such integrated scheme about education was formulated for the ruling class.

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DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

Appointment of Qualified Ministers

Kautilya's Arthashastra Lays down strict norms of conduct and control for the heads of the departments and officers of the government. If an officer fails while discharging his responsibilities then he would be fined twice the amount of his pay. Chief Officer will be given the responsibility of keeping the watch over the performance of each person under his charge. Therefore, in order to maintain the probity in public service and to obviate chances of corruption among officers the confiscation of the wealth i.e., earned through unfair means is prescribed by Kautilya.

Conclusion

However, elements of good governance are not solely dependent on the function of the government itself, but it equally hinges on the mutual cooperation, understanding and involvement of the majority of the citizens and organisations. From the above facts it can be concluded that Kautilya advises the king to place the interests of his subjects above his own. He should take the responsibility to make his subjects prosperous, impartially deliver justice, and never try to misuse his power. In other words, the king should not give any chance to his subjects to be disappointed with him. It can be possible through selfless and dedicated service that the king wins the love of his subjects, and secures his royal position. In the end it can be said that Arthashastra covers many aspects and propounds principles of universal applicability. The use of these principles will enhance our present day efforts towards fulfilling the national agenda for good governance undoubtedly.

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DOI: http://ijmer.in.doi./2023/12.02.31
www.ijmer.in

RELEVANCE OF UN IN THE CONTEMPORARY WORLD ORDER

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Introduction

UN and its role, always remains a subject of discussion, debate and criticism among the students, statesmen, policy makers and academicians since its origin. United Nations Organisation came into existence after the Second World War in 1945.It was an effort of the world leaders to protect the coming generation from the destruction of Third World War. It was formed to maintain the international peace and security, to develop friendly relations among nations, to develop international cooperation among the states, for the solution of socio-economic, political, cultural and other problems and respect for human rights and other freedoms.

To achieve these objectives UN Charter was framed by the founder members. Basically it was a positive reaction of states involved in the Second World War. When the UN Charter was signed in 1945 there was clear consensus on the organization's role, but the world has changed dramatically since then. Member states need to contemplate how global developments have affected the relevance of the United Nations and decide how it can be changed so that it can serve better in the contemporary world scenario. The paper is an attempt to analyse the relevance of UN in the cotemporary world order especially in the context of Russia-Ukraine war.

With the end of the Cold War and other drastic changes in 1990's, the emergence of the United States as the most powerful country made the world order Uni-polar. This shift in the world order has only added various opportunities and problems especially in the context of political instability in different parts of the world. The economic globalisation, which has swept the world like a hurricane, has only introduced income inequalities among peoples and countries. The contemporary world order is a subject of discussion and debate especially after the out- break of Russia and Ukraine war. But it can stated that it is changing from multi centric to more bi-polar world order, as Russia with China are emerging as another centre of power against US and its allied powers in the world. In this changed world scenario it is important to analyse the significant role and relevance of UN.

Origin of UN

With the long process of meetings and conferences of Heads of the state/Government UN came into existence after Second World War in 1940's. On 1 January 1942, President Franklin Delano Roosevelt of the United States convened a conference of 26 'Allied' Nations in Washington D.C. The conference issued the 'Declaration by United Nations'. The intention of the Declaration was to create structures of global governance under the broad framework of the UN. These structures would secure, and sustain the peace that would follow the war.

Sustaining post-war peace by creating a holistic triad of structures to deal with financial, developmental, and trade policies became a priority for the participants of the Washington conference. Their broad goal was to overcome economic nationalism, which had given rise to conditions leading to the Second World War, and to facilitate the flow of capital for investments in reconstruction and development, especially of infrastructure, to sustain economic growth.[i]

After a long process of conferences and meetings UN came into existence in October 1945. During the process of making UN, various principles were decided for the smooth working of the organisation. It was decided that UN will work on fundamental principles of sovereignty of the states, peaceful settlement of disputes, no use of force, committed for assistance of the state by other states, no interference in domestic jurisdiction of the member states. It has own UN charter and constitutional mechanism was developed to guide UN in its working. Different agencies were created for the functioning of the International Organisation.

UN is not relevant

It is being argued by the critics that UN is not playing its role in changed international scenario and it is not relevant. It is not capable to face the threats to world peace and security. It has no its own military and economic power for collective security operations. Due to lack of its own security mechanism it has to remain dependent on other states. It is also being argued that it is puppet in the hand of super powers. The veto powers countries misuse their powers for their own national interests. Like other wars and conflicts in the history, during the Russia Ukraine war, it has been proved that UN and its agency are not able to resolve the arms conflict and disputes,

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DOI: http://ijmer.in.doi./2023/12.02.31
www.ijmer.in

especially where the veto power country is involved. Russian Ukraine war is still going on after various efforts by the international organizations and European and other countries of the world.

Failure to check arms race in the world

Arms race and existence of nuclear, chemical atomic and other weapons of mass destruction is the biggest challenge to world peace and security. For their security and economic gains countries are involved in making of destructive weapons in the world. Sale of weapons is the important base of their economy and economic market. Basically, they are the threat to peace and security. Complete disarmament and arms control are the fundamental goals of UN. It has developed various mechanisms for keeping check on arms race. Various treaties were made (such as NPT, CTBT, SALT 1, SALT 2, PNTBT, Outer Space Treaty, Chemical Weapon Convention, Arms Trade Treaty etc.) to keep check on the making, testing and development of the weapons of mass destruction. But the agency is failed in checking the arms race in the world. Weapons of mass discretions are being developed by all the powerful countries in the world. Due to its economic and other limitations UN is not able to impose its policies and decisions on member states who are involved in arms race. The existence of these weapons are challenging the harmonious and cordial relations among the states and creating conflicts. Nuclear states are using weapons as instruments for their foreign policy goals. They are influencing the small and weak states and promoting and protecting their interests at the international level.

Failure in the pacific settlement of disputes

Pacific settlement of disputes is one of the important tools for the maintenance of peace and security by the UN. As per UN charter UN secretary general has been assigned duty to work as watchdog of world peace and security when any conflict arises, it is the prime duty of UN and its agencies to trace the problem and use all the methods mentioned in the UN charter for the pacific settlement of international disputes.

The international organisation is not able to resolve the issues between the states and they convert in arms conflicts. Member states are bound to follow the decisions of UN but practically they try to avoid UN decisions for their national interest. Fundamentally, foreign policy of any state is bound to fulfil the national interests than obligations of regional or international organisations. Russia-Ukraine military conflict is the latest example of arms conflict where UN agencies failed to settle the dispute well in time. Now it has been converted in one of the longest war of the world. There is a need to strengthen UN and its agencies for effective application regarding the provision of the pacific settlement of disputes.

Failure in the maintenance of peace and security

World peace and security was the core issue behind the origin of the UN after the Second World War. Special provisions were made for coercive methods for maintenance of peace and security in chapter 7 of the UN charter. It has carried out collective security actions during Korean war in 1951and Gulf war in 1990's. but it was hijacked by the then superpowers for their own national interests. Even the activities were carried out under the leadership of UN practically it was carried out by America (Superpower) and its allied countries. Even the flags of UN were not used in the Gulf War. Only few countries participated in the activity of collective security and send their military for the military operations, as per their national interests. In fact, UN does not have its own defence mechanism (military and weapons) and resources. The organisation always remains dependent on its member countries for military and financial assistance. Therefore, the success of UN collective security activity depends on the will and interests of member countries specially the superpowers.

Unable to manage socio-economic crisis in the world

UN was created for keeping check on armed conflicts and wars in the world. It was a strategic and diplomatic objective but it cannot be fulfilled without economic conditions. Economy is the sub-structure of society which determines the political conditions and national interest of a state. They play important role in building peace and security in the world. For this purpose, UN established social economic council to take care the socio-economic problems of the world. The council has framed various policies and programmes for the solution of socio-economic problems and welfare of the masses in the world. It has played an important role in the identification of the problems but not able to provide solution and remedies to the problems. After the origin of UN, the world is facing the problem of illiteracy poverty, hunger, unemployment, starvation etc. In fact, UN activities depend on the economic support of the member countries. It does not have its own economic and financial mechanism for the financial support to the member counties. It depends on the donations given by the economically rich countries. As when and how they donate UN use the same donation for its socio-economic objectives.
UN member countries are facing serious social and economic problems. Economic crisis of Sri Lanka is one of the examples of worst economic condition of a member of UN. UN is helpless and not able to cooperate and support the Sri Lankan government at this time

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DOI: http://ijmer.in.doi./2023/12.02.31
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of grave economic conditions. In fact, UN agencies didn't play any role to protect the lives from hunger and starvation. There is need to economically strengthen UN and its agencies so that lives of poor people can be saved.

Failure of UNO in the resolution of environmental issues

Due to technological and other development the world is facing serious environmental issues in the world. In fact, scientific and technological development has provided comfort and facilities for better life. They have speed up the industrial, commercial, and economic growth in the world. On the other hand they have destroyed the ecological balance and environment. After the industrial revolution the world is facing the problem of global warming, climate change, depletion of ozone layer, environmental pollution, acid rain and other problems related to environment.

UN has framed various policies and programmes for the production of environment. It has organised various conferences on global warming and climate change at international level but the world is still facing the serious problems related to environment which are directly related to the human life. In fact, the world is divided between north and south on this issue. North (Rich) are blaming South (Poor) for environmental pollution. They are arguing that 70% of the world population is living in south. They are consuming and exploiting more natural resources than north. South is blaming north for producing maximum carbon-dioxide and other poisonous gases through the use of refrigerators, air conditioners and other household equipments for better living standard. They are polluting more. Polluters must pay for the environmental protection.

Due to this North South controversy, UN is not able to play any constructive role to protect the human life from environmental pollution. There is a need to strengthen UN and its agencies for effective outcomes of its environmental agenda.

Lack of democratisation of UN and its structure

Democracy and democratisation of the regional and international organisations is of utmost importance in the modern age. Democratisation stands for equal right and participation of the member countries. UN is not a democratic organisation. it is unequally organised and structured. Few countries are having more shares in the distribution of power. Especially the super powers have been given veto power. They are dominating the whole working of the organisation. Rest are depending on the cooperation of veto power countries. The undemocratic structure and working of UN has created questions on its role and relevance in the contemporary world order.

Due to undemocratization member countries are not cooperating UN in its working. It has become an instrument of fulfilling foreign policy goals of the rich and powerful countries. It is unfortunate on the part of the UN that it is dominated and hijacked by the veto power countries for their national interest. If even one veto power country is against the decision of the ogranisation. It can create hurdle and avoid UN to take necessary actions in particular conditions specially clash of interests. The powerful countries dominate the other developing countries and try to impose their own decisions on these weak countries. It is one of the biggest hurdles in the democratic decision making process of the UN. There is a need to democratisation of UN and its agencies for the healthy working of the UN in the contemporary world order. Basically, it was a condition at the time of origin of the UN to give special status to the powerful countries. In fact imbalance in UN is the base of its survival and growth. If no special powers were given to these countries than UN cannot come in existence after the Second World War. Even in the present scenario, it is the survival condition for the world body.

Provision of non- interference in domestic affairs of the member states

To protect the sovereignty and identity of member states, special provision was made under the article 2(7), of the UN charter. According this article UN has no authority to interfere in the domestic affairs of the state. It was an effort to maintain balance between UN and state sovereignty so that UN can function in coordination with member states.

Initially, it was respected by all members, later on it was misused by the states as an instrument against UN actions. Basically, it is a subjective phenomenon. Sometimes, it is difficult to explain what domestic matter is and what is not domestic. Due to subjectivity, state can claim any issue as domestic issue. Due to this structural and functional dimension of UN and the existing international scenario, it is difficult for UN to play any role in the pacific settlement of the dispute. The failure and success of UN depends on approach towards the issue.

Emerging trends and issues in the cotemporary world order are posing challenge to the working of the UN. Basically, there is a conflicting situation between the international organisation and national interests of the member states. In Russia-Ukraine war UN is

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not able to play any constructive role due to Russia as a Veto Power member. Russia is using veto power and dominating decision making process in the security council of UN.

Security threats from the violent arms conflicts between states (such as Russia and Ukraine conflict). Violation of human rights in the different parts of the world is emerging as one of the crucial humanitarian problems for UN. Terrorism, religious fundamentalism, communalism, violent riots in the different parts of the world are posing another threat to world peace and security. Arms race and existence of weapons of mass destruction (Atomic, nuclear weapon, chemical weapon, Hydrogen bomb, biological weapons, Inter Ballistic Missiles etc) are another challenge for maintenance of world peace and security.

There has been the existence of political and non-political controversial issues among the states. (Especially the border disputes such as Indo-Pak and Indo-China border disputes). Beside this, threats from violence and massive human rights violations within states, threats from terrorism, threats from organized crime, threats from the proliferation of weapons - particularly WMD, but also conventional.[ii]

UN is still relevant in the contemporary world order

Though UN is facing various challenges and problems regarding world peace and security but that do not mean that UN is not relevant. The world body has played significant role in the peaceful settlement of international disputes. It has played very significant roll abolition of imperialism and colonialism, racialism from the world after the Second World War. It has successfully created awareness about the non- political issues such as environmental protection, human right, welfare and security of women, protection of children rights. It provided an important for platform for building of international cooperation, coordination and harmonious relations among the member states. UN has increased recognition and respect for international law in the world by its efforts for establishment of various institutions.

Moreover, it has created awareness about international issues and conflicts among the states at international level and try to avoid conflicting situations among the states. Always try to keep check arms race and the different types of weapons of mass destruction in the world. It was an effort towards weapon free world. During COVID- 19 Pandemic UN played significant role in awareness on the pandemic and saved the human generation from the menace of the pandemic.

It needs reforms in its basic structure and functioning. There is need to restructure and expend UN Security Council so that it can provide representation to all the major powerful countries.[iii] It is important for the democratic functioning of the Security Council that use of veto power should be minimized. It should work as a leading agency of UN for the maintenance of world peace and security. Moreover, it is important to democratise the structure and functioning of UN so that it can really work as world body and represent the whole world. There is need to create some mechanism for military and financial assistance for UN peace operations so that it should not remain depend on the member states for its peace operations and other activities regarding the implementations of its provisions for the same. It is important that UN should develop a mechanism for arms control through its agencies so that world can be saved from the destruction of the weapons. There is further need to strengthen the efforts for the protection of ecological environment in the world so that life can be saved from the threat of global warming, climate change, and other environmental threats.

In the changed scenario social and economic security is one of the major challenges for the developing and poor countries of the world, through social and economic council can help these countries to come out from hunger and starvation. Despite crippled by Bretton Woods Institutions, UN has played limited but effective role on the economic matters. Supported the North-South dialogue and aspired for emergence of new international economic order.[iv] Moreover, there is need to create an atmosphere of trust and mutual faith among the member states. The whole working of UN depends on the cooperation of member states specially the superpowers. For the cooperation, mutual faith among member states is must. There is a need to give importance to international issues than national interests by the states.

Conclusion

Role and relevance of UN always remain a topic of debate and criticism among academicians, statesmen and foreign policy makers during all world orders. During bi-polar world order, it was used and misused by the bloc politics. After the end of cold war in 1990's UN and its actions were dominated by US and its allied countries. Recently it was not able to play any constructive role in Russia-Ukraine conflict. It was framed in the post second war era to save the coming generation from the third world war. Various organs and agencies were created for its functioning. UN plays a prominent role in settlement of various disputes and maintenance of peace and security at international level. UN always remains dependent on its member states specially the superpowers for their financial

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assistance and military cooperation during peace operations and activities in the world. There is a need to restructure and democratise UN and its agencies to make it more powerful so that it can play significant role in the contemporary world order dominated by Russia-Ukraine war.

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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:1(5), January: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd January 2023
Publication Date:10th February 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

GOOD GOVERNANCE WITH SPECIAL REFERENCE TO KAUTILYA'S ARTHASHASTRA

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Abstract

Good governance is a term which has become an exercise and agenda of almost all institutions. Good governance has different implications to different organizations and institutions. But Kautilya's Arthashastra is a crossover treatise which deals with the art of government, its functions, the duties and responsibilities of the leader/ king and his ministers and above all the concerns of his citizens. As a good number of works has been completed on the issues related to good governance. The idea of good governance has been undergoing expansion and acquiring new features and elements over centuries in human history. Great thinkers, scholars and philosopher have raised the issue of good governance from time immemorial in political discourses, treatise and produced many writings over the centuries. They have conceptualized many aspects, dimensions and characteristics of good governance in the process. In the background of all these facts this paper aims at exploring the various aspects of good governance with special reference to Kautilya's Arthashastra.

Keywords: Good Governance, Arthashastra, Exploring, Functions.

Introduction

Governance is a dire need of many countries in the world today. The intellectuals, bureaucrats and civil society members have been accepting this need day by day. The concept of governance is not new. In fact, the concept of governance is as old as human civilization. It is widely accepted that good governance First and for most presupposes justice. The well-established hallmarks of good governance include

* Justice for All citizens
* Justice between state/government officials
* Justice between men, women and individuals
* Justice between minority groups and majority groups

Now the question arises is what justice is and how it can be secured or administered. These specific questions constitute the subject matter of Plato's "The Republic". Plato's conception of good governance is evident in his theory of ideal state in which philosophers i.e. after undergoing years of training and learning to distinguish well from evil would be kings. The Development and promotion of the mental and physical health of the citizens would be the responsibility of the state and the citizens would perform well-defined public duties.

If good governance is another name of democratic government then Aristotle should be considered as the strongest defender of it. As per Aristotle's theory the Citizenship signifies participatory Citizenship by which citizens actively participate in governmental /administrative decision making processes. The citizen shares in the administration of justice and in the holding of public office. Aristotle highlighted the need for observance of ethics in public life i.e. Moral code of conduct for politicians and administrators for the First time. Good governance calls for Corruption free management of public affairs and promotion of public good. Adherence to High Moral standard by members of political class and the bureaucracy and finally peace+ prosperity for the public. So, the credit for stressing the importance of public service ethics in his self-titled works goes to Aristotle. As this master philosopher reminded the humanity that good governance can be possible only if the government shows Great concern for equality of all the citizens because the existence of inequality in society is bound to trigger revolution as per this theory of revolution.

After the Second World War and decolonisation, the emergency of new nations i.e. third world acted as a big impetus for further evolution of the term good governance for developing nations. The issue of Development became a question of life and death. Good government meant removal of ill health, poverty, unemployment, illiteracy, ignorance and good governance became synonyms. In India, since 1991 the government has effected a paradigm shift in its development policy. The state led modal i.e. command model has been substituted by people participative, democratic and decentralized Model of Development which is based on the policy of privatization, liberalization and globalization.

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DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

The need for sustainable Development and sustainable human rights including child development women empowerment rehabilitation of the displaced and disabled and below poverty line people have been brought with in the ambit of good governance since 1975. Good governance means dedicated participation and total commitment of the state civil society, NGO's citizens and individuals everywhere working from the grass roots to the national and international level to oppose, supress and root out the terrorism and other heinous crimes against humanity.

THE CONCEPT OF GOOD GOVERNANCE: KAUTILYA'S ARTHASHASTRA

Kautilya's Arthashastra is a "treasure- trove filled with precious gems of wisdom". Irrespective of the forms of government the concepts and principles of Kautilya have relevance to administrators of society. It is necessary to relate his principles of good governance to modern times and concepts and today's problems in each political system. Rather than focusing on the theories of origin and functions of the state Kautilya's Arthashastra deals with the subject of statecraft i.e. policy of the state administration. It may be said that Arthashastra is the first comprehensive treatise on politics in ancient India. As Artha means material wellbeing of the people living on the earth in this reflection Arthashastra may be defined as the Shasta which shows how acquisition and protection of earth and its inhabitants may be carried out. Generally, it is the responsibility of the state.

According to Kautilya's Arthashastra, good governance has three basic objectives:

1. The provision for national security and infrastructure- such as roads to facilitate and promote commerce should be ensured by the king.
2. The king should ensure the formulation of efficient policies and their effective implementation, he should make arrangements for removal of all obstructions to economic growth which encourage capital formation.
3. The king should ensure a fair and clean administration.

The concept of good governance refers to a political system in which the king exercises his power in utilizing the economic and social resources for the welfare of his citizens. Arthashastra states: "In the happiness of his subject lies his happiness; in their welfare his welfare; whatever pleases himself he shall not consider as good, but whatever makes his subjects happy, he shall consider good". The work of government is threefold- that what the king sees with his own eyes, that what he knows indirectly through reports submitted to him and that what he infers about work not done by knowing about work that has been done. A King should be bound to guarantee that the common good (dharma) is preserved. So, good governance works as foundation for the sustainability of human development.

Collaboration of different factors in society/community

According to the Kautilya's Arthashastra, good governance comes with peace and order in the system and it can be achieved through the collaboration of various different factors in a community. The first of among these factors is the leader. The leader is the one who takes responsibility for everything what is happening in a community. The leader or the king who leads the nation plays a very significant role in Indian society. Therefore, a leader must be manifested with a lot of virtues. Because the Kautilya's Arthashastra's main goal is to nurture the welfare of the people, which is called Yoga-kshema, the King or the leader of the society must work for achieving this goal. Kautilya says, in the most categorical pronouncement of the concept of good governance, in the 19th chapter of book1:

In the happiness of his people lies his happiness,

In their welfare his welfare,

Whatever pleases himself he shall not consider as good, but

Whatever pleases his people he shall consider as good.

Thus, Kautilya is for a welfare state where the welfare and prosperity of the people are the supreme concern of the ruler. This welfare goal must be the most important and prime concern even against the King/ leader's own needs. This is the true service of a leader.

The king's deference to the law

Apart from rendering his welfare services to the people, the king's deference to the law is the second most important factor. The king of the state must be righteous and must become an example for his citizens. As what is written in the first chapter of the

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DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

Arthashastra, "as when the king is active the servants become active following his example. If he is too remiss, they too become remiss along with him." So, if the king himself humbly follows rules and regulations, so will the people. Therefore, the king must be morally upright in every way. The Arthashastra talks about various qualities of a good leader and stresses on honesty and trustworthiness of the King. Kautilya gave much emphasis on the issue of corruption as it was ravaged in the Indian society during that time. Even though with the passage of time corruption still remains in the present society.

Political stability and sagacity of the king

Kautilya's views on politics were largely based on secular politics. He believed that politics must be considered as an independence science. As per Kautilya's Arthashastra, intelligence and knowledge were the most important factors in maintaining the system of the kingdom. Kautilya occupies pre-eminence position among the famous ancient Indian political thinkers because of his ideas on politics and ethics. His analysis of the relationship of politics and ethics reflects the most enlightened view-point of one of the most renowned political thinkers of ancient India. Artha is an imposing element in this world and politics is also related to it. This idea is in complete similarity to materialistic philosophy of Karl Marx, according to which political power is based on economic factors. His Arthashastra is "truly an anthology of political wisdom and art of statecraft. Thus, Kautilya declared that politics was the supreme art and supreme science. The prosperity and utility of all sciences hinged on the wellbeing of the politics.

The main duty of the king

The first and form of duty of the king is to ensure the protection of the people. Dakshina aur Palna mint primary the protection of the people and property of the state. The ruler most maintain order of the society disorder and offences against the law must be repressed without and you severity or leniency. Discipline lays at the route of the success. Kautilya's whole concept of the duties of the rulers is positive and explicitly suggested improvement of every aspect of social life. According to Kautilya justice is the Bedrock of the society. The king must follow the views and wishes of the friends and leaders of the people. If the ruler works against the wishes of the people or if he fails to keep his promises to the people, he shall become unreliable and untrustworthy. The ruler must also find his own prosperity in the prosperity of the people and he must give priority to provide them security and wellbeing. He should help the miserable helpless and deceased persons. According to Kautilya's Arthashastra a good ruler is expected to attend the problems himself faced by women, the aged, and the afflicted and helpless person. The ruler should be bound by his vow to be always in readiness for action in the discharge of his duties.

Alertness of ruler

According to Kautilya's Arthashastra if the ruler is strong and full of energy the people will be equally so. It is necessary that the ruler is always wide awake and alert. With the help of the prime Minister, he is expected to keep an examining watch over the character and conduct of his ministers. He says that a ruler must devote 18th his time each day for attending the problems and concerns of the common people. Kautilya says that a ruler or King must never cause his petitioners to weight at the door.

Kautilya on Economics

According to Arthashastra, there is a strong relation between good governance and the economy of a nation. As stated in the Summary on Kautilya's Arthashastra: It's Contemporary Relevance, "the end is economic governance while political governance is the means" (Chapter 2). It means that good management and good governance has an effect on the economic aspect of a nation. The Arthashastra then serves as basic guidelines for the proper management of the economy of the country. Having the knowledge about how to manage resources, such as monetary funds, can contribute to the development of the economy.

And since, the political governance is the means for economic governance, there is a need for good political governance. Kautilya noted, "Hence the king shall be ever active in the management of the economy. The root of wealth is economic activity and lack of it brings material distress. In the absence of fruitful economic activity, both current prosperity and future growth are in danger of destruction. A king can achieve the desired objectives and abundance of riches by undertaking productive economic activity"

Views regarding need of Education

Kautilya also recognise the need of education of the king and administrator for developing their skills in state apart from giving guidelines on good governance. He focused on the overall development of the personality of each person so that he may be able to make reasonable judgments. His focus was not only limited to the education of politics. He also imposed upon the ruler strict moral discipline as well as control of the senses. It was for the first time that such integrated scheme about education was formulated for the ruling class.

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Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/01.01.99
www.ijmer.in

Appointment of Qualified Ministers

Kautilya's Arthashastra Lays down strict norms of conduct and control for the heads of the departments and officers of the government. If an officer fails while discharging his responsibilities then he would be fined twice the amount of his pay. Chief Officer will be given the responsibility of keeping the watch over the performance of each person under his charge. Therefore, in order to maintain the probity in public service and to obviate chances of corruption among officers the confiscation of the wealth i.e., earned through unfair means is prescribed by Kautilya.

Conclusion

However, elements of good governance are not solely dependent on the function of the government itself, but it equally hinges on the mutual cooperation, understanding and involvement of the majority of the citizens and organisations**.** From the above facts it can be concluded that Kautilya advises the king to place the interests of his subjects above his own. He should take the responsibility to make his subjects prosperous, impartially deliver justice, and never try to misuse his power. In other words, the king should not give any chance to his subjects to be disappointed with him. It can be possible through selfless and dedicated service that the king wins the love of his subjects, and secures his royal position. In the end it can be said that Arthashastra covers many aspects and propounds principles of universal applicability. The use of these principles will enhance our present day efforts towards fulfilling the national agenda for good governance undoubtedly.

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ISSN : 0974-0066

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Vol-83 No.03
January - June : 2023

आज़ादी का
अमृत महोत्सव

मध्य भारती

मानविकी एवं समाजविज्ञान की द्विभाषी शोध-पत्रिका

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ISSN 0974-0066

UGC Care List, Group-C (Multi disciplinary), Sl.no.-15

विशेषांक

राष्ट्रीय शिक्षा नीति-2020 : परिप्रेक्ष्य और परिवर्त्य

डॉक्टर हरीसिंह गौर विश्वविद्यालय

सागर (मध्यप्रदेश) - 470003

CONTENT

GANDHIAN PHILOSOPHY ON PEACE AND RELIGIOUS HARMONY

Dr. Rakesh Kumar, Assistant Professor, Department of Political Science, GMN (PG) College, Ambala Cantt. : drrakesh311@gmail.com

Abstract

Mahatma Gandhi was the greatest thinker of the twentieth century. Gandhiji was multidimensional personality. He was a mature political leader. He was a great social reformer. He was a saint, an educationist a humanist and a great philosopher. He won the hearts of the people across the globe was known as a saint. Being the leader of the popular and mass struggle like Indian freedom movement, anti-colonialism, anti-imperialism, anti-racism Gandhi ji adopted unique methods of Ahimsa (Non-violence) satyagraha (force of truth), trusteeship, Non-cooperation, peace and harmony. There is no peace for treachery, hate, cowardness and selfishness in the Gandhian Philosophy. On the other hand Gandhian philosophy to life and politics needs a lot of hard work, purity of soul, self sacrifice and tolerance or patience. He believed in equality, social justice freedom, peace and religious harmony.

Gandhi ji believed in peace and religious harmony because it is a situation or a period of time in which there is no war or violence in a country or area. In other words the peace is a concept of societal friendship and harmony in the absence of hostility and violence. In a social sense, peace is commonly used to mean a lack of conflict and freedom from fear of violence between Individuals or groups. In Joban Galtung words, "Peace is the absence of physical and structural violence." Albert Einstein says about peace, "Peace is not merely the absence of war but the presence of justice of law, of order – in short, of Government." On the other hand communal harmony means that people of different religion, castes, creeds, sex and different background live together in the society with love and peace. According to Gandhi, the main aim of religion is to make a one-on-one interaction between God and humans. He believed God is identical with truth. He perceived God through the service of humanity, because God lives in the heart of every human being or for that matter is every one of his creations. So, Gandhian philosophy communicates that the peace and harmony are the base of development, growth, social justice and stability in the globe.

Key Words : Thinker, Human, Leader, Peace, Harmony Religion, Society, Creation, Growth, World.

Gandhian Philosophy on Peace and Religious Harmony

Mohandas Karamchand Gandhi was born on 2 October 1869 at Porbandar, Kathiawar. His parents were followers of Jainism which regards ahinsa as one of its basic principles. At nineteen he was sent to England for the study of law. He returned to India in 1891. In 1893, he proceeded to South Africa as a legal counsel to an Indian firm. He stayed in South Africa for about twenty years. There he encountered the problem of racial discrimination. Indians were treated too badly for words, there were many oppressive and unjust laws against them at that time. In 1906, the government of South Africa passed a new law the Asiatic Registration Act. This law laid down that all Asiatics must register their names with the government and allow their fingerprints to be taken just as if they were criminals. Under the leadership of Gandhiji, the Asians in South Africa started a non-violent movement of civil resistance. There were thrown into prison by the thousands. Gandhiji was himself imprisoned. Ultimately, force had to bow before the heroic gentleness, and the Act was withdrawn.

In the world history it was first ever that the many people's including women, Youth and children have participated in the Indian freedom movement and defeated the British Empire peacefully. It was under the Gadhi's moral and political leadership quality that the people were not trained to practice Satyagraha and non-violence but they were also made a realize the power of truth. Gandhi said that all the problems come in society due to violence and only are solution of these problems peace and harmony.

"Emerging Trends & Challenges in Humanities, Education, Science and Social Sciences"

Published by:

International Council for Education, Research and Training

Dr. Rakesh Kumar

ISBN: 978-93-95789-05-9

March 25, 2023

Conference Proceeding

8th International Multidisciplinary Conference

"Emerging Trends & Challenges in Humanities, Education, Science and Social Sciences"

March 25, 2023

Editor:

Dr. Rakesh Kumar

ISBN: 978-93-95789-05-9

ICERT

International Council for Education Research and Training

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Branch office: 116, Shyam Ji Complex, Delhi NCR-Bahadurgarh, India

Website: www.icert.org.in

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Medicine in Omics 8 (2023) 100020

Contents lists available at ScienceDirect

Medicine in Omics

journal homepage: www.elsevier.com/locate/meomic

Review

Physiological Pathway, diagnosis and nanotechnology based treatment strategies for ovarian Cancer: A review

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ARTICLE INFO

Keywords:
Ovarian cancer
Diagnosis
Biomarkers
Nanotechnology
Novel drug delivery system

ABSTRACT

Ovarian cancer is a fatal disease requiring great attention from the scientific community to find novel ways for diagnosis and treatment. Most ovarian malignancies, or 90% of cases, are epithelial ovarian cancers. HGSC (High Grade Ovarian Cancer) is the most prevalent subtype, and the majority of women who are diagnosed with it eventually develop resistance to standard treatments. Expression of genes linked to these immune pathways and increased cytotoxic immune cell infiltration is primarily associated with HGSC cancer that shows DNA damage repair gene deficiency and high chromosomal instability. Currently, chemotherapy, radiation, and cytoreduction are the most common forms of treatment; nevertheless, in the absence of targeted therapies, patients experience unfavorable side effects and develop drug resistance. It is critical to understand the intricate biology of the disease and find relevant biomarkers in order to make an early diagnosis or anticipate how a patient will respond to a given treatment. Interferon activating medicines have been studied as a potential means of enhancing anti-tumor immunity in ovarian cancer. Chemical and biological nano-sensors have also been developed to detect different types of cancers, including ovarian cancer. Further, the application of nanotechnology for diagnostic and therapeutic purposes makes it a more convenient, target-specific, and side effect free delivery system for ovarian cancer treatment. A combination of nanotechnology with the physiological biomarkers and therapeutic agents created a novel system of nano theranostics, which have the potential of real-time monitoring and diagnosis and simultaneous delivery of the therapeutic agent for the treatment of ovarian cancer.

Contents

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https://doi.org/10.1016/j.meomic.2023.100020
Received 8 November 2022; Revised 26 December 2022; Accepted 29 March 2023
Available online 4 April 2023

Introduction

Ovarian cancer is the most prevailing cause of women world wide's fatality, being noticeable only at an advanced phase of the disease. In the early phases of the disease, patients display some general indications due to a dearth of efficient diagnosis approaches [1]. Ovarian epithelium generally results in huge ovarian cancer cases in contrast to stromal or germ cells [2]. These types of ovarian cancer parade assorted morphological as well as symptomatic changes [3,4]. Contemporary techniques employed for diagnosing ovarian cancer are amounts of CA-125 (cancer antigen 125) in the serum, MRI, CT, transvaginal ultrasonography, etc. Each method possesses its pros and cons depending upon the disease stage as CA-125 is not much helpful in examining the early stages and depends on the simultaneous use of other imaging techniques like MRI, CT. Initially, chemotherapy based on platinum was employed, then taxanes like paclitaxel were utilized, and presently the intraperitoneal chemotherapy approach is gaining more attention. Diagnosis of the disease at an early stage and specific treatment approaches can help in alleviating the disease. Further, the drug delivery approach's selection plays a crucial role in the target-specific transport of the cytotoxic agents. Advancements in nanotechnology aid in the treatment of ovarian cancer. The therapeutic agents can be modified using various ligands to make their complexes with the drug delivery system, for instance, carrier-conjugated complexes; self-assembling composites with polymers/lipids, using carbon nanotubes, quantum dots, metal–organic complexes, etc. [5-8]. The nano-sized colloidal drug delivery systems like liposomes, nanoparticles, and dendrimers are widely employed for targeted drug delivery in cancer treatment [9-11]. These drug delivery systems offer a controlled release of drugs with advanced pharmacokinetics and reduced toxic systemic consequences [12]. The nanoparticles gain access to the target cells through small apertures of the tumor cells' vascular lining [13]. The smaller size and surficial modification of nanoparticles facilitate their utility as drug delivery carriers as this helps avoid clearance by the reticuloendothelial system. Techniques like opsonization by using polyethylene glycol can be employed to circumvent clearance [12]. The advanced forms of nanoparticles like metal nanoparticles, i.e., gold, silver, copper, etc. nanoparticles, and magnetic nanoparticles, abet their targeting ability. The metal nanoparticles also facilitate the therapeutic effect of drugs used in the treatment [14,15]. Hence, the effectiveness of nano-carriers for cancer treatment is increasing recently due to their constructive assets in the treatment. However, the impediment in the execution of desired particle size dispersion and scale-up studies limits their commercial development [16]. The current manuscript will expound on the diverse aspects of the nano-carrier drug delivery system to treat ovarian cancer, along with its prospects.

Ovarian cancer pathophysiology

Tumors of the ovary are grouped primarily as surface epithelial-stromal, sex cord-stromal, and germ cell tumors, depending on the anatomical arrangement of tumor origination. In some cases, tumors are formed of different types hence known as mixed tumors. The tumors of the sex cord-stromal group are derived from mesonephric alongside mesenchymal source. While tumors of the group germ cells are found to be of similar origin to tumors of testicular germ cells, the germ cells that have gone off the target while drifting among the yolk sac and emerging gonads may result in tumors of germ cells outer to gonads [17]. Nearly 90 % of ovarian cancers are comprised of surface epithelial carcinomas. In 1972, Sir Spencer Wells identified the ovary's primary function surface epithelium in melanoma [18]. These tumors comprise diverse morphological classes such as mucinous, serous, clear cell, endometrioid, squamous, transitional, undifferentiated, and mixed depending on the epithelial manifestation of tumors. This type of tumor may primarily be resulted from the ovarian surface epithelium and intraparenchymal sacs or analogous benevolent injuries of the ovary and are constituted of mesothelial cells lining the ovarian surface [19,20]. The precursor of surface epithelium for ovarian cancer can be categorized and analyzed as described in Fig. 1.

Distinct conduits support the growth of the varied ovarian cancers; the low type serous carcinoma encompasses transmutations in KRAS and BRAF, the high grade involves the transmutations of TP 53 alongside malfunctioning of BRCA1/ 2 or both. Adenoma- borderline tumor-carcinoma series having alterations of KRAS are responsible for mucinous carcinoma. Endometriosis embracing alterations in CTNNB1 and PTEN are the main basis of endometrioid carcinomas; it is also instigated by genetic variations of the immaculate cellular carcinoma. This implies that high-grade ovarian carcinoma develops shorn of any certainly discernable precursor abrasion while the low-grade type develops through antecedent endometriosis [19]. The tumors of BRCA1 and 2 are supposed to be comparatively violent with meager diagnosis potentials [21,22]. Pal et al. 2005 described that the occurrence of genetic ovarian cancer due to *BRCA1* and 2 are prevalent as compared to the previously reported statistics; the transmutations of BRAC 2 are credited with a greater number of inherited ovarian carcinoma; around 30% of patients presented obscure ancestral record for implying vulnerability to inherited carcinoma [23]. Despite the number of investigations, the exact pathology of ovarian cancer is still unclear and needs more investigation to locate the proper conduit [24].

Role of mitochondria in ovarian cancer

The two processes of mitochondrial fusion and fission make up mitochondrial dynamics. Mitochondria have the ability to continually fuse together and fission, or split into two mitochondria. Small and fragmented mitochondria are produced during the fission process, and these mitochondria can produce reactive oxygen species, trigger mitophagy, or speed up cell proliferation in response to nutritional overuse and cellular malfunction. There has been evidence of an increase in mitochondrial fission in a number of human disorders, including numerous different cancer cell types. Drp1, a modulator of

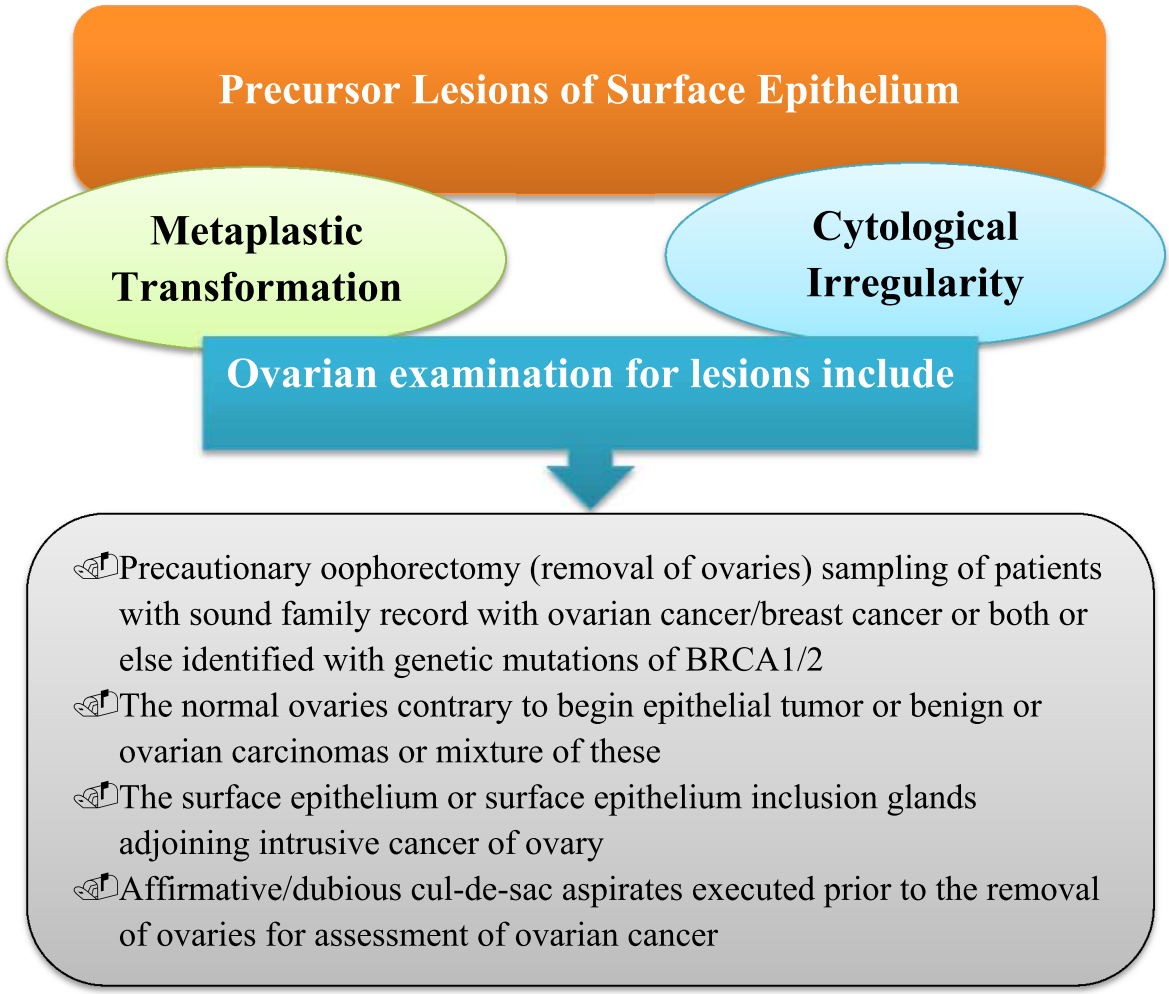

Fig. 1. Surface epithelium precursors and examination of ovarian cancer.

mitochondrial fission, may play a role in carcinogenesis in ovarian cancer, as evidenced by the rise in mitochondrial fragmentation, Drp1 protein, and mRNA levels in ovarian cancer cells [25,26]. A significant gentic incident linked to the development of ovarian cancer has been discovered to be changed in mtDNA copy number or mt DNA strength, measured as the amount of mtDNA comparable to normal DNA in the cell [27].

Ovarian cancer pathways

The epithelial ovarian cancer is the most lethal malignancy of females responsible for the highest (i.e., 5th ranked) death rates. The aforementioned categories of cancers are generally illustrated through mutations of KRAS, PTEN, BRAF, and Beta-catenin (CTNNB1), and a regular chromosomal constitution. The Wnt/beta-catenin signaling pathway mainly controls the regular differentiation, cellular equilibrium, physiological practices, and embryonic growth in comprehensive stem cell niche and exhibit crucial function in ovarian cancers (Fig. 2) [28,29].

Wnt signaling pathway

The Wnt pathway comprises of Wnt proteins composed of 19 glycoproteins opulent in cysteine [30]. Wnt-2b, Wnt-5a, Wnt-11 are the main three transcripts of Wnt that are generally found in the cell lines of ovarian cancer descended from ovarian cancers of humans (Fig. 3).

Planar cell polarity pathway

The complex of Wnt–Frizzled binds to Daam1 (Dsh-associated activator of morphogenesis) in Planar Cell Polarity Pathway. This chain of actions results in stimulation of GTPases, i.e., Rhoa and Rac, which further facilitate cell polarity.

Ca^{2+} ion pathway

In this pathway, the complex of Wnt-Fzd-Dsh binds to Ror ½ (G-protein), resulting in stimulation of CaMKII (calmodulin-dependent kinase II), PKC (protein kinase C), along with phosphatase calcineurin. These reactions encourage the augmentation of the calcium's intracellular amount, which incites further signaling pathways [31-33].

Canonical (Wnt/β-catenin) signaling pathway

Two phases occur for this pathway in phase 1 is the “Off” occurs either due to the absence of Wnt ligand, receptor, or blockage of the receptor. In this state, the transmembrane receptors, i.e., Fzd or Lipoprotein-related proteins (LRP5/6) occupied by DKK1–4 (Dikkopf family) promptly, inhibiting the Wnt binding. Further, WIF-1 (Wnt-inhibitory factor), along with a family of SFRP1-5 (secreted Fzd receptor proteins), binds with Wnt and averts its binding transmembrane receptors. Axin, APC (adenomatous polyposis coli), and GSK3β (glycogen synthase kinase 3β) make a complex for degrading the β-catenin in

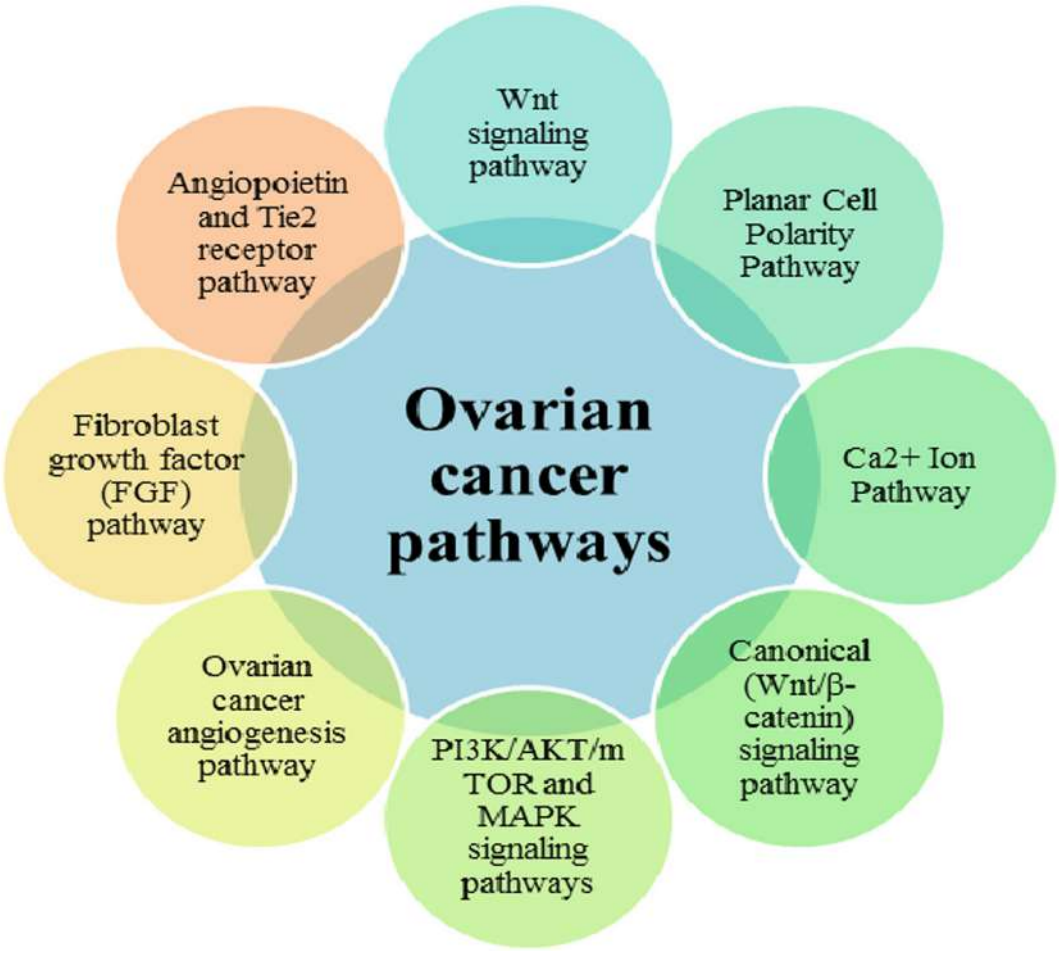

Fig. 2. Different pathways involved in ovarian cancer.

lack of binding of the ligand Wnt. The kinases like GSK3β and CK1 (casein kinase1) conducts the phosphorylation of β-Catenin; afterward, 26 S proteasome carries the ubiquitination alongside proteasomal degradation. This stumpy amount of β-catenin makes Groucho (coprocessor) available for the transcription factors LEF/TCF (lymphoid enhanced factor/ T-cell factor), resulting in blockage of activation of target genes, confirming the transcriptional suppression.

Phase 2 starts with the pathway's activation by binding the ligand Wnt to receptors Fzd and LRP5-6 (low-density lipoprotein receptor-related proteins), stabilizing the β-catenin. The kinases, i.e., GSK3β and CK1, conduct phosphorylation of LRP5/6; the Dsh got engaged with plasma membrane for interaction to Fzd. The destruction complex gets inactivated along with inhibition of β-catenin available degradation by binding of Axin to Dsh and phosphorylated LRP5/6. This makes β-catenin available for activating the Wnt target gene by binding with TCF/LEF through Groucho's displacement; interaction to the coactivators like BCL9/LGS (B-cell lymphoma 9/Legles) and pygopus for promoting the target gene transcription. This leads to the formation of transcriptional activation complex by binding of coactivators with β-catenin in the nucleus. The transcription of genes is facilitating survival and propagation through β-catenin [34-36].

In the canonical pathway, the downstream proteins like cyclin D1, c-MYC, BIRC5 (survivin), Axin2, and MMPs (matrix metalloproteinases) get activated as a consequence of binding transcriptional factors and β-catenin. Around 100 above target genes have been recognized that are controlled by the Wnt pathway; in the case of ovarian cancer, about 23 of the target genes were indicated to be overexpressed [37,38].

Progranulin (PGRN) regulating PI3K/AKT/mTOR and MAPK signaling pathways

PGRN protein partakes in the growth, development, metastatic movement, and chemoresistance of epithelial cancer of the ovary and cisplatin resistance. The expression of PGRN is controlled by diverse signaling pathways like PI3K (Phosphoinositol 3 kinase), AKT, mTOR (Mammalian Target of Rapamycin), ERK1/2, protein kinase C (PKC), and mitogen-activated protein kinase (MAPK) in distinct types of cells. As the mTOR and ERK1/2 pathways regulate PGRN in Ovarian clear cell carcinoma, it can be correlated that it is highly resilient to cisplatin treatment. Hence PGRN serves as a biomarker for the therapeutic response to multi-kinase and mTOR inhibitors. The signaling pathway cAMP (EPAC) - ERK1/2 regulates PGRN by activation of exchange protein for cAMP in cell lines of ovarian cancer, i.e., OVCAR-3 and HEY-A8. The expression of PGRN gets reduced by restraining the signaling pathways ERK1/2 and PI3K/ AKT/mTOR. Hence, PGRN may serve as a principal biomarker for carrying therapeutic responses in ovarian clear cell carcinoma [39-42].

In a study by Batool, it was evident that CD83 progressed growth propagation, colonies and spheroid, and *in vivo* tumorigenesis in ovarian cancerous cells. It remarkably restrained the movement alongside the infiltration ability of the cancerous cells. Further, it controls the factors of proliferation, namely cyclin-CDKs, CD44/KIT, in a constructive manner; on the other hand, MMP1 & 7 (matrix metallopeptidases) are controlled negatively. CD83 performs the downstream regulation of the signaling pathways FOXO1/p21/CDK2/CCNB1 and STAT3/ DKK1 through the instigation of the chute MAP3K7-MEK1/2-

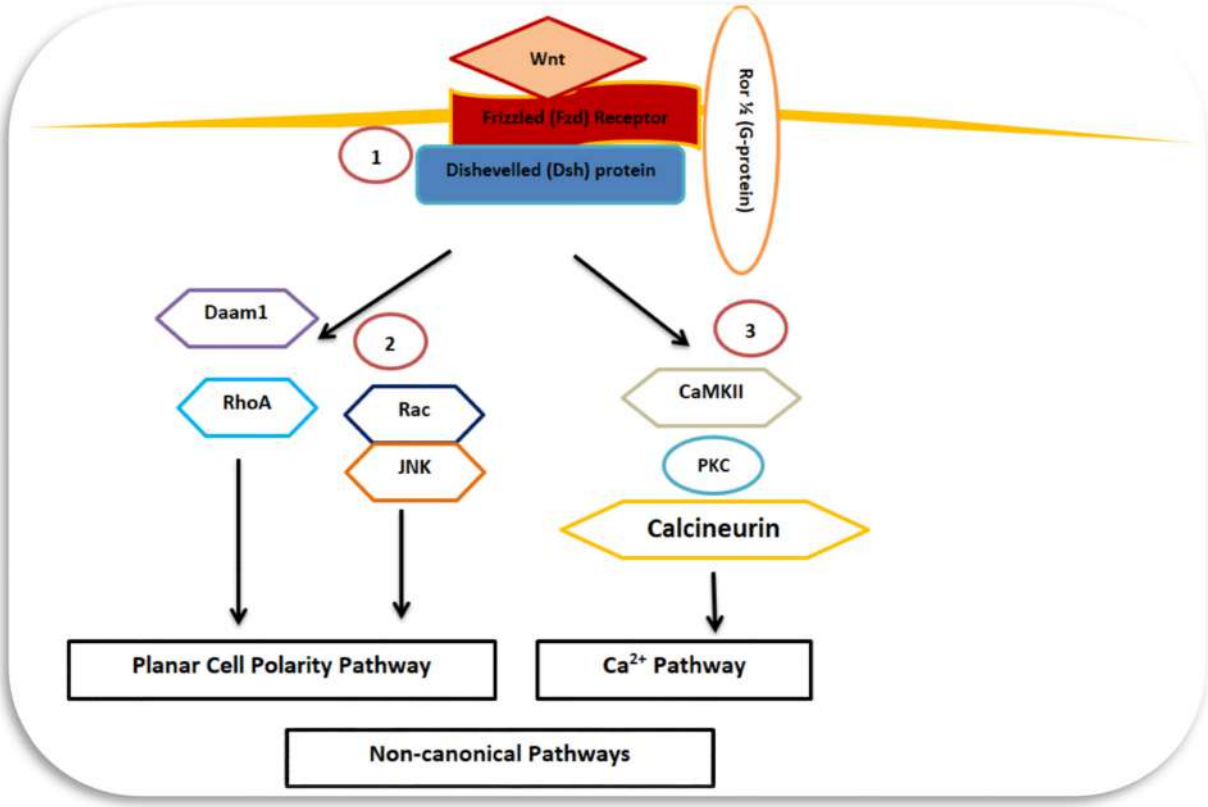

Fig. 3. Wnt Non-canonical pathways.

ERK1/2, hence respectively triggering the development of spheroid and propagation of ovarian cancerous cells. Similarly, it revealed the role of the CD83-MAPK pathway in regulating the propagation and differentiation of ovarian cancerous cells [43,44]. In addition to this, epithelial membrane protein 1 (EMP1) stimulates the propagation and penetration of the ovarian cancerous cells by employing the signaling pathway RAS/RAF/MAPK/c-JUN [45]. Repression of the signaling pathway transforming growth factor-β (TGF- β) upon aging causes epigenetic changes resulting in ovarian cancers [46].

Ovarian cancer angiogenesis pathway

Angiogenesis is the characteristic activity resulting in the propagation of cancer. The pathways allied to angiogenesis occur through binding among the molecules (platelet-derived growth factor, fibroblast growth factor, vascular endothelial growth factor) and their corresponding receptors (Tie2 receptor).

The vascular endothelial growth factor (VEGF) comprises of a family unit of seven proteins, i.e., VEGF A–E proteins and placental growth factor 1 and 2 (PIGF-1 and PIGF-2); these proteins exhibit their action by signaling by way of tyrosine kinase receptor expressed on the endothelial cell surface known as VEGFR 1–3 (vascular endothelial growth factor receptors). Signaling is activated by binding of a ligand to the receptor causing dimerization of the receptor. VEGF signaling in ovarian cancer embraces the molecules like PI-3 kinases, MAP kinases, and the JAK-STAT pathway components. Other proteins implicated in signaling interaction besides VEGF/VEGFR complex are src kinases and phospholipase C that augment the vascular permeability and interact with Erk/MAPK molecules.

The platelet-derived growth factor (PDGF) comprises four isomeric units, i.e., PDGF A-D, which bind to particular receptors, namely PDGFR-α or PDGFR-β. Consequent to the beginning of the PDGF pathway, the signaling proceeds through PI-3 K/Akt composite pathway alongside MAPK molecules with proteins related to the src group and phospholipase C- γ. Ras protein, STAT protein, and guanine-5′-triphosphate (GTPase) activating protein are also involved in PDGF signaling. Around 5–6 times rise in the amount of PDGF came about in ovarian cancer compared to the natural ovarian epithelium. Expression of PDGFR occurs in ovarian cancer. PDGF also exhibits its action in cooperation with VEGF to uphold the formation and stabilization of vessels; hence PDGF plays an imperative role in angiogenesis and oncogenesis.

Fibroblast growth factor (FGF) pathway

It is an important pathway related to embryogenesis; FGF occurs in around 23 isomeric forms, and a total of five receptors (FGFR 1–5) with structural similarity alongside two domains, namely intracellular tyrosine kinase and extracellular immunoglobulin-like domains have been identified. The proteins from the PI-3 K/ AKT course and MAPK are involved in the signaling conduit of FGF. IP3 and phospholipase –C also controls the downstream FGF signaling.

Angiopoietin and Tie2 receptor pathway

Angiopoietin with two isomorphs angiopoietin-1 & 2 (Ang-1 & 2) interacts with the Tie 2 receptors to carry formation and stabilize vessels. It can act in conjugation with pro-angiogenic factors like VEGF and PI-3Kprotein, protein kinase B, molecules like MAPK/Erk. Hence, it may also exhibit a role in ovarian cancer proliferation [47,48].

Diagnosis of ovarian cancer

Several research analyses have been conducted to identify the basic mechanism behind ovarian cancer and its prognosis in recent decades. Besides discovering serum biomarkers related to distinct pathways,

there is a need to explore more as no single biomarker is adequate to envisage disparities of an assorted disorder, namely ovarian cancer (Table 1) [49,50]. Nanosensors are another strategy to develop diagnosis tool for ovarian cancer. Both serum biomarkers and nanosensors play an important role in cancer diagnosis (Fig. 4).

The miRNA can be employed as a biomarker for the prognosis and recognition of ovarian cancer; in ovary cancer, miR-9 has been observed to be under-expressed. While miR- 21/ 15a/ miR-92 is under upregulation in the case of ovary cancer. Early-stage ovarian cancer can be represented by a lesser expression of miR-31. The final stage has been recognized by downregulation of miR- 34 a/b/c, miR-449b, miR-503, and miR-507. Further, the family of miR-200 and let-7 are observed to be overexpressed and under-expressed, respectively, in patients of ovarian cancer [75,76]. Human epididymis protein (HE)4 gene acts as a latent biomarker for identifying early-stage endometrial and ovarian cancer in high-risk patients [77].

Role of sensors in ovarian cancer

Reliable early detection of ovarian cancer by measuring the biomarker in the serum needs methods whose detection limits (DLs) are below the normal patient concentration with adequate sensitiveness [78].

Nano-sensors are instruments that detect a nanoscale force, either chemical or biological when a sensor's share is working. In general, nano-sensors remain focused on nanoparticles, which remain conjugated to an affecting ligand wherever the ligand identifies the explicit marker of relevance, providing the nano-sensors specificity the nanoparticles serve, for instance, the generator or detector of a signal that determines sensitivity. Nanoparticles can identify a high concentration of markers at exceedingly small concentrations of the instance due to their high aspect ratio. The attachment of proteins to the antibody on the microcantilever's surface contributed to the nanomechanical structural deformation of the microcantilever [79].

The electrocatalytic behavior of the carbon nanotubes was linked to “topological defects.” A higher charge density is developed than the normal hexagonal network in the pentagonal domains present at the hemispheric ends of the graphite cylinder, which improve the electroactivity of carbon nanotubes [80]. For these purposes, they were commonly used as electrode materials, and a large number of electrochemical biosensors were introduced using carbon nanotubes as a platform for both biomolecule immobilization and electrochemical transduction [81].

DNA aptamer, a special single-strand DNA (ssDNA) isolated from a random sequence of DNA/RNA libraries, utilizing the *in vitro* collection practice, notorious as the systematic progress of ligands by exponential enrichment (SELEX) [82].

Wei et al., 2018 synthesized nanocomposites of Gold nanoparticles (AuNPs)/ abridged graphene oxide (rGO)/thionine (THI), which remain coated on working electrodes to immobilize the DNA aptamer sample. They stated the exceptional conductivity of AuNPs and rGO similarly play an important role in the transfer of electrons [83].

Multi-wall carbon nanotubes (MWCNTs) have been optimized to convey thousands of alkaline phosphatase enzyme molecules per carbon nanotube and secondary antibodies to reach an FM protein DL in buffers. Kavosi et al. advanced a sensitive electrochemical immune sensor for the exposure of Antigen based on the covalent immobilization of redox mediator (thionine) on polyamidoamine dendrimer (AuNPs-PAMAM) embedded in gold nanoparticles and multi-walled carbon nanotubes/ionic liquid/chitosan nanocomposite (MWCNTs / IL / Chit) as substrate [84].

Lu et al., 2013, reported that Au nanoparticles (AuNPs) dotted carbon nanotubes (MWCNTs)–graphene composite for the high-performance electrochemical immune sensor, which was immobilized on the working electrode [85].

Table 1
List of biomarkers for epithelial ovarian cancer.

S. No.	Name of Biomarker	Description	Ref.
	Abnormal spindle-like microcephaly-associated protein (ASPM)	It is critical for the natural functioning of the mitotic spindle in the course of cell division Its level gets increased in ovarian cancer	[51,52]
	Cell division cycle associated 8 (CDCA8)	It codes a constituent of the chromosomal passenger complex. Its overexpression occurs in ovarian cancer; promotes cancer growth.	[53]
	Centromere protein M (CENPM) also identified as a proliferation-associated nuclear element	Exhibit a chief part in the assemblage of kinetochore proteins, effective mitotic development, and chromosome arrangement	[54]
	Centrosomal Protein 55 (CEP55), also known as scaffold proteins	It regulates mitotic spindle as well as microtubule organization It is being overexpressed during cancer and is related to genomic instability	[55,56]
	Hyaluronan Mediated Motility Receptor (HMMR)	Essential for the regulation of cell cycle regulation; or activating signaling pathways, it makes a complex with CD44 and hyaluronan Its overexpression results in tumor succession, violent phenotype, and reduced diagnosis in multiple cancers	[57–60]
	Ras GTPase-activating protein 1 (RACGAP1)	Regulates the processes like cytokinesis, transformation, intrusive movement along with metastasis	[61,62]
	Targeting protein for Xenopus kinesin-like protein 2 (TPX2)	It is essential for the assembly of microtubule and growth through the M phase. TPX2/AURK signaling employed as a possible target for gnomically unsteady ovarian cancer as well as breast cancer	[63,64]
	Ubiquitin-conjugating enzyme E2C (UBE2C)	It is employed for ubiquitination which, alters anomalous or short-lived proteins with ubiquitin further direct for degradation	[65,66]
	ZWINT a protein having interaction with ZW10	It is a component of kinetochore complex is needed for kinetochore-microtubule connection	[67,68]
	Kinase insert domain receptor (KDR) is the vascular endothelial growth factor receptor 2	It is crucial for VEGF-generated regulation of endothelial propagation, migration, and budding; hence supports angiogenesis needed for cancer development.	[69–71]
	Epithelial cell adhesion molecule (EPCAM)	It is an epithelium-specific intercellular adhesion molecule conducting Ca^{2+}- free homophilic cell–cell adhesion. It has been employed as a therapeutic as well as a prophetic marker for several epithelial tumors like ovarian, breast, non-small cell lung, and urothelial carcinomas	[72]
	Mammalian Forkhead Box (FOXM1) transcription factor	FOXM1 diminution can increase sensitivity for ovarian cancer cells to cisplatin	[73]
	KIF11 (Kinesin)	KIF11 codes kinesin Eg5, a motor protein essential for microtubule antiparallel slipping through mitosis, which is being used for targeting clinically. It is needed for the maintenance of tumor cell viability	[74]

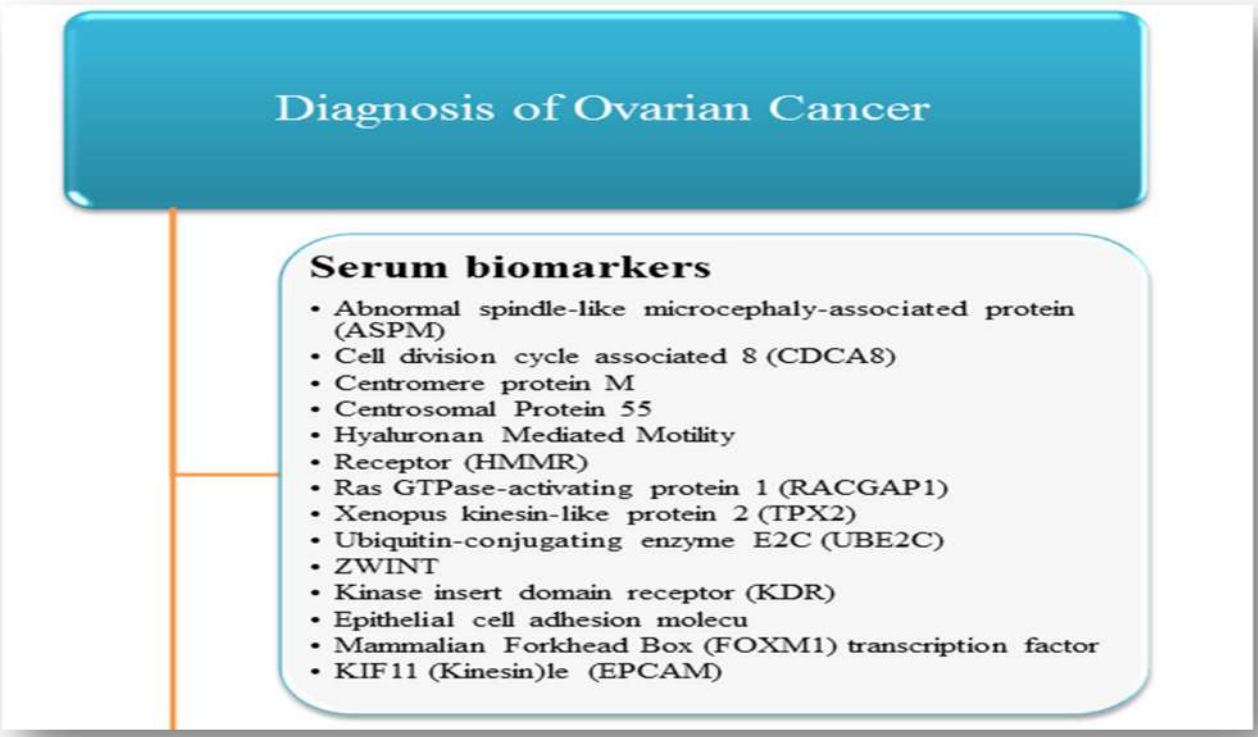

Fig. 4. Serum biomarkers and nanosensors for diagnosis of ovarian cancer.

In the immune sensor area, AuNPs, being the most commonly used nano-carriers, become particularly attractive. AuNPs have many unique features, such as ideal biocompatibility, good electrical conductivity, and large surface-to-volume ratios [86].

The Silver nanoparticles (AgNPs) are subject to modern innovative techniques resulting in extremely novel morphologies and characteristics. These nanoparticles hold numerous strengths, enabling much easier to transfer electrons and accommodate more active sites on their surface [87].

Thunkhamrak et al., 2020 have successfully established an important voltammetric immune sensing framework for sensitive PSA detection. The probe and transducer were prepared utilizing the hybrid of graphene oxide modified screen-printed carbon electrode (SPCE) and silver nanoparticles (AgNPs) [88].

Argoubi et al. came up with the design of a novel label-free aptasensing electrochemical structure for PSA detection. It utilized the sensing gold electrodes coated with mesoporous silica film which prevents the diffusion of $[Fe(CN)_6]^{3/4-}$ redox probe across the nanochannels of the mesoporous film [89].

Ovarian cancer treatments

Numerous treatment strategies are available for ovarian cancer, which can be in conjunction with surgery, chemotherapy, and neoadjuvant therapy to lower the tumor size. Carboplatin, along with paclitaxel, is given in combination as a primary chemotherapeutic treatment for ovarian cancer. PARP (poly (ADP-ribose) polymerase) inhibitors are a novel treatment for ovarian cancer; these help in self-renewal of the damaged cells alongside recovering the impairment caused throughout chemotherapy. Olaparib, rucaparib, alongside niraparib are the new agents under this category [90]. Patients diagnosed with a higher stage of ovarian cancer are generally endorsed for debulking surgery. Accomplishing the maximum benefit of debulking surgery is done by carrying investigative laparoscopic surgery [91]. Several other biological agents can be utilized, which proved beneficial for the treatment of ovarian cancer. These are named cytotoxic agents are described in Table 2.

Therapy through targeting represents biological agents possessing interference with biochemical and molecular pathways causing cancerous propagation, angiogenesis, infiltration, metastasis, and reduced apoptosis. These therapies have been evaluated for mono as well as combined therapy with chemotherapeutic agents. Stimulation of epidermal growth factor receptor causes reduced diagnosis as several cellular progressions are related to cancerous phenotype. Both inhibitions of enzyme EFGR tyrosine kinase by molecules like erlotinib/ gefitinib and binding of the receptor to monoclonal antibodies can be

Table 2
Description of various cytotoxic agents of ovarian cancer.

S. No.	Cytotoxic agent	Description	Ref.
	Epothilones	These microtubular agents are effective for paclitaxel resilient cell-lines.	[92]
	TLK 286	It is a glutathione equivalent prodrug that is actuated by using glutathione S-transferase π (GST π). It causes apoptosis through the release of reactive electrophile via a metabolic process by GST π.	[93]
	Yondelis (ET7.3)	It is a derivative of an organism from the Mediterranean marine. It binds to a marginal furrow of DNA and produces cytotoxicity.	[94]
	Pemetrexed	It is an antifolate targeting dihydrofolate reductase, thymidylate synthase, 5-aminoimidazole-4 carboxamide ribonucleotide formyltransferase, and glycinamide ribonucleotide formyltransferase	[95–97]

employed for impeding the signal transduction [98]. Another small molecule, cetuximab, which can be used with carboplatin, produced moderate action in patients having positive EFGR reverted platinum-sensitive primary peritoneal or ovarian cancer. Acneiform eruptions and sporadic hypersensitivity are caused by cetuximab in a high number of patients [99]. A new EGFR inhibitor, lapatinib, an inhibitor of erbB1 and erbB2, impedes tyrosine kinase activity for both [100]. Bevacizumab was employed as an angiogenic inhibitor in monotherapy and combined therapy with carboplatin (for platinum resilient carcinoma) and cyclophosphamides [101-103]. Ovarian cancer continues to be a challenge; it is fatal for a large number of patients.

Nanotechnology based formulation for ovarian cancer treatment

Indeed, several novel drug delivery systems (NDDS) in clinical trials remain designed to be advanced auxiliarious to integrate vigorous targeting. Ovarian Cancer is significantly uttered cell-surface proteins embraced through claudins, mucins, integrins, folate receptor, luteinizing hormone Receptor, Epidermal Growth Factor Receptor (EGFR). Though, the utmost current approaches comprise targeting of these by peptides and antibodies, through which novel magnetic targeting remains similarly on the prospect for tumor localization [104]. Liposomes, microspheres, dendrimers, nanoconjugates, and nanostructured lipid formulations are only a few of the nanomaterials-based drug delivery systems that have been developed. Numerous benefits are offered by nanobased vehicle systems to support therapeutic drug delivery, including biocompatibility, non-toxicity, biodegradability, improved therapeutic impact compared to free drug, non-inflammatory effects, and scale-up production options [105]. Few of these are abridged beneath (Fig. 5):

Microspheres

Guilford Pharmaceuticals Inc. established Paclimer®, microspheres of biodegradable nature made up of polyphosphoester. The microspheres were in the size range of ~ 53 μm, comprising 10% (w/w) paclitaxel intended to be released over a period of 90 days with *in vitro* release rate of 1–2% per day. Armstrong et al., a metronomic medicating correspondent reported that the phase I trials showed that intraperitoneal paclitaxel microsphere continuously discharged the paclitaxel (doses extending from 120 to 900 mg/ml) in ovarian cancer patients for at least eight weeks [106]. Nevertheless, biocompatibility concerns designated by the existence of enduring polymer filaments seven months afterward, the management of signified the noticeable inflammatory reaction and gentle deprivation of the polymer. Yang et al. reported that advanced paclitaxel-loaded microspheres poised of di-block copolymers of poly (ethylene glycol) and poly(sebacic acid) (PEG-PSA) with a despicable diameter of 14.2 μm that delivered significant loading (~13% w/w) and persistent drug discharge for 13 days. Paclitaxel in Cremophor EL ethanol (50:50) (Taxol®) preparation, once managed intraperitoneal, stood vacant by systemic absorption within hours. In distinction, a single dose of IP paclitaxel/PEG-PSA particles efficiently circumvented systemic outflow and curbed tumor progress intended for further than 40 days in a murine ovarian cancer model and extended the median survival time to 75 days, associated through 47 days survival with Taxol® management and 34 days per IP placebo constituents, thus presenting an encouraging prospective as IP delivery approach [107]. As a type of sustained-release drug carrier, sustained-release drug microspheres provide a wide range of development possibilities. Different gelatin-based microspheres have been created using novel technologies to act as sustained delivery systems for genetic material, pharmaceuticals, and favorable bacteria. Additionally, these microspheres have shown promise as cell carriers and 3D scaffold components in tissue engineering and regenerative medicine. They not only have good injectability but can also be incorporated into a macroscale construct with the loaded cells [108,109]. To assess the invasion of cancer cells *in vitro,* a co-culture system with 3D mesenchymal stem cells 2 aggregates containing gelatin microspheres and cancer cells appears promising [110].

Nanoparticles

Nanotechnology can remain as an outcome for the hindrances of ovarian cancer management. The current revisions stood appraised, laterally through the improvements in the nano-biological discipline. Nanotechnology remains institute to obligate comprehensively probed for molecular imaging, drug conveyance, management, and tumor targeting [111]. Nano-carrier systems such as polymeric micelles, solid

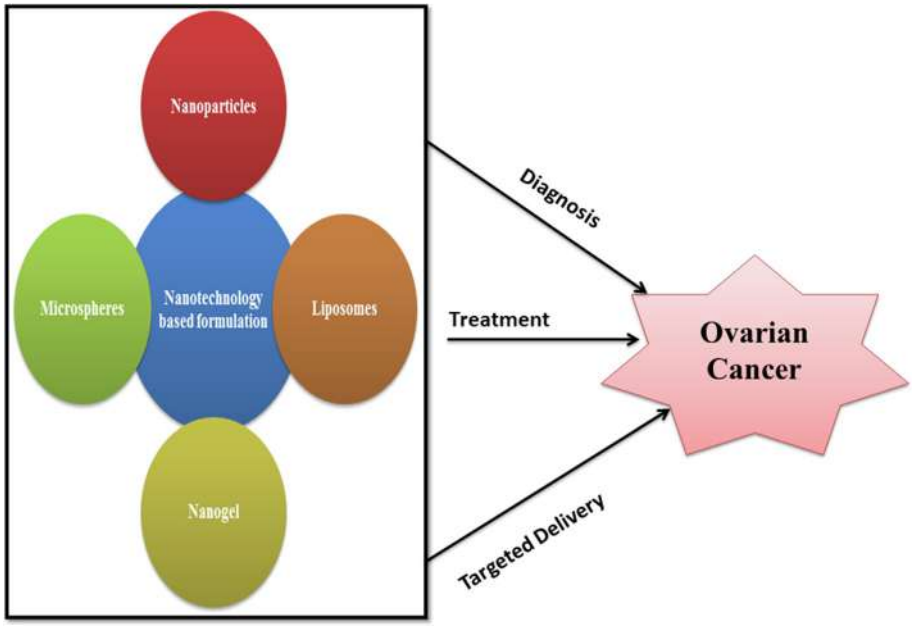

Fig. 5. Nanotechnology based formulations for treatment of ovarian cancer.

lipid nanoparticles, liposomes, and niosomes are good approaches to traverse through tumor locality and targeting the tumors due to their nano size, well-designed surfaces and constancy which carry and release the hydrophobic drugs for prolonged period in systemic circulation [112]. Generally, old chemotherapy techniques showed immediate clearance, degradation, detrimental effects of drug distribution to vigorous tissues, poor localization of drug in tumor tissues owing to poor penetration capability, and multidrug resistance [113]. So, the novel nano-particulate delivery systems reported to augment the distribution of chemotherapeutics. Due to improved uptake in cancer cells, nanoparticle preparations can proceed through the benefit of the improved permeability and retention outcome (EPR) to localize in tumors deprived of any precise targeting [114]. Paralleled to systemic management, nanoparticles through précised chemotherapeutics can enable augmented therapeutic delivery to the tumor surroundings.

Further than the EPR consequence, adorning the elements through cancer cell-specific ligands deals with a surplus path to distribute a high drug quantity in a directed approach. Prominently designed for the patient, encapsulation and site-specific transport can diminish the toxicity outlined the numerous therapeutics previously acknowledged for the clinic's practice. The enormous assortment of diverse nanostructures and empathies presented permits the strategy of derivatized constituents that integrate diagnostic, imaging, and drug-delivery properties, currently labeled theranostics [115].

Polymeric nanostructures can withstand the variations in intracellular microenvironment, comprising pH and enzyme action and release the drug at desired site in regulated manner. Recently a hydrophobic core was enclosed in cross linked polymeric shell and a protective PEG corona made of triblock ethylene glycol-glutamic acid-phenylalanine [116]. These micelles stood co-loaded through both paclitaxel and cisplatin that show higher cytotoxicity. The degradation enzymes, such as cathepsin B, destroys the polymer and releases both medications [117]. The *in vivo* study showed that combination therapy was found more effective as compared to free drugs or micelles comprising one therapeutic in multidrug-resistant ovarian cancer tumors [104]. The use of nanoparticles is designed to transport immune-stimulatory and immune-suppressive particles in amalgamation through chemo or radiotherapy or as adjuvants to added immunotherapies [118]. Nanoparticles of obliging correspondingly have been considered to generate vaccines to stimulate T cell response. Besides tumor growth [119], are consenting for the co-delivery of antigens and adjuvants [120], subsidizing to the insertion of multiple antigens to activate DC targets [115] and ensuring the persistent discharge of antigens for a protracted immune stimulation [121,122].

The most effective methods that can be employed to enhance ovarian cancer therapy and aid in chemoresistance evasion are RNAi and nanotechnology. When delivered *in-vivo,* siRNA is thought to be particularly effective at facilitating the knockdown of particular genes upon their entry into the cytosol by preventing mRNA expression necessary for their translation. This process is known as RNAi (RNA interference) [123]**.** Magnetic nanoparticles have been widely used in biomedical areas because of their special characteristics. Targeted delivery was improved by image guided nanoparticles used along with non-invasive magnetic resonance imaging contrast agents [124].

Liposomes

Liposomes are spherical lipid bilayers and comparable to nano micelles and can capture the both lipid and water-soluble drugs. Lipophilic drugs integrate into the lipid bilayer and hydrophilic drugs inside the vesicle. These lipophilic nano-carriers can remain employed intended for imaging and precise targeting of tumor tissues, though, the ligands specific to the phospholipid bilayer, cellular uptake consequently allowing a distinct remedial outcome to the directed specific-sites [125-129].

Different in-vivo studies showed the promising outcomes of targeted liposomal preparations for ovarian cancer. Cholesterol liposomes loaded with docetaxel targets a luteinizing hormone-releasing hormone analog, LHRHa and within 60 min administration, the targeted liposome accumulates 9 times higher than free docetaxel at the ovarian tumor and declined undesirable accumulation in the liver and spleen [130]. Alternative targeting constituent explored intended for micellar drug carriers is the “OA02″ peptide, having affinity for the α-3 integrin receptor overexpressed on tumor cell surfaces. This not only deals with the optimization for clinical efficiency, but also declined toxicity reducing the chemotherapeutic complexities [131].

Thermosensitive phospholipids were used for grafting WSGFPGVWGASVK, an ovarian targeted ligand, to create liposomes that would target ovarian cancer [132].

Nanogel

Nukolova et al. developed a folate-targeted nano gel for ovarian cancer by utilizing a cross-linked di-block copolymer delivering the cisplatin or doxorubicin at tumor-specific site and studied antitumor action in a murine ovarian cancer model [133]. Another study showed encouraging outcomes from nano gel loaded with cisplatin targeted for luteinizing hormone-releasing hormone. This marker is overexpressed, not only in ovaries; but also in breast [134]. In nano gel synthesis outlines, the 3D bulk substantial is the main contrived, trailed through the remedial agent's design. This inadequate management of the drug sustains pharmaceutical reliability.

Solid lipid nanoparticles (SLNs) similarly show comparable properties to nano micelles. Lipophilic drugs are encapsulated in the hydrophobic interior of SLNs and the release of such drugs delayed as membrane destabilization takes time. Though, utmost liposomal and SLNs are overhead 90 nm in size due to inherent structural constraints that ominously constrain delivery to ovarian tumor tissue [135].

Nano micelles are deliberated as forthcoming carriers for diagnostic and therapeutic agents owing to this prolonged circulatory time, amended drug stability, precise targeting, and propagation obsessed by tumor material.

In doxorubicin-loaded poly (L-histidine) micelles, folate is utilized as a targeting ligand and aimed to release the drug in controlled manner in acidic pH whereas non-targeted micelles produced 5 times higher the plasma half-life than free doxorubicin. The *in vivo* study on human ovarian carcinoma A2780 showed that these micelles favorably accumulated at the tumor [136]. The respective group, too, intended an analogous micelle, adapted through folate consenting for receptor-mediated endocytosis and enriched drug uptake. In mice, these targeted mixed micelles subdued the progress of multidrug-resistant tumors with nominal weight loss to the animal [137]. Modifying the pH sensitivity to the initial endosomal pH range of 6.0 is intended for even more significant action, subduing tumor growth in mice for at least 50 days [138].

Nanotheranostics in treatment of ovarian cancer

Versatility of nano-particles allow them to carry various moieties simultaneously intended for multiple functions such targeting ligands, drugs molecules and diagnostic agents which enables it for targeting, therapeutic, diagnostic and real-time drug tracing. This approach of co-delivery of multiple ligands for multiple functions is known as nano theranostics [139]. Nanotheranostics offers a encouraging approach to monitor the real time drug distribution inside the body and the cancer pathophysiological process simultaneously which there by helps in identification of tumors and predict the therapeutic potential of drugs [140]. Ganta et al. delivered the docetaxel via folate targeted gadolin-

ium attached theranostic nanoemulsion which target the chemoresistant efflux transporters of ovarian cancer. The cellular uptake studies showed that folate receptor-positive SKOV3 ovarian cancer cell line exhibited the time dependent uptake of theranostic nanoemulsion which was higher than non-targeted nanoparticles. The IC_{50} value of chemoresistant SKOV3TR decreased 270-fold as compared to free drug in MTT study. In magnetic resonance imaging study it was found that folate targeted theranostic nanoparticles accumulated over a period of 24 h at the tumor site [141].

Discussion

Ovarian cancer is a deadly disease with shallow detection potential in the early stages. It is the major cause of death among women throughout the world, and the situation worsens when it is found that the early-stage detection of this disease is infrequent, and around 60% of cases of ovarian cancer are detected at stage-III or later stages of the disease. Therefore, it is very much appropriate to review and understand this disease's pathophysiology to find out the strategies for early diagnosis and treatment. The majority of the ovary tumors have been found to originate from the surface epithelial and germ cells, depending on the anatomical arrangement of tumor origination. This cancer is generally found to occur through mutations of KRAS, PTEN, BRAF, and Beta-catenin (CTNNB1) following different pathways. So, the mechanism of diagnosis of ovarian cancer could be based on identifying different biomarkers from the above-mentioned pathways and biosensors as suggested by Eftkahri et al, 2019 and Ahmadian et al, 2022 [142,143]. Early detection of these biomarkers makes ovarian cancer diagnosed at early stages. The application of nanotechnology in the diagnosis and treatment approaches makes them more effective with high sensitivity. Nano-sensors help scan and detect chemical and biological markers at very lower concentrations where other conventional techniques fail to do so. These nano-sensors have high selectivity, specificity, and sensitivity toward cancer biomarkers. They can be incorporated into the novel nanotechnology-based dosages forms such as microsphere, nanoparticles, liposomes, nanogel, and others, making it a potential multifunctional theranostic entity for diagnosis and treatment of ovarian cancer at its early stages. Further, with the advancement in science, a novel concept of pharmacogenomics has great potential for the future for early diagnosis and effective treatment of different diseases, including ovarian cancer. However, this system is nascent and requires much research to become a reality [144].

Future Prospects: Development of newer diagnostic techniques is the need of time for early detection and diagnosis of life-threatening diseases like cancer including ovarian cancer. Development in the diagnostic science is still in its nascent stage and a lot of efforts are required in future. Application of newer techniques such as biosensors, biomarkers, and functional genomics may become the future diagnostic tools integrated in the novel drug delivery systems to actually act as the theranostic approach of disease treatment in future.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare that there is no conflicts of interest.

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General Relativity and Gravitation (2023) 55:46
https://doi.org/10.1007/s10714-023-03097-5

RESEARCH

New agegraphic dark energy in Brans–Dicke theory with sign changeable interaction for flat universe

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Received: 9 September 2022 / Accepted: 27 February 2023

Abstract

In the present study, we discuss a cosmological model considering interaction between new agegraphic dark energy and dark matter with sign changeable interaction term within the framework of Brans–Dicke theory of gravity for a flat universe. We assume the well motivated logarithmic form of Brans–Dicke scalar field in terms of the scale factor to find the cosmological parameters such as equation of state parameter, deceleration parameter and plot graphs to discuss their evolution against redshift parameter z. It is shown that the equation of state parameter may behave like cosmological constant for suitable values of parameters but it shows quintessence like behavior for different values of model parameters in future. The deceleration parameter shows observationally verified recent phase transition and accelerated expansion of the universe in future. The physical significance of well-known cosmological planes i.e. $w - w'$ and statefinder diagnostic is also explored for our model. The statefinder diagnostic shows that new agegraphic dark energy behaves like chaplygin gas in early time and behaves like quintessence in future. Moreover, for suitable values of parameters it behaves like cosmological constant at present. The analysis of $w - w'$ plane shows that our model shows freezing region and reaches in the vicinity of ΛCDM model in future. Further, we apply thermodynamic analysis and found that the generalized second law of thermodynamics is satisfied with in the model.

Keywords New agegraphic dark energy · Cosmological parameters · Sign changeable interaction · Statefinder analysis · Thermodynamic analysis

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Published online: 08 March 2023

Contents

1 Introduction

It is not a matter of investigation now that our universe is going under an accelerated expansion which was predicted in 1998 by two teams headed by Perlmutter [1] and Riess [2], separately using the observations of supernovae Ia. It has been confirmed by a number of observations like Baryon Acoustic Oscillations [3], Wilkinson Microwave Anisotropy Probe (WMAP) [4, 5], Large Scale Structure [6], Cosmic Microwave Background Radiation [7] and recently by the observations of Planck Probe [8, 9]. The matter of investigation is to find the cause of present time accelerated expansion. If Einstein's general relativity (GR) is the ultimate theory of the cosmic evolution then it is expected that something must exist in the universe which have negative pressure. This unknown energy content having negative pressure is named dark energy (DE) [10, 11] which contains approximately 70% of the universe. In the literature, a lot of DE models have been proposed to explain this unknown type of energy content, but the most accepted model is a non zero cosmological constant [12]. However, it faces two serious problems: fine tuning and cosmic coincidence problem [13]. Recent problem the model facing is the H_0 tension [14, 15] which is related to the present value of Hubble parameter. The DE concept may be in essence an issue of quantum gravity. But till now a complete theory of quantum gravity has not been established. This issue motivates us to look the effect of gravity in effective quantum field theory in which basic principle of quantum gravity must be included. One of DE candidates which contains some notable properties of quantum gravity is agegraphic dark energy.

Agegraphic dark energy (ADE) model takes into account the uncertainty relation of quantum mechanics together with gravitational effect in GR. On the line of quantum fluctuation of space time, Karolyhazy and his collaborators [16, 17] observed that the distance t in Minkowaski space-time can not be known to a better accuracy than $\delta t = \gamma t_p^{2/3} t^{1/3}$, where γ is dimensionless constant of order one, t_p denotes the reduced Planck time. Throughout this paper, we use $h = k_b = c = 1$. The Karolyhazy relation together with time energy uncertainty relation ensure one to take a quantum energy density of metric fluctuation of space time. Therefore, the energy density of ADE can be obtained as [18]

$$\rho_D = \frac{3n^2 M_p^2}{T^2}, \tag{1}$$

where M_p is the reduced Planck mass, T is the age of the universe and the factor $3n^2$ is taken to avoid some uncertainties like species of quantum field in the universe, the effect of curved space time. But, this model of ADE suffers from some internal inconsistencies [19]. To alleviate these inconsistencies, a new form of model was proposed by Wei and Cai [20] in which the cosmic age T is replaced by cosmic conformal age η for the time scale in equation (1). This new version of DE is known as new agegraphic dark energy (NADE) [21–23]. Following [20], the energy density of NADE is given by

$$\rho_D = 3n^2 M_p^2 \eta^{-2} \tag{2}$$

where η is defined as

$$\eta = \int_0^t \frac{dt}{a} = \int_0^a \frac{da}{Ha^2}. \tag{3}$$

Here, a is the cosmic scale factor and H is known as Hubble parameter. The NADE models have been studied extensively in the literature and also fit the observation data well [24–31].

Apart form GR, the another way to explain this accelerated expansion is modified theories of gravity which modify the Einstein–Hilbert action of GR. These theories are also able to explain other cosmological aspects of cosmic evolution. One of the most important modified theories is Brans-Dicke (BD) theory which is a scalar-tensor theory of gravity. BD theory was proposed by Brans and Dicke [32] in 1961 and presents a natural extension of Einstein's GR. This theory is a successful theory nowadays because of its association with string theory and extra dimensional theory. It is solely based on dimensionless arguments and with the matter lagrangian being minimally coupled. In BD theory, Newton's gravitational constant G is not presumed to be constant but is proportional to the inverse of the scalar field ϕ, which can vary from place to place and with time. The literature on BD theory is vast and rich in all aspects. Cosmological models based on BD theory have studied almost all fields of the cosmic evolution [33–42].

A number of authors have discussed NADE models in BD theory [43–45] but most of them consider the power law form of BD scalar field which faces the constant deceleration parameter problem (see Ref. [46] and Refs. therein). Recently, Kumar and Singh [47] proposed a logarithmic form of BD scalar field to counter the constant deceleration parameter problem of power law form. Authors studied NADE model in BD theory with this logarithmic form of BD scalar field and also investigated the scenario by taking interaction between NADE and dark matter. Taking logarithmic form of BD scalar field, a number of models have been discussed in BD theory [48–52]. In some recent works, NADE model has been considered in $f(G, T)$ gravity [53] and in $f(T, B)$ gravity [54]. It is suggested from observations that the sign of interaction changes between dark sector during cosmic evolution in the redshift range $0.45 \leq z \leq 0.9$ [55]. The sign changeable interaction term was proposed by [56, 57] as $Q = q(\alpha\dot{\rho} + 3\beta H\rho)$ where α and β are constants and Q changes its sign as the deceleration parameter q changes its sign during evolution of the universe. Therefore, it is significant to discuss DE models considering the sigh changeable interaction term. The NADE model with sign changeable interaction in GR has been studied [58]. In the present study we extend the work of Kumar and Singh [47] by taking sign

changeable interaction term as $Q = 3b^2Hq\rho_D$ in BD theory. We provide a more detailed study in the present paper as we discuss statefinder analysis, $w - w'$ analysis and thermodynamic analysis for our model which was lacking in [47].

The statefinder analysis was proposed by Sahni et al. [65] to discriminate among DE models. The statefinder is geometrical in nature as it obtained using scale factor and its time derivatives only. This analysis can be applied in a model-independent manner and allows us to characterize the properties of DE. The statefinder analysis is able to successfully discriminate DE models namely the cosmological constant, quintessence, k-essence, Chaplygin gas, braneworld models etc. The Statefinder analysis plays a particularly important role for modified gravity theories such as scalar–tensor models and braneworld models of DE, for which the equation of state is not a fundamental physical entity. Another important analysis is the $w_D - w'_D$ analysis which attained recognition for examining the DE models. Caldwell and Linder [66] proposed this analysis for analyzing the quintessence scalar field model. The $w_D - w'_D$ plane explains the accelerated expansion region of the universe. Thermodynamic analysis has become an important part of gravitation theories. Jacobson [67] and Padmanabhan [68] established connection between gravitation theories and thermodynamics which provided a background for thermodynamic study in gravitation theories. The generalized second law of thermodynamics has become a point of interest for cosmologists in recent years. The NADE model in context of generalized second law of thermodynamics has been studied in the framework of GR [69]. We apply all above mentioned analysis to examine our model.

The paper is organized as follows. In Sects. 2 and 3, we present the field equations of BD theory where we consider interacting NADE and logarithmic form of BD scalar field. We obtain cosmological parameters, the equation of state (EoS) parameter and deceleration parameter (DP) in Sect. 4 to study the possible evolution of the universe. In Sect. 5, we apply statefinder analysis to compare our model with existing models. In Sect. 6, the trajectories of $w - w'$ analysis are plotted for different values of model parameters. Section 7 is devoted to thermodynamic analysis of the model. In Sect. 8, we present a summary of our results.

2 Model and field equations

The action of BD theory with matter Lagrangian density $\mathcal{L}_m$ in Jordan frame is given by

$$S = \int d^4x\sqrt{-g}\left[\frac{1}{2}\left(-\phi R + \frac{\omega}{\phi}g^{\mu\nu}\partial_\mu\phi\ \partial_\nu\phi\right) + \mathcal{L}_m\right], \tag{4}$$

where R represents Ricci scalar curvature, g is the determinant of metric tensor $g_{\mu\nu}$ and ω is the dimensionless Brans-Dicke coupling parameter between scalar field and gravity. Here, ϕ denotes time dependent scalar field known as BD scalar field given by $\phi = (8\pi G)^{-1}$. The scalar field and matter field in Jordan frame do not interact and matter minimally couples to the metric. We consider a homogeneous and isotropic Friedmann-Robertson-Walker (FRW) universe, which is given by the line element

$$ds^2 = dt^2 - a^2(t)\left[\frac{dr^2}{1-kr^2} + r^2(d\theta^2 + r^2 sin^2\theta d\phi^2)\right], \tag{5}$$

where $a(t)$ is the cosmic scale factor of the universe, k is the curvature parameter having values 0, 1 or -1 describing the nature of space curvature. The observations suggest that the universe is almost flat at the present time. As we are interested to study the late time evolution of the universe, therefore, we consider a flat universe for which $k = 0$.

The field equations corresponding to the action (4) and line element (5) taking $k = 0$ are given by

$$H^2 + H\frac{\dot{\phi}}{\phi} - \frac{\omega}{6}\frac{\dot{\phi}^2}{\phi^2} = \frac{\rho_m + \rho_D}{3\phi}, \tag{6}$$

$$2\frac{\ddot{a}}{a} + H^2 + 2H\frac{\dot{\phi}}{\phi} + \frac{\omega}{2}\frac{\dot{\phi}^2}{\phi^2} + \frac{\ddot{\phi}}{\phi} = -\frac{p_D}{\phi}, \tag{7}$$

$$\ddot{\phi} + 3H\dot{\phi} = \frac{\rho_m + \rho_D - 3p_D}{2\omega + 3}, \tag{8}$$

where ρ_m is energy density of DM, ρ_D is energy density of NADE and p_D denotes the pressure of NADE. Here, the over dot denotes the derivative with respect to time. We consider a well motivated logarithmic form of BD scalar field [47], which was proposed to counter constant DP problem associated with power law form. The logarithmic form of BD scalar field is given as

$$\phi = \phi_0 \ln(\alpha + \beta a) \tag{9}$$

where ϕ_0, $\alpha > 1$ and $\beta > 0$ are constants. From Eq. (2), the energy density of NADE takes the following form

$$\rho_D = 3n^2\eta^{-2}\phi, \tag{10}$$

because in BD theory the value of ϕ is taken as $\phi = (8\pi G)^{(-1)}$ which gives $\phi = M_p^2$.

3 Interacting NADE model with sign changeable interaction

The interaction between DM and DE is a matter of concern. There is no confirmation of interaction between these two dark components, however, some cosmological observations [70, 71] suggest existence of it. Several cosmological models of interaction between DM and DE have been studied in the literature [58, 72–76]. Taking the advantage of observational support to interaction, we consider interaction between DM and NADE. The continuity equations in the presence of interaction between DM and NADE can be written as

$$\dot{\rho}_m + 3H\rho_m = Q, \tag{11}$$

$$\dot{\rho}_D + 3H(1 + w_D)\rho_D = -Q, \tag{12}$$

where $w_D = \frac{p_D}{\rho_D}$ denotes EoS parameter of NADE. The term Q denotes interaction term between DM and NADE. The sign of interaction term Q defines the direction of the energy transfer i.e. for $Q > 0$, there is an energy transfer from NADE to DM, and for $Q < 0$, there is an energy transfer from DM to NADE.

In the available literature [77–79], the interaction term in general has been taken as $Q = 3c\rho H$, where ρ is energy density of dark sector and c is transfer strength. As c is constant and energy density and Hubble's parameter are positive, therefore this interaction term does not change it's sign. It is suggested by observations that the sign of interaction between dark sector changes during cosmic evolution in the redshift range $0.45 \leq z \leq 0.9$ [55]. To accommodate the observational evidence, the sign changeable interaction term was proposed by Wei [56, 57] as $Q = q(\alpha\dot{\rho} + 3\beta H\rho)$, where α and β are constants, which contains DP q. The interaction term Q changes its sign as q changes its sign during evolution of the universe. Therefore, we consider sign changeable interaction term to study the interaction between DM and NADE in the framework of BD theory with logarithmic form of BD scalar field in present study. We have considered a simple sign changeable interaction term which is given as

$$Q = 3b^2 Hq\rho_D, \tag{13}$$

where b^2 is coupling constant.

Sometimes it is beneficial to use dimensionless density parameters for simplification of system of equations and involved calculation. It also helps to explain physical quantities in more elaborative way. The critical energy density and dimensionless density parameters take the form

$$\rho_{cr} = 3\phi H^2, \tag{14}$$

$$\Omega_m = \frac{\rho_m}{\rho_{cr}} = \frac{\rho_m}{3\phi H^2}, \tag{15}$$

$$\Omega_D = \frac{\rho_D}{\rho_{cr}} = \frac{n^2}{\eta^2 H^2}. \tag{16}$$

Differentiating (16) with respect to cosmic time t and using $\Omega'_D = \frac{\dot{\Omega}_D}{H}$ we get

$$\Omega'_D = -2\Omega_D \left(\frac{\dot{H}}{H^2} + \frac{\Omega_D^{1/2}}{na} \right), \tag{17}$$

where prime denotes derivative with respect to $\ln a$.

4 Equation of state and deceleration parameter

To discuss the evolution of the universe, there are several cosmological parameters which are important, some of which are the DP q and EoS parameter w_D. We will discuss the evolution of the universe through the EoS parameter and DP for our model.

The EoS parameter defines about accelerated and decelerated expansions of the universe. It is defined as the ratio of pressure p_D and energy density ρ_D, and looks like $w_D = p_D/\rho_D$. The value of w_D is classified as follows, for $w_D = 1$, it represents stiff fluid, $w_D = 1/3$ shows radiation dominated phase whereas $w_D = 0$ shows matter dominated phase, $-1 < w_D < -1/3$ shows DE dominated accelerated phase with quintessence, $w = -1$ shows the cosmological constant and $w_D < -1$ presents phantom era of the universe. The EoS parameter of NADE can be obtained by using Eqs. (9), (10), (13) and (16) in (12) as

$$w_D = -1 - b^2 q + \frac{2\sqrt{\Omega_D}}{3na} - \frac{\beta a}{3(\alpha+\beta a)\ln(\alpha+\beta a)}, \tag{18}$$

where q is DP defined by $q = -\frac{\ddot{a}}{aH^2} = -1 - \frac{\dot{H}}{H^2}$. It is worth mentioning that for $\beta = 0$ the EoS parameter approaches to it's respective form in GR [58]. Here, we have different second term in comparison of [47] due to sign changeable interaction term. We observe that $w_D \approx -1 - b^2 q$ as $a \to \infty$. Thus, w_D will never cross the phantom divide line if $q < 0$ in far future whereas it crosses phantom divide line in far future in the work [47]. The phantom crossing of interacting NADE also observed in GR [20] and in BD theory (using power-law form of BD scalar field) [44]. For the case $b = 0$ (non-interacting NADE), w_D approaches to cosmological constant ($w_D = -1$) in far future as observed in [20, 47]. It is evident from the expression of w_D given by (18) that to explain the evolution of w_D we require q. In addition, we also require q to explain the evolution of the universe. The sign of q describes the accelerating or decelerating rate of expansion and change in sign represents the phase transition of the universe. The value of q can be obtained by using Eqs. (9), (10) and (16) in equation (7) as

$$q = \frac{1 + 3\Omega_D\omega_D + \frac{2\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} - \frac{\beta^2 a^2}{(\alpha+\beta a)^2\ln(\alpha+\beta a)} + \frac{\omega\beta^2 a^2}{2(\alpha+\beta a)^2[\ln(\alpha+\beta a)]^2}}{2 + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)}}. \tag{19}$$

Here, we observe that the expression of q contains w_D as expression of w_D contains q. Now, we want to find their values independent of each other. We have two Eqs. (18) and (19) with two variable w_D and q which are of our interest. Therefore, we can easily obtain final expressions of w_D and q using the Eqs. (18) and (19) which are as follows

$$\begin{aligned} w_D = \frac{1}{2 + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} + 3b^2\Omega_D}\Bigg[& -2 + \left(\frac{2\sqrt{\Omega_D}}{3na} - \frac{5}{3} - 2b^2\right) \\ & \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} - b^2 + \frac{b^2\beta^2 a^2}{(\alpha+\beta a)^2\ln(\alpha+\beta a)} \\ & + \left(\frac{-\omega b^2}{2} - \frac{1}{3}\right)\frac{\beta^2 a^2}{(\alpha+\beta a)^2[\ln(\alpha+\beta a)]^2} + \frac{4\sqrt{\Omega_D}}{3na}\Bigg] \end{aligned} \tag{20}$$

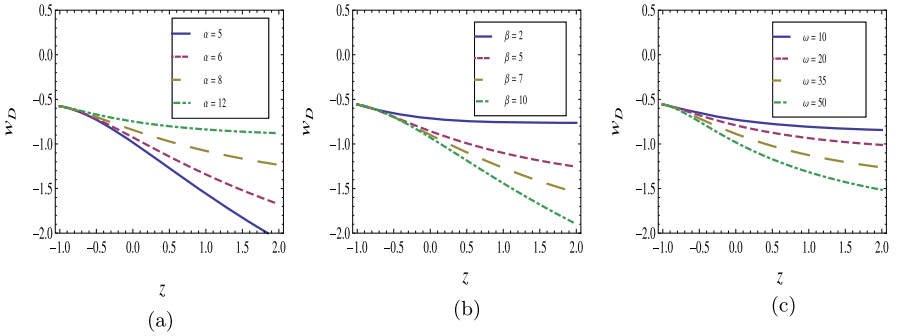

Fig. 1 We have taken $n = 5$, $b = 2$ and $\Omega = 0.70$ to plot EoS parameter w_D against the redshift parameter z. **a** is plotted for fixed values $\omega = 25$, $\beta = 9$ and for various values of α. **b** is plotted for fixed values $\omega = 25$, $\alpha = 5.5$ and for various values of β. **c** is plotted for fixed values $\alpha = 5.5$, $\beta = 4$ and for various values of ω

$$q = \frac{1 - 3\Omega_D + \frac{2\Omega_D^{3/2}}{na} + \frac{\beta a(2-\Omega_D)}{(\alpha+\beta a)\ln(\alpha+\beta a)} - \frac{\beta^2 a^2}{(\alpha+\beta a)^2\ln(\alpha+\beta a)} + \frac{\omega\beta^2 a^2}{2(\alpha+\beta a)^2[\ln(\alpha+\beta a)]^2}}{2 + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} + 3b^2\Omega_D} \tag{21}$$

Now, we have expressions of w_D and q which are given by Eqs. (20) and (21), respectively. Let us discuss the evolution of both the cosmological parameters one by one. We first discuss the evolution of w_D against redshift z. It is more appropriate to discuss the evolution of cosmological parameters against redshift z in comparison of scale factor a. Therefore, using $a = (1+z)^{-1}$ in normalized unit, we plot the evolution of w_D versus z in Fig. 1. The graphs have been plotted for various values of α, β and ω in Fig. 1a–c respectively for fixing the other parameters. It is observed from the figure that all the evolution trajectories showing quintessence behavior of w_D in future and converge to one fixed point. It is interesting to note that NADE behaves differently in the present model in comparison of works [20, 44, 47] where NADE behaves like phantom in far future. It is observed that for smaller values of α and large values of β and ω the phantom divide line ($w_D = -1$) can be crossed during the evolution. For large values of α and small values of β and ω, the trajectory remains almost constant lying in quintessence region in recent time and future. It can be observed that α has just reverse effects on the evolution of the EoS parameter w_D in comparison of β and ω. It can also be seen from the figure that for suitable values of parameters we can achieve cosmological constant type behavior ($w_D = -1$) at present time.

It may be observed form the expression of q that $q \approx \frac{1-3\Omega_D}{2+3b^2\Omega_D}$ as $a \to \infty$. Thus, the universe will observe an accelerated expansion in far future if $\Omega_D > 1/3$ otherwise it will decelerate. Now, let us discuss the evolution of q against the redshift z. We have plotted the trajectories of q versus redshift z in Fig. 2. The effects of the model parameters α, β and ω can be seen in Fig. 2a–c respectively. A feasible model of cosmic evolution must show the recent phase transition rather just showing acceleration. Our model shows recent phase transition of cosmic evolution for all values of model parameters. It is observed that for smaller values of model parameter

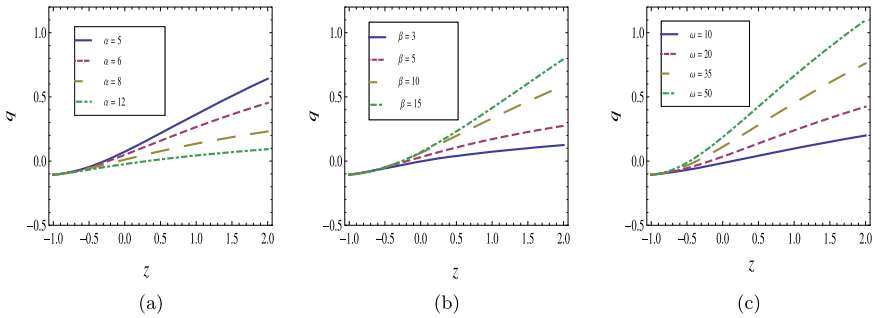

Fig. 2 We have taken $n = 5$, $b = 2$ and $\Omega = 0.70$ to plot q against the redshift parameter z. **a** Is plotted for fixed values $\omega = 25$, $\beta = 9$ and for various values of α. **b** Is plotted for fixed values $\omega = 25$, $\alpha = 5.5$ and for various values of β. **c** Is plotted for fixed values $\alpha = 5.5$, $\beta = 4$ and for various values of ω

α and larger values of the parameters β and ω, the model shows phase transition very late in future not in present time and for larger values of model parameter α and smaller values of the parameters β and ω, the model shows phase transition very early not in present time. Therefore, very small or very large values of the model parameters α, β and ω are not favourable to observe the recent phase transition of the universe (see Fig. 1). Our model shows accelerated expansion of the universe in future, i.e., after recent phase transition universe will accelerate through out the evolution.

The BD parameter ω plays an important role in BD theory and has been constrained by solar system experiments and cosmological observations. In the parameterized post Newtonian formalism, solar system experiment Cassini put a very high bound $\omega > 40{,}000$ [59, 60]. On the other hand, cosmological observations put relatively very low bounds on BD parameter ω. Even, these observations predict negative values of ω and put negative bounds on it. In 2005, Acquaviva and collaborators [61] found that $\omega > 120$ and, Wu and Chen in 2010 [62] found $\omega < -120$ or $\omega > 97.8$ at 95% confidence level. Using the data of WMAP and Planck Probe, Li and collaborators in 2013 [63] obtained $\omega > 181.65$ at 95% confidence level. Recently, Nui and collaborators have found constraints $\omega \geq 40$ using the data of gravitational wave observations [64]. The theoretical studies of cosmological scale properties of the universe also predict small values of ω to explain the known phenomena of cosmic evolution [46, 85]. In the present study, we are interested to discuss the cosmological scale properties of the universe like it's dynamic evolution. Therefore, cosmological constraints on ω are preferable than the solar system constraints. Therefore, we have used small values of ω to plot graphs which are able to explain the recent phase transition of the cosmic evolution.

5 The statefinder analysis

Although a lot of DE models are available in literature which explain the cosmic acceleration of the universe but to distinguish between different dark energy models, a geometrical diagnostic pair known as statefinder diagnostic $\{r, s\}$ was introduced by

Sahni et al. [65]. The statefinder pair is constructed directly from space time metric and includes the scale factor $a(t)$ and its time derivative upto third order. The statefinder pair $\{r, s\}$ is defined as follows

$$r = \frac{\dddot{a}}{aH^3} = 1 + 3\frac{\dot{H}}{H^2} + \frac{\ddot{H}}{H^3}, \quad s = \frac{r-1}{3(q-1/2)}. \tag{22}$$

The trajectories of different DE models show different behavior in $r - s$ plane which gives a reliable way to differentiate among DE models. The statefinder pair $\{r, s\} = \{1, 0\}$ correspond to ΛCDM model, $\{r < 1, s > 0\}$ shows quintessence like behaviour of DE model and $\{r > 1, s < 0\}$ shows chapygin gas like behaviour of DE. The $SCDM$ model correspond to statefinder pair $\{r, s\} = \{1, 1\}$. As a DE model departs from the fixed point $\{r, s\} = \{1, 0\}$, it suggests a reliable way to differentiate this model from ΛCDM model. To calculate r, we need values of $\frac{\dot{H}}{H^2}$ and $\frac{\ddot{H}}{H^3}$. We know that $q = -1 - \frac{\dot{H}}{H^2}$, thus, we get $\frac{\dot{H}}{H^2} = -1 - q$. Now, using the value of q given by (21), we get the value

$$\frac{\dot{H}}{H^2} = \frac{-3 + 3\Omega_D(1-b^2) - \frac{2\Omega_D^{3/2}}{na} - \frac{\beta a(3-\Omega_D)}{(\alpha+\beta a)\ln(\alpha+\beta a)} + \frac{\beta^2 a^2}{(\alpha+\beta a)^2\ln(\alpha+\beta a)} - \frac{\omega\beta^2 a^2}{2(\alpha+\beta a)^2\ln(\alpha+\beta a)^2}}{2 + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} + 3b^2\Omega_D} \tag{23}$$

and differentiating equation (23) w.r.t t, we obtain the value as follows

$$\begin{aligned}
\frac{\ddot{H}}{H^3} = {} & 2\left(\frac{\dot{H}}{H^2}\right)^2 + \Bigg[\left(3(1-b^2) - \frac{3\sqrt{\Omega_D}}{na}\right)\Omega_D' + \frac{2\Omega_D^{3/2}}{na} \\
& + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)}(\Omega_D' - 3 + \Omega_D) \\
& + \frac{\beta^2 a^2}{(\alpha+\beta a)^2[\ln(\alpha+\beta a)]^2}(3 - \Omega_D - \omega) + \frac{\beta^2 a^2}{(\alpha+\beta a)^2\ln(\alpha+\beta a)}(5 - \Omega_D) \\
& + \frac{\beta^3 a^3}{(\alpha+\beta a)^3[\ln(\alpha+\beta a)]^2}(\omega - 1) - \frac{2\beta^3 a^3}{(\alpha+\beta a)^3\ln(\alpha+\beta a)} \\
& + \frac{\omega\beta^3 a^3}{(\alpha+\beta a)^3[\ln(\alpha+\beta a)]^3} \\
& - \frac{\dot{H}}{H^2}\left(3b^2\Omega_D' + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} - \frac{\beta^2 a^2}{(\alpha+\beta a)^2[\ln(\alpha+\beta a)]^2}\right. \\
& \left. - \frac{\beta^2 a^2}{(\alpha+\beta a)^2\ln(\alpha+\beta a)}\right)\Bigg] \\
& \left[2 + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} + 3b^2\Omega_D\right]^{-1}
\end{aligned} \tag{24}$$

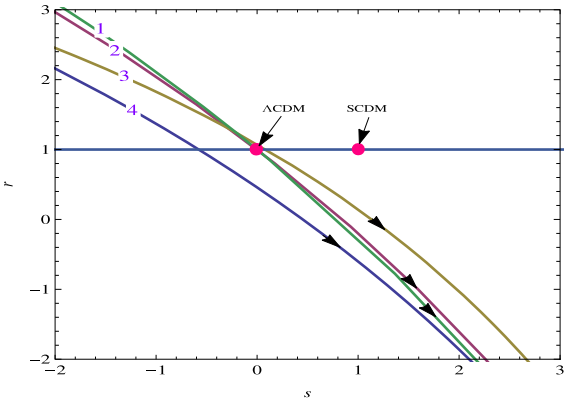

Fig. 3 To plotted the $r - s$ trajectories, we have taken $\Omega_D = 0.70$, $n = 5$ and $b = 0.1$. The trajectory 1 is plotted for $\alpha = 5$, $\beta = 4$ and $\omega = 25$, the trajectory 2 is plotted for $\alpha = 5$, $\beta = 4$ and $\omega = 35$, the trajectory 3 is plotted for $\alpha = 4.5$, $\beta = 4$ and $\omega = 25$ and the trajectory 4 is plotted for $\alpha = 4.5$, $\beta = 4.5$ and $\omega = 25$

Now, using the values given by Eqs. (23) and (24) we can obtain the expression of r and then the expression of s. We have plotted the trajectories in $r - s$ plane to explore the behavior of our model which have been shown in Fig. 3. The points plotted on the straight line in the figure represent the values corresponding to ΛCDM model which is $\{1, 0\}$ and for the $SCDM$ model which is $\{1, 1\}$. We observe that the trajectories start from chaplygin gas region and ends in quintessence region. It matches with predictions of EoS analysis we have done in Sec. 4 where w_D showed quintessence like behavior in future. In the framework of GR, it was observed that interacting NADE behave almost like quintessence throughout the evolution and approaches to ΛCDM model [80]. Thus, the NADE model in BD theory behaves differently in comparison to its behavior in GR as also observed in the analysis of EoS parameter w_D. We observed that the trajectories meet ΛCDM model during the evolution for suitable values of the parameters but do not approaches to SCDM model. Further, We have observed that increasing the value of parameter β deviates the trajectory more and more from ΛCDM model. The statefinder analysis of our model shows that NADE shows chaplygin gas type behavior in early time, cosmological constant type behavior in present time and behave like quintessence in future.

6 The $w - w'$ analysis

In this section, we study the $w_D - w'_D$ dynamical analysis for NADE model. The $w_D - w'_D$ analysis attained recognition for examining the DE models. The $w_D - w'_D$ (where prime denotes the differentiation with respect to $\ln a$) plane explains the accelerated expansion region of the universe. Caldwell and Linder [66] firstly used $w_D - w'_D$ analysis to study the quintessence scalar field. It's investigation gives us a technique without any unreliability for classification of the model using the model parameters. Two different classes have been characterized known as thawing region $w'_D > 0$ and freezing region $w'_D < 0$ on $w_D - w'_D$ plane.

For NADE model, the expression for w'_D looks as $w'_D = \frac{\dot{w}_D}{H}$. The expression of w'_D can be obtained as

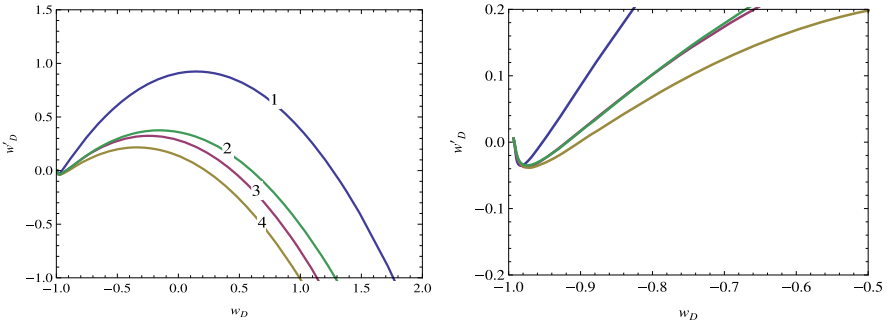

Fig. 4 To plot trajectories, we have taken $\Omega_D = 0.70$, $n = 5$ and $b = 0.1$. The trajectory 1 is plotted for $\alpha = 5$, $\beta = 4$ and $\omega = 25$, the trajectory 2 is plotted for $\alpha = 5$, $\beta = 4$ and $\omega = 35$, the trajectory 3 is plotted for $\alpha = 4.5$, $\beta = 4$ and $\omega = 25$ and the trajectory 4 is plotted for $\alpha = 4.5$, $\beta = 4.5$ and $\omega = 25$. The figure (**b**) is zoomed version of (**a**)

$$
\begin{aligned}
w'_D = \Bigg[& -3w_D b^2 \Omega'_D + \left(\frac{\Omega'_D}{3na\sqrt{\Omega_D}} - w_D - 2b^2 - \frac{5}{3} \right) \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} \\
& + \left(\frac{-2\sqrt{\Omega_D}}{3na} + w_D + 2b^2 - \omega b^2 + 1 \right) \frac{\beta^2 a^2}{(\alpha+\beta a)^2 [\ln(\alpha+\beta a)]^2} \\
& + \left(\frac{-2\sqrt{\Omega_D}}{3na} + w_D + 4b^2 + \frac{5}{3} \right) \frac{\beta^2 a^2}{(\alpha+\beta a)^2 \ln(\alpha+\beta a)} \\
& + \left(-b^2 + \omega b^2 + \frac{2}{3} \right) \frac{\beta^3 a^3}{(\alpha+\beta a)^3 [\ln(\alpha+\beta a)]^2} \\
& - \frac{-2\beta^3 a^3 b^2}{(\alpha+\beta a)^3 \ln(\alpha+\beta a)} + \left(\frac{\omega b^2}{2} + \frac{1}{3} \right) \frac{2\beta^3 a^3}{(\alpha+\beta a)^3 [\ln(\alpha+\beta a)^3]} \\
& + \frac{2\Omega'_D}{3na\sqrt{\Omega_D}} - \frac{4\sqrt{\Omega_D}}{3na} \Bigg] \\
& \left[2 + \frac{\beta a}{(\alpha+\beta a)\ln(\alpha+\beta a)} + 3b^2 \Omega_D \right]^{-1}. \qquad (25)
\end{aligned}
$$

We have plotted the evolution trajectories in $w_D - w'_D$ plane for different values of model parameters in Fig. 5a. The Fig. 5b is zoomed version of Fig. 5a to observe the behavior of trajectories in the vicinity of ΛCDM model, which has values $w_D = -1$, $w'_D = 0$. It is observed that all the trajectories shows thawing region in present and begin to show freezing region in future. It is observed that the behavior of $w_D - w'_D$ plane analysis shows freezing region which match with today's observations. It can be observed clearly from the figure that all the trajectories meets at one single point in late time signalling freezing region. It seems that the behavior of trajectories in $w_D - w'_D$ analysis depend on the value of model parameters. It is observed from the figure that the trajectories reach in vicinity of ΛCDM model in future, which has values $w_D = -1$, $w'_D = 0$, shown clearly in Fig. 5b.

7 Thermodynamic analysis

Thermodynamics and gravity theories are independently well established branches of science. Jacobson established a fascinating connection between gravity and thermodynamics by deriving Einstein's field equations using only thermodynamical considerations [67]. Padmanabhan [68] derived the first law of thermodynamics starting from the Einstein's field equations for a static and spherically symmetric space-time. In another work, authors recovered the field equations of f(R) theory using only thermodynamical considerations [81]. These seminal works have inspired a number of cosmologists to work on so-called thermodynamics of space-time. Many aspects of thermodynamics have been studied in gravity theories one of which is generalized second law of thermodynamics (GSL). According to GSL, total entropy of the universe (horizon entropy and entropy of the matter inside the horizon) is an increasing function of the time. The GSL has been discussed in both Einstein's theory and modified theories of gravity [69, 82–84].

Recently, GSL has been investigated in original BD theory and authors have obtained expression of rate of change of total entropy of the flat FRW universe [85]. In the present work, we have also considered original BD theory in a flat FRW universe. Therefore, we have considered the same expression of rate of change of total entropy of the flat FRW universe for our investigation of validity of GSL in our model. To be noted that we have taken NADE in our model while they have not considered any DE component. The total entropy of the universe is given by

$$S_{tot} = S_h + S_{in}, \tag{26}$$

where, S_{tot} denotes total entropy, S_h denotes horizon entropy and, S_{in} denotes entropy of total fluid inside the horizon. The rate of change of total entropy S_{tot} can be obtained as

$$\dot{S}_{tot} = \dot{S}_h + \dot{S}_{in}, \tag{27}$$

where dot represents the time derivative. Authors [85] have considered the entropy of dynamical apparent horizon rather than teleological event horizon which seems more relevant to the study. The entropy of the apparent horizon is given by the relation $S_h = 2\pi A$, where $A = 4\pi R_h^2$ denotes the area of the apparent horizon. Here, R_h denotes radius of the apparent horizon which is related to the Hubble parameter in the flat FRW universe as $R_h = \frac{1}{H}$. Thus, the entropy of the apparent horizon takes the form as $S_h = \frac{8\pi^2}{H^2}$ and it's rate of change is given by

$$\dot{S}_h = -16\pi^2 \frac{\dot{H}}{H^3}. \tag{28}$$

The Gibbs law of thermodynamics for fluid inside the horizon produces the relation

$$T_{in} dS_{in} = dE_{in} + p_t dV_h, \tag{29}$$

where the subscript t denotes the total quantity and the volume $V_h = \frac{4}{3}\pi R_h^3$. Now, we can obtain the rate of change in entropy of the fluid inside the horizon as

$$\dot{S}_{in} = \frac{(\rho_t + p_t)\dot{V}_h + \dot{\rho}_t V_h}{T_{in}}. \tag{30}$$

If we consider that the fluid is in thermal equilibrium with the horizon then temperature of fluid inside horizon (T_{in}) and temperature of dynamical apparent horizon (T_h) are same (see [85] and references therein). It is known as Hayward-Kodama temperature which is given by

$$T_h = \frac{2H^2 + \dot{H}}{4\pi H}. \tag{31}$$

It may be observed that this temperature reduces to the Hawking temperature $T_{Hawking} = \frac{H}{2\pi}$ [86] in de Sitter space where $\dot{H} = 0$. Now, the rate of change of entropy of fluid inside the horizon can be obtained using Eqs. (30) and (31) as

$$\dot{S}_{in} = 16\pi^2 \frac{\dot{H}}{H^3}\left(1 + \frac{\dot{H}}{2H^2 + \dot{H}}\right). \tag{32}$$

Now using Eqs. (27), (28) and (32), one can get the rate of change of total entropy as [85]

$$\dot{S}_{tot} = \frac{\left(\frac{4\pi\dot{H}}{H^2}\right)^2}{H\left(\frac{\dot{H}}{H^2} + 2\right)}. \tag{33}$$

If GSL is satisfied with in the model then we must observe $\dot{S}_{tot} \geq 0$ otherwise model will violate GSL. We observe that the inequality $\dot{S}_{tot} \geq 0$ is followed if $\frac{\dot{H}}{H^2} + 2 \geq 0$ because H is a positive quantity as we have shown accelerated expansion in present and future epochs. Thus, GSL is satisfied with in our model if we observe $\frac{\dot{H}}{H^2} \geq -2$. Here, we are considering only present epoch and future evolution because the model is devoted to explain the late time evolution of the universe. We have plotted graphs of $\dot{S}_{tot}$ against redshift z in Fig. 5. We observe that GSL is satisfied during present evolution and in future for various combination of model parameters. It has also been observed in the framework of GR that NADE model satisfies GSL [69]. Authors also considered dynamical apparent horizon for their study as we have considered in the present model. Further, we observe that $\dot{S}_{tot}$ freezing to zero in far future, i.e., $\dot{S}_{tot} \to 0$ and $z \to -1$. It is interesting to observe that S_{tot} becomes almost constant in far future.

8 Closing remarks

The present day accelerated expansion of the universe has become more interesting with the passing of time. In the present work, we have studied NADE model with sign changeable interaction term in BD theory of gravitation. BD theory of gravity is a natural extension of GR and arose interest in modern cosmology. We demonstrated that how the NADE evolve with cosmic evolution in BD theory. The EoS parameter of interacting NADE has been plotted for different values of model parameters. It is found that all the evolution trajectories converge to one single point showing quintessence

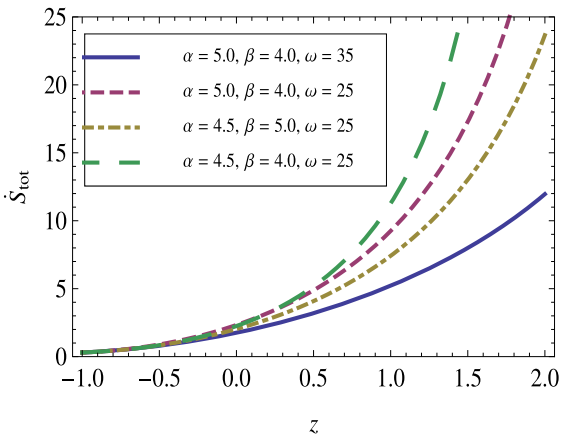

Fig. 5 To plotted the $\dot{S}_{tot}$ against redshift z, we have taken $\Omega_D = 0.70$, $H = 70$, $n = 5$ and $b = 0.1$. The trajectories are plotted for various values of parameters α, β and ω

behavior in future whereas it shows phantom type behavior in works [20, 44, 47]. The phantom divide line ($w_D = -1$) can also be crossed for different values of model parameters during the evolution. The plots of q show recent phase transition (decelerated expansion to accelerated expansion) of the universe which makes our model more realistic. It is also observed that very small or very large values of the model parameters α, β and ω are not favourable to observe the recent phase transition of the universe.

We observed that the $r - s$ trajectories start from chaplygin gas region and end in quintessence region. The trajectories meet ΛCDM model during the evolution for suitable values of parameters. Thus, interacting NADE behaves like chaplygin gas in starting, behaves like ΛCDM during the evolution and in last behaves like quintessence whereas it behaves like quintessence throughout the evolution in GR and approaches to ΛCDM in last [80]. It is also observed that increasing the value of parameter β deviates the trajectory more and more from ΛCDM model. The trajectories in $w_D - w'_D$ plane show thawing region in present and freezing region in future. We also observed that the trajectories reach in vicinity of ΛCDM model in future, which has values $w_D = -1$, $w'_D = 0$. We have applied thermodynamic analysis to check the validity of GSL in our model. We have plotted graphs of $\dot{S}_{tot}$ against redshift z to observe its behavior during the present epoch and in future. It is observed that the GSL is satisfied for various combination of model parameters. Thus, the model passes the thermodynamic test successfully.

Acknowledgements The authors are thankful to the referee for constructive suggestions to improve the manuscript.

Data Availability There is no data associated with this article.

Declarations

Competing interests Authors have no financial interests.

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Eur. Phys. J. C (2022) 82:143
https://doi.org/10.1140/epjc/s10052-022-10093-7

THE EUROPEAN PHYSICAL JOURNAL C

Regular Article - Theoretical Physics

Reconsidering holographic dark energy in Brans–Dicke theory

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Received: 4 September 2021 / Accepted: 6 February 2022 / Published online: 15 February 2022

Abstract In this paper, we reconsider the concept of holographic dark energy in the framework of Brans–Dicke theory in the formalism of the flat Friedmann–Lemaitre–Robertson–Walker metric. Firstly, we demonstrate how the assumption $\phi \propto a^n$, where ϕ and a stand for the Brans–Dicke scalar field and scale factor, respectively, naturally leads to a constant deceleration parameter, irrespective of the energy content of the universe. Secondly, we consider interacting holographic dark energy with Hubble horizon as IR cut-off, and find the value of the Hubble parameter and corresponding value of the scale factor. Further, we find the value of the Brans–Dicke scalar field ϕ for the obtained value of the Hubble parameter and holographic dark energy. We obtain the corresponding value of the deceleration parameter and show that it can explain the phase transition of the universe. Moreover, statefinder diagnostics has been applied to compare the model with existing models. On the other hand, we consider the viscous behavior of holographic dark energy and show that the viscous holographic dark energy can play the role of interacting holographic dark energy as it is able to explain the phase transition of the universe. Further, we find the value of the Brans–Dicke scalar field ϕ for this viscous holographic dark energy. In this model also, we apply the statefinder diagnostic.

1 Introduction

The study of distant Type Ia supernovae [1–4] has changed our perception about the evolution of the universe drastically and it was found that the universe is going through an accelerated expansion at present. The observations of the cosmic microwave background radiation [5,6], large-scale structure [7,8], baryon acoustic oscillations [9] and Planck data [10,11] confirm the accelerated expansion of the universe. These observations suggest that about 70% of the energy content of the universe is in the form of a mysterious component, which violates the strong energy condition, called dark energy (DE). A number of DE models have been proposed in the literature but the ΛCDM model is the simplest and most successful model of DE. However, despite excellent features, it has some theoretical problems like the coincidence and fine-tuning problems [12]. The major problem with the ΛCDM model based on Einstein's general relativity arises when it is applied at the quantum level. According to Einstein, space time is warped by the matter and energy in it, but quantum physics says the matter and energy exists in multiple states simultaneously. So, where is the gravitational field? In recent papers [13,14], the authors have shown that the ΛCDM model has some major tension with recent observations and it may not be the best description of our universe. Therefore, it is time to explore all the possibilities for a better understanding of the universe.

Modified theories of gravity have been considered as an alternative to general relativity (GR) and play an important role in the study of the evolution of the universe. A natural extension of GR is Brans–Dicke (BD) theory, introduced by Brans and Dicke [15] in 1961. In this theory, the gravitational constant G is no longer a constant, and is replaced by a scalar field $\phi(t) = (8\pi G)^{-1}$, called the BD scalar field. The BD scalar field plays a vital role in BD theory, and various cosmological phenomena have been studied using a power law form of the BD scalar field [16–20]. In the literature, it has been shown by a number of authors [21–23] that the power law form $\phi \propto a^n$, where a represents the cosmic scale factor, of the BD scalar field leads to a constant deceleration parameter (DP). Therefore, the well known phase transition of the universe from deceleration to acceleration cannot be achieved using a power law form. However, a number of authors [17–19,24,25] have obtained a time-dependent DP for the same assumption of ϕ. Kumar and Singh [26] discussed this prob-

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lem of constant DP associated with the power law form, and proposed a logarithmic form of the BD scalar field. A number of authors have considered the logarithmic form to explain various aspects of cosmological evolution [27–31]. In the present paper, we are interested to discuss the evolution of the universe in BD theory without taking any specific form of BD scalar field and also to find a suitable form of BD scalar field for the model under consideration in the paper.

Dynamical DE candidates, which contains some significant properties of quantum gravity, are able to present a unified model of the universe and explain some problems of modern cosmology like the coincidence problem [32]. The dynamical DE models based on the holographic principle, introduced by 't Hooft [33] and further discussed by Susskind [34], have garnered a lot of attention to explain the accelerated expansion and problems of the ΛCDM model. These models of DE are known as holographic dark energy (HDE) models. It was shown [35] that the formation of black holes puts an upper bound on the DE density in the formalism of quantum field theory. According to Li [36], if ρ_h is the quantum zero-point energy density caused by a short distance cut-off, the total energy in a region of size L should not exceed the mass of a black hole of the same size. Taking the largest IR cut-off L to saturate the inequality, the author obtained $\rho_h = 3c^2 M_p^2 L^{-2}$, where c is a dimensionless constant and M_p stands for the reduced Planck mass. A number of authors have discussed various aspects of HDE [37–43].

In the present paper, we consider the concept of HDE is the framework of BD theory. First, we demonstrate how the assumption $\phi \propto a^n$ naturally leads to a constant DP which is not able to describe the phase transition. Further, without using any specific form of the BD scalar field, we obtain the value of the scale factor and corresponding value of ϕ for HDE model. We obtain the value of the DP by using the value of the scale factor and discuss the possible evolution of the universe. Further, we are interested to discuss dissipative behavior of HDE in BD theory. Therefore, we consider viscous HDE. More precisely, we are interested to know whether the non-interacting viscous HDE may play the role of interacting HDE.

Dissipation is a natural phenomenon of the cosmic fluid and plays a significant role in the evolution of the universe. Scalar dissipative processes of the cosmic fluid may be treated via the relativistic theory of bulk viscosity. The phenomenon of bulk viscosity arises in the cosmological fluid when the fluid expands (contracts) too fast, due to which the system is out of thermal equilibrium. Then, the effective pressure become negative to restore the thermal equilibrium [44]. Therefore, it is natural to consider bulk viscosity in an accelerating universe. In the literature, bulk viscosity has been discussed to study early time inflation, as well as late time acceleration due to its ability to produce an effective negative pressure [45–52]. We show that the non-interacting viscous HDE with Hubble length as IR cuf-off can explain the phase transition (deceleration to acceleration) in contrast to non-interacting non-viscous HDE [17,18]. This also shows that viscous HDE may play the role of interacting HDE in the framework of BD theory.

Sahni et al. [53] introduced a robust analysis to discriminate among DE models, known as statefinder diagnosis. The statefinder is a geometrical diagnostic, and allows us to characterize the properties of DE in a model-independent manner. It is dimensionless and is constructed from the scale factor of the universe and its time derivatives only. The statefinder diagnosis is able to successfully discriminate between a wide range of DE models, including the cosmological constant, quintessence, k-essence, Chaplygin gas and braneworld models. Statefinder diagnosis plays a particularly important role for modified gravity theories such as scalar–tensor models and braneworld models of DE, for which the equation of state is not a fundamental physical entity. Thus, it is important to compare our DE models with existing ones. Therefore, we obtain the value of the statefinder parameters r and s, and plot $r-s$ and $r-q$ trajectories to compare both of our models, interacting HDE and non-interacting viscous HDE, with existing models.

The paper is organized as follows. HDE with Hubble horizon as an IR cut-off is the subject of the next section in the framework of BD theory. We demonstrate that $\phi \propto a^n$ is not a suitable choice to explain the phase transition of the universe. Further, we find the scale factor a and the corresponding value of ϕ. We obtain the expression of the DP q to study the possible evolution of the universe. Further, we apply statefinder diagnostics to compare our model with existing models. Section 3 is devoted to the study of viscous HDE. We obtain the value of a, ϕ and q as in Sect. 2. Further, we discuss statefinder diagnostics in the model. In Sect. 4, we present a summary of our results.

2 Holographic dark energy in Brans–Dicke theory

The action for BD theory in the Jordan frame is given by

$$S = \int d^4x\sqrt{-g}\left[\frac{1}{2}\left(-\phi R + \frac{\omega}{\phi}g^{\mu\nu}\partial_\mu\phi\ \partial_\nu\phi\right) + \mathcal{L}_m\right], \tag{1}$$

where R, g and $\mathcal{L}_m$ represent the Ricci scalar curvature, determinant of the metric tensor $g_{\mu\nu}$ and the matter Lagrangian density, respectively. Here, ϕ and ω denote the BD scalar field and BD coupling parameter, respectively. From a theoretical point of view, there are no constraints on the coupling parameter ω. Observational constraints vary depending upon the method being used. Solar system bounds are $\omega > 40{,}000$ [54] whereas cosmological scale bounds are very low [55–57]. In this theory, the Newtonian constant G is replaced with

a time dependent scalar field ϕ such that $\phi = (8\pi G)^{-1}$. The dimensionless parameter ω is introduced to represent a coupling between the scalar field and gravity. In the Jordan frame, the scalar field and matter field do not interact, and matter minimally couples to the metric.

We assume a homogeneous and isotropic flat Friedmann–Lemaitre–Robertson–Walker (FLRW) space-time to discuss the evolution of the Universe. The FLRW line element is given by

$$ds^2 = dt^2 - a^2(t)\left[\frac{dr^2}{1-r^2} + r^2(d\theta^2 + \sin^2\theta\, d\phi^2)\right], \quad (2)$$

where a is the cosmic scale factor. We assume that the universe is filled with pressureless dark matter (DM) and HDE. We exclude baryonic matter and radiation due to their negligible contribution to the total energy budget during late time evolution. In the above formalism, the Brans–Dicke field equations take the form

$$H^2 + H\frac{\dot{\phi}}{\phi} - \frac{\omega}{6}\frac{\dot{\phi}^2}{\phi^2} = \frac{\rho_m + \rho_h}{3\phi}, \quad (3)$$

$$2\frac{\ddot{a}}{a} + H^2 + 2H\frac{\dot{\phi}}{\phi} + \frac{\omega}{2}\frac{\dot{\phi}^2}{\phi^2} + \frac{\ddot{\phi}}{\phi} = \frac{-p_h}{\phi}, \quad (4)$$

where ρ_m, ρ_h and p_h stand for the energy density of DM, energy density of HDE and pressure of HDE, respectively. The evolution of the BD scalar field ϕ follows the wave equation

$$\ddot{\phi} + 3H\dot{\phi} = \frac{\rho_m + \rho_h - 3p_h}{2\omega + 3}. \quad (5)$$

Using Eqs. (3)–(5), we obtain the combined energy conservation equation as follows [16,17]:

$$\dot{\rho} + 3H(\rho + p) = 0, \quad (6)$$

where $\rho = \rho_m + \rho_h$ and $p = p_h$.

2.1 Power law form and constant deceleration parameter

To discuss DE models including HDE and models without DE in the framework of BD theory, there is a general tendency to assume $\phi \propto a^n$. Now, we will show that this assumption leads to a constant value of DP which does not depend on which candidate of DE is being considered. It does also not depend on whether it is non-interacting or interacting DE. Combining the Eqs. (3)–(5) and using the assumption $\phi \propto a^n$, we obtain a single equation as follow:

$$2\frac{\ddot{a}}{a}(3 - n\omega) + H^2(6 - 4n\omega - n^2\omega) = 0. \quad (7)$$

In cosmology, the DP is defined as $q = -\frac{\ddot{a}}{aH^2}$. Therefore, one can find the value of q from Eq. (7) directly as

$$q = -\frac{\ddot{a}}{aH^2} = \frac{(6 - 4n\omega - n^2\omega)}{2(3 - n\omega)}. \quad (8)$$

It is evident that a constant DP is obtained irrespective of the matter content of the universe for the assumption $\phi \propto a^n$. One can observe, whatever the candidate of DE we choose, non-interacting or interacting, we will get the same Eq. (7) and the same value of DP. However, a number of authors [17–19,24,25] have obtained a time-dependent DP for the same assumption of ϕ, taking different forms of DE. This problem of the power law form was addressed in the paper [26], and the authors proposed a logarithmic form of the BD scalar field which is free from this problem.

2.2 Interacting HDE, BD scalar field and deceleration parameter

In the present paper, we are not going to take any one of the available forms of the BD scalar field. Rather, we are interested to find an appropriate form of it for the HDE model. We assume interaction between the pressureless DM and HDE as taken by [17]. Now, the conservation equations of pressureless DM and HDE are given by

$$\dot{\rho}_m + 3H\rho_m = Q, \quad (9)$$

$$\dot{\rho}_h + 3H(\rho_h + p_h) = -Q, \quad (10)$$

where Q denotes the interaction term between DM and HDE. For $Q > 0$, there is energy transfer from HDE to DM and there is energy transfer from DM to HDE for $Q < 0$. In the literature, Q has been assumed to be proportional to the Hubble parameter, i.e., $Q \propto H$ to maintain the interaction term Q as a function of a quantity with units of inverse of time multiplied with the energy density. Therefore, we assume $Q = \Gamma\rho_h$ as taken by [17,19], where Γ stands for the interaction rate. Here, we do not restrict Γ to be a positive quantity as in the above cited papers. We are interested to study the effects of the energy transfer from both sides on the evolution of the universe, i.e., energy transfer from HDE to DM as well as from DM to HDE. Therefore, for this requirement, we have taken Γ such that it can take both the sign, positive and negative. We consider HDE with Hubble length $L^{-1} = H$ as IR cut-off as taken by [17,19]:

$$\rho_h = 3c^2M_p^2H^2, \quad (11)$$

where c is a dimensionless constant and M_p stands for the reduced Planck mass, and has the value $M_p^2 = \frac{1}{8\pi G}$. In BD theory, we know that Newton's gravitational constant G is no more a constant, but we assume M_p as a constant in HDE (11) as taken in papers [17,19]. It may be noticed from the equation for ρ_h that there are no theoretical restrictions on the constant c, except that it has to be of the order of the reduced Planck mass M_p. We consider the equation of state (EoS) of HDE as $p_h = w\rho_h$, where w is the EoS parameter. Now, we are interested to find the value of ϕ rather than assuming it. One way is that we assume the scale factor like a

power or exponential law, and using Eqs. (3)–(5) separately or together, find the value of ϕ. But, this will be just a reconstruction of the BD scalar field and then there will be no significance to the HDE. Conversely, if we use HDE (11) in Eqs. (3)–(5), then also we will not get any fruitful result. Therefore, we find the scale factor from the energy conservation equation (10) of the HDE using the form (11) for the HDE.

Using the value of Q and Eq. (11), Eq. (10) reduces to

$$\dot{H} + \frac{3}{2}(1+w)H^2 + \Gamma H = 0. \tag{12}$$

Here, we obtain a single evolution equation for the Hubble parameter. It may be seen from this equation that the constant c from Eq. (11) does not appear. Hence there are no further restrictions on c. On solving Eq. (12), we get the value of H in terms of cosmic time t as

$$H = \frac{e^{-\frac{\Gamma}{2}t}}{c_0 - \frac{3(1+w)}{\Gamma}e^{-\frac{\Gamma}{2}t}}, \tag{13}$$

where c_0 is a constant of integration. On solving Eq. (13), one can obtain scale factor as

$$a = c_1\left[c_0 - \frac{3(1+w)}{\Gamma}e^{-\frac{\Gamma}{2}t}\right]^{\frac{2}{3(1+w)}}, \tag{14}$$

where $c_1 > 0$ is an another constant of integration. The non-negative of c_1 is the consequence of calculation because it appears under the logarithmic function which is not defined for negative values. We can rewrite the scale factor as

$$a = a_0\left[1 - \frac{3(1+w)H_0}{\Gamma}(e^{-\frac{\Gamma}{2}(t-t_0)} - 1)\right]^{\frac{2}{3(1+w)}}, \tag{15}$$

where a_0 and H_0 denote the present value of the Hubble parameter and the scale factor at the time t_0, the present value of time when the HDE begins to dominate. From Eq. (9), we obtain the value of the DM energy density as

$$\rho_m = \frac{M_p^2\, c^2\, \Gamma}{\frac{3(1+w)}{\Gamma} - (\frac{1}{H_0} + \frac{3(1+w)}{\Gamma})e^{\frac{\Gamma}{2}t}} - \frac{3\,t}{e^{\frac{3(1+w)}{\Gamma} - (\frac{1}{H_0} + \frac{3(1+w)}{\Gamma})e^{\frac{\Gamma}{2}t}}}\, c_2, \tag{16}$$

where c_2 is a constant of integration. Now, using the values of ρ_h and ρ_m in the wave equation (5), one can find the value of the BD scalar field ϕ. For simplicity we let $c_2 = 0$, however, the solution also exists for a nonzero value of c_2. On solving Eq. (5), we obtain the value of ϕ as

$$\phi(t) = -\frac{M_p^2\, c^2\, \Gamma}{3}\, t + \frac{M_p^2\, c^2\, (1-3w)}{(2\omega+3)\left[\left(\frac{1}{H} + \frac{3(1+w)}{\Gamma}\right)e^{\frac{\Gamma}{2}t} - \frac{3(1+w)}{\Gamma}\right]}\, t$$

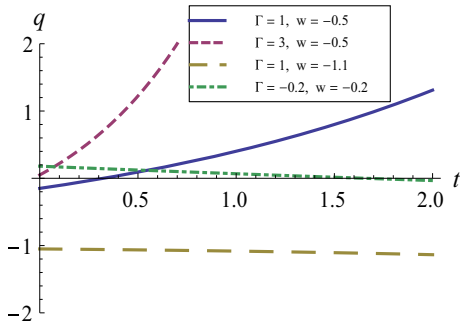

Fig. 1 The figure of deceleration parameter q against cosmic time t has been plotted for $H_0 = 10$

$$-\frac{3}{2}t\left[\left(\frac{1}{H} + \frac{3(1+w)}{\Gamma}\right)e^{\frac{\Gamma}{2}t} - \frac{3(1+w)}{\Gamma}\right] \times e^{\left[\left(\frac{1}{H} + \frac{3(1+w)}{\Gamma}\right)e^{\frac{\Gamma}{2}t} - \frac{3(1+w)}{\Gamma}\right]}\, c_3 + c_4, \tag{17}$$

where c_3 and c_4 are constants of integration. We have obtained the scale factor a and the BD scalar field ϕ corresponding to HDE with Hubble length as IR cut-off. Now, using the value of the scale factor, we obtain the value of the DP (q) to discuss the evolution of the universe. The expression of the DP for the scale factor (15) is as follows:

$$q = \left[\frac{\Gamma}{2H_0} + \frac{3(1+w)}{2}\right]e^{\frac{\Gamma}{2}(t-t_0)} - 1. \tag{18}$$

Here, we obtain a dynamic DP whose value depends on the interaction rate (Γ) and the EoS parameter of HDE (w). Both the parameters Γ and w play an important role in the evolution of the universe. If $\Gamma > 0$ and w lies in the quintessence region, i.e. $-1 < w < -1/3$, then there are two possible evolutions of the universe. We obtain a phase transition from early time acceleration (inflation) to deceleration for $(\frac{\Gamma}{2H_0} + \frac{3(1+w)}{2}) < 1$, and decelerated expansion through out the evolution for $(\frac{\Gamma}{2H_0} + \frac{3(1+w)}{2}) > 1$. If w lies in the phantom region, i.e. $w < -1$, then there are three possible evolutions of the universe. Two possible evolutions are same as in the quintessence region with the same conditions. In the third possibility, we obtain a negative value of q throughout the evolution with the condition $(\frac{\Gamma}{2H_0} + \frac{3(1+w)}{2}) < 0$, i.e. $\Gamma < -3(1+w)H_0$. Thus, we get recent accelerated expansion of the universe which is not possible in the quintessence region. Here, our results show that $\Gamma > 0$ is not an appropriate choice to discuss the recent phase transition of the universe, however, we can obtain the recent accelerated expansion in the phantom region.

A viable model must accommodate the phase transition rather than only showing acceleration. It seems that the assumption $\Gamma > 0$ has been considered by several authors to deal with an important issue, viz., why is the observational value of the vacuum energy (DE) 120 orders smaller than the

value predicted by quantum physics? Let us now discuss the case when $\Gamma < 0$. In this case, we obtain recent accelerated expansion for both the quintessence and the phantom HDE. To achieve the recent phase transition, the parameters must satisfy the condition $(\frac{\Gamma}{2H_0}+\frac{3(1+w)}{2}) > 1$, i.e. $w > -\frac{1}{3}-\frac{\Gamma}{3H_0}$. As we have taken $\Gamma < 0$, we obtain $w > -\frac{1}{3}$. The energy transfer from DM to HDE, due to the choice $\Gamma < 0$, is responsible for the result $w > -\frac{1}{3}$ required to explain the recent phase transition. The value of Γ should be very small ($\Gamma <<< 1$) to obtain the phase transition at late times. Therefore, we can achieve $w \approx -\frac{1}{3}$. We observe that phantom HDE is not suitable to accommodate the phase transitions of the universe for both positive and negative values of Γ. Further, we observe that the quintessence HDE successfully describes the phase transition from early time acceleration (inflation) to deceleration for $\Gamma > 0$. However, to accommodate the recent phase transition (deceleration to acceleration) for $\Gamma < 0$, the EoS parameter has the value below the quintessence divide ($w > -\frac{1}{3}$). The evolution of the DP for various values of the model parameters has been shown in Fig. 1. It may be seen that the desired behaviour of a transition from deceleration to acceleration is obtained for $\Gamma < 0$ and $w > -\frac{1}{3}$.

2.3 Statefinder Diagnosis

Sahni et al. [53] introduced a robust analysis to discriminate among DE models known as statefinder diagnosis which was further discussed by Alam et al. [58] in detail. The authors presented a new diagnostic pair $\{r, s\}$ which is geometrical in nature as it is constructed from the space-time metric directly. The pair r and s are known as statefinder parameters. The statefinder pair $\{r, s\}$ provides a very comprehensive description of the dynamics of the universe and consequently the nature of DE. For the ΛCDM model and the SCDM model, the statefinder pair $\{r, s\}$ has the fixed value $\{1, 0\}$ and $\{1, 1\}$, respectively. The statefinder pair $\{r, s\}$ is defined as, respectively,

$$r = \frac{\dddot{a}}{aH^3}, \quad s = \frac{r-1}{3(q-1/2)}. \tag{19}$$

In our model, we obtain the value of the statefinder parameters using the value of the scale factor a given by (15) and the value of the DP q given by (18) as follows

$$\begin{aligned} r = {} & \frac{e^{\Gamma(t-t_0)}}{4H_0^2}\Big[(1-3H_0(1+w)(e^{-\frac{\Gamma(t-t_0)}{2}}-1)) \\ & \times(\Gamma+3H_0(1+w)+6H_0w(e^{-\frac{\Gamma(t-t_0)}{2}})) \\ & +2H_0(1+3w)(2+3w)e^{-\Gamma(t-t_0)}\Big], \\ s = {} & \frac{e^{\Gamma(t-t_0)}}{(2\Gamma H_0+6(1+w)H_0^2)e^{\frac{\Gamma(t-t_0)}{2}}-18H_0^2} \\ & \times\Big[\Big\{(1-3H_0(1+w)(e^{-\frac{\Gamma(t-t_0)}{2}}-1)) \\ & \times(\Gamma+3H_0(1+w)+6H_0w(e^{-\frac{\Gamma(t-t_0)}{2}})) \\ & +2H_0(1+3w)(2+3w)e^{-\Gamma(t-t_0)}\Big\}-1\Big]. \end{aligned} \tag{20, 21}$$

We have plotted the $r-s$ trajectories in Fig. 2. The points plotted on upper boundary of the figure represent the values of $\{r, s\}$ for the ΛCDM model which is $\{1, 0\}$ and for the SCDM model which is $\{1, 1\}$. In Fig. 2a, we have plotted $r-s$ trajectories for a negative interaction rate $\Gamma = -0.1$ and in Fig. 2b, we have plotted $r-s$ trajectories for a positive interaction rate $\Gamma = 0.005$. We observe in Fig. 2a, where $\lambda < 0$, that in the late time evolution, the trajectories show quintessence-like behavior and approache the ΛCDM model (See Alam et al. [58]). In Fig. 2b, where $\lambda > 0$, we observe that the trajectories for $w > -1$ look like those in Fig. 2a, but start with higher negative values of r and higher positive values of s. Further, the trajectories move towards the ΛCDM model at late times, but never reach there. The trajectories of phantom HDE ($w < -1$) in Fig. 2b behave differently and approach the ΛCDM model at late times, which is shown by trajectory (5) in the figure.

The $r-q$ trajectories have been plotted in Fig. 3. The straight line in the figure has been plotted to show the evolution of the $r-q$ trajectory for the ΛCDM model and the fixed point values of $\{r, q\}$ for the steady-state (SS) model and the SCDM model having the values $\{1, -1\}$ and $\{1, 0.5\}$, respectively. We have plotted $r-q$ trajectories for a negative interaction rate $\Gamma = -0.1$ in Fig. 3a and for a positive interaction rate $\Gamma = 0.01$ in Fig. 3a. It has been observed in Fig. 3a that for smaller negative values of the EoS parameter w, the trajectories behave like quintessence during late time evolution. But as we increase the negative value of w, the trajectories deviate more and more from quintessence behavior. In Fig. 3b, we observe that for small negative values of w, the trajectories behave almost like quintessence at early times, but do not approach the SS model at late times, whereas the quintessence model approaches the SS model at late times. As we increase the negative values of w, greater deviation from quintessence has been observed. Even, phantom HDE shows totally different behavior from the quintessence HDE shown by trajectory (5) in the figure.

3 Viscous holographic dark energy in Brans–Dicke theory

In an isotropic and homogeneous FLRW model, the dissipative process may be treated via the relativistic theory of bulk viscosity proposed by Eckart [59] and later on pursued by Landau and Lifshitz [60]. It has been found that only the bulk viscous fluid remains compatible with the assumption of

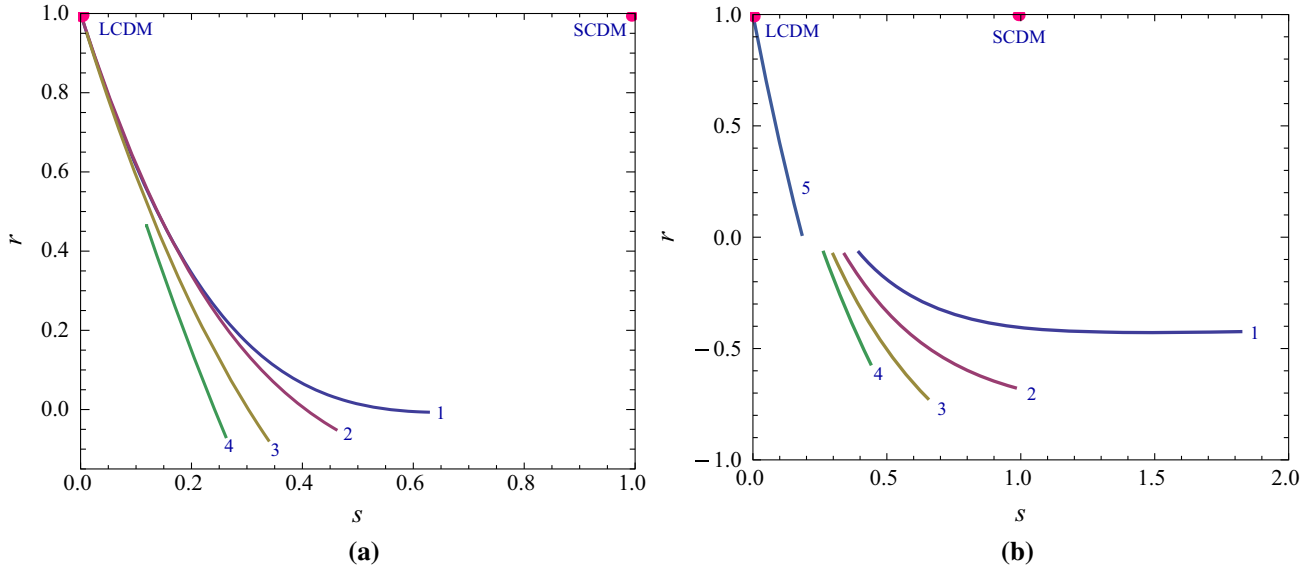

Fig. 2 The $r - s$ trajectories has been plotted in the figure. We have taken $H_0 = 10$ and $t_0 = 1$ to plot the trajectories. The figure **a** is plotted for negative value of interaction rate $\Gamma = -0.1$ and the figure **b** is plotted for positive value of interaction rate $\Gamma = 0.005$. In figure (**a**), the trajectories 1, 2, 3, and 4 are plotted for $w = -0.35$, $w = -0.5$, $w = -0.7$, and $w = -0.9$, respectively. In figure (**b**), the trajectories 1, 2, 3, 4 and 5 are plotted for $w = -0.6$, $w = -0.7$, $w = -0.8$, $w = -0.9$ and $w = -1.2$, respectively

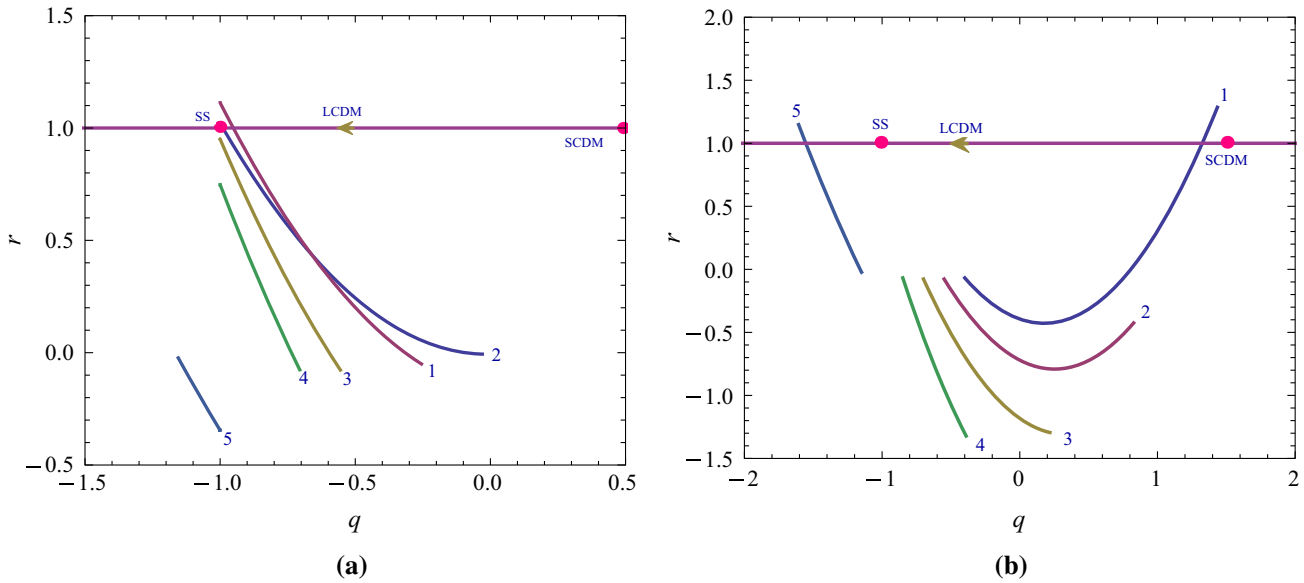

Fig. 3 The $r - q$ trajectories has been plotted in the figure. We have taken $H_0 = 10$ and $t_0 = 1$ to plot the trajectories. The figure **a** is plotted for negative value of interaction rate $\Gamma = -0.1$ and the figure **b** is plotted for positive value of interaction rate $\Gamma = 0.01$. In figure (**a**), the trajectories 1, 2, 3, 4 and 5 are plotted for $w = -0.35$, $w = -0.5$, $w = -0.7$, $w = -0.8$ and $w = -1.1$, respectively. In figure (**b**), the trajectories 1, 2, 3, 4 and 5 are plotted for $w = -0.6$, $w = -0.7$, $w = -0.8$, $w = -0.9$ and $w = -1.1$, respectively

large scale homogeneity and isotropy. The other processes, like shear and heat conduction, are directional mechanisms and they decay as the universe expands. Bulk viscosity has been studied in the literature to discuss inflation as well as the recent acceleration of the universe. It also plays an important role to explain the phase transition of the universe, particle creation, the photon to baryon ratio in the universe and to avoid the initial singularity [45–52]. We do not know the exact nature of DE yet except its negative pressure and it might be viscous in nature. Therefore, it will be interesting to discuss the bulk viscous nature of HDE. The main purpose behind considering bulk viscous HDE in BD theory is to find the answer to the question: Can the non-interacting bulk viscous HDE play the role of interacting HDE? The concept of viscous DE has been discussed extensively in the literature, e.g., [39,61–63]. Due to viscous effects, the effective pressure of HDE is given by $P_{eff} = p_h + \Pi$, where Π denotes the change due to bulk viscosity. According to Eckart theory [59], $\Pi = -3\zeta H$, where ζ stands for the coefficient of bulk viscosity. Here, we consider $\zeta > 0$ to satisfy the second law of thermodynamics. Now, we have the Friedmann equations and the wave equation for BD theory as follows:

$$H^2 + H\frac{\dot{\phi}}{\phi} - \frac{\omega}{6}\frac{\dot{\phi}^2}{\phi^2} = \frac{\rho_m + \rho_h}{3\phi}, \tag{22}$$

$$2\frac{\ddot{a}}{a} + H^2 + 2H\frac{\dot{\phi}}{\phi} + \frac{\omega}{2}\frac{\dot{\phi}^2}{\phi^2} + \frac{\ddot{\phi}}{\phi} = \frac{-P_{eff}}{\phi}, \tag{23}$$

$$\ddot{\phi} + 3H\dot{\phi} = \frac{\rho_m + \rho_h - 3P_{eff}}{2\omega + 3}. \tag{24}$$

3.1 Non-interacting HDE, BD scalar field and deceleration parameter

In Sect. 2, we have discussed non-viscous HDE interacting with DM. In this section, we assume that there is no interaction between DM and viscous HDE. Therefore, the energy density of viscous HDE and DM conserve separately. The conservation equations of the viscous HDE and DM are given by

$$\dot{\rho}_m + 3H\rho_m = 0, \tag{25}$$

$$\dot{\rho}_h + 3H(\rho_h + P_{eff}) = 0. \tag{26}$$

There are a number of choices available for bulk viscosity coefficient ζ in the literature. One of the important forms of it is $\zeta = \zeta_0 + \zeta_1 H$ which is a combination of two forms ζ_0 (constant) and $\zeta \propto H$. This motivation can be traced in fluid mechanics where the transport/viscosity phenomenon is involved with velocity $\dot{a}$ which is related to the expansion rate H. As the system of field equations of BD theory is complex, therefore, for simplicity we choose a constant coefficient of bulk viscosity, i.e., $\zeta = \zeta_0$, where $\zeta_1 = 0$. In the paper [64], the authors have shown using observations that $0 < \zeta_0 < 3$ when $\zeta_1 = 0$. Therefore, firstly, we have taken $\zeta_0 > 0$ in our model as this is required to satisfy the second law of thermodynamics as mentioned above. Secondly, in [64], it has been shown that $0 < \zeta_0 < 3$ to satisfy observations, and we adopt this condition here also. With the help of Eq. (11), Eq. (26) reduces to

$$\dot{H} + \frac{3}{2}(1+w)H^2 - \frac{3\zeta_0}{2c^2M_p^2}H = 0. \tag{27}$$

On solving Eq. (27), we get the value of H in terms of cosmic time t as

$$H = \frac{e^{\frac{3\zeta_0}{2\,M_p^2\,c^2}t}}{c_5 + \frac{M_p^2\,c^2\,(1+w)}{\zeta_0}e^{\frac{3\zeta_0}{2\,M_p^2\,c^2}t}}, \tag{28}$$

where c_5 is a constant of integration. On solving Eq. (28), one can obtain the scale factor as

$$a = c_6\left[c_5 + \frac{M_p^2\,c^2(1+w)}{\zeta_0}\,e^{\frac{3\zeta_0}{2\,M_p^2\,c^2}t}\right]^{\frac{2}{3(1+w)}}, \tag{29}$$

where $c_6 > 0$ is an another constant of integration. The non-negative of c_6 is the consequence of calculation because it appears under the logarithmic function which is not defined for negative values. We can rewrite the scale factor as

$$a = a_0\left[1 + \frac{M_p^2\,c^2(1+w)H_0}{\zeta_0}\,(e^{\frac{3\zeta_0}{2\,M_p^2\,c^2}(t-t_0)} - 1)\right]^{\frac{2}{3(1+w)}}, \tag{30}$$

where a_0 and H_0 denotes the present value of the scale factor and the Hubble parameter at time t_0, the present value of time where the HDE begins to dominate. We obtain the scale factor in exponential form as in the case of non-viscous interacting HDE in Sect. 2. From Eq. (22), we obtain the value of the energy density of the DM $\rho = \frac{A}{a^3}$, where A is a constant of integration. Now, we obtain the value of ϕ from the wave equation (21) for the scale factor given by Eq. (27) as follows:

$$\begin{aligned}\phi = {} & \frac{1}{3}e^{-\frac{3\zeta_0}{M_p^2\,c^2}t}\left[\frac{1}{H_0} + \frac{M_p^2\,c^2(1+w)}{\zeta_0}(e^{\frac{3\zeta_0}{M_p^2\,c^2}t} - 1)\right]^{-\frac{2(2+w)}{1+w}} \\ & \times t\left[\frac{(\zeta_0 - M_p^2\,c^2(1+w)H_0)^3 A}{(2\omega+3)\zeta_0^3\,a_0^3\,H_0^{\frac{5+3w}{1+w}}}\right. \\ & + \frac{3A\,M_p^2\,c^2(1+w)(\zeta_0 - M_p^2\,c^2(1+w)H_0)^2}{(2\omega+3)\zeta_0^3\,a_0^3\,H_0^{\frac{2(2+w)}{1+w}}}e^{\frac{3\zeta_0}{M_p^2\,c^2}t}\,t \\ & + \frac{A\,M_p^6\,c^6(1+w)^3}{(2\omega+3)\zeta_0^3\,a_0^3\,H_0^{\frac{2}{1+w}}}e^{\frac{9\zeta_0}{M_p^2\,c^2}t}\,t \\ & \left. + \frac{3\,M_p^2\,c^2(w^2+2w+3)}{(2\omega+3)\zeta_0}e^{\frac{9\zeta_0}{M_p^2\,c^2}t}\right. \\ & \times\left(\frac{1}{H_0} + \frac{M_p^2\,c^2(1+w)}{\zeta_0}(e^{\frac{3\zeta_0}{M_p^2\,c^2}t} - 1)\right)^{\frac{2}{1+w}}\,t\end{aligned}$$

$$+\frac{(\zeta_0 - M_p^2\, c^2(1+w)H_0)}{\zeta_0 H_0} e^{\frac{6\zeta_0}{M_p^2\, c^2}t}$$
$$\times\Big(\frac{3A\; M_p^4\, c^4(1+w)^2}{(2\omega+3)\zeta_0^2\; a_0^3\; H_0^{\frac{2}{1+w}}}+\frac{3M_p^2\, c^2(1-3w)}{(2\omega+3)}$$
$$\times\Big(\frac{1}{H_0}+\frac{M_p^2\, c^2(1+w)}{\zeta_0}\times(e^{\frac{3\zeta_0}{M_p^2\, c^2}t}-1)\Big)^{-\frac{2)}{1+w}}\Big)t$$
$$-\Big(\frac{1}{H_0}+\frac{M_p^2\, c^2(1+w)}{\zeta_0}$$
$$\times\, c_7\,(e^{\frac{3\zeta_0}{M_p^2\, c^2}t}-1)\Big)^{\frac{(5+3w)}{1+w}} t\;\; e^{\left(\frac{\frac{-3\zeta_0 t}{M_p^2 c^2}t}{-3e^{\frac{1}{H_0}+\frac{M_p^2\, c^2(1+w)}{\zeta_0}(e^{\frac{3\zeta_0}{M_p^2\, c^2}t}-1)}}\right)}$$
$$+3\zeta_0 e^{\frac{3\zeta_0}{M_p^2\, c^2}t}\left(\frac{1}{H_0}+\frac{M_p^2\, c^2(1+w)}{\zeta_0}(e^{\frac{3\zeta_0}{M_p^2\, c^2}t}-1)\right)^{\frac{2(2+w)}{1+w}}$$
$$\times t+c_8\Big], \tag{31}$$

where c_7 and c_8 are integration constants. This is the form of ϕ for viscous non-interacting HDE.

The DP for this model is given by

$$q=\frac{3\left[1+w-\frac{\zeta_0}{M_p^2\, c^2\, H_0}\right]}{2\, e^{\frac{3\zeta_0}{2M_p^2\, c^2}(t-t_0)}}-1. \tag{32}$$

Here, we obtain a time dependent DP as obtained in the case of non-viscous interacting HDE. We observe that the sufficiently large value of the bulk viscous coefficient (ζ_0) can accelerate the expansion of the universe irrespective of whether the HDE is of quintessence type or of phantom type. Further, if the HDE is of phantom type, i.e., $w<-1$, then this is sufficient to accelerate the expansion irrespective of the bulk viscous coefficient (ζ_0). To accommodate the recent phase transition from deceleration to acceleration, the parameters must satisfy the condition $\frac{3}{2}\left[1+w-\frac{\zeta_0}{M_p^2\, c^2\, H_0}\right]>1$, i.e., $w>-\frac{1}{3}+\frac{\zeta_0}{M_p^2\, c^2\, H_0}$. We observe that non-interacting bulk viscous HDE produces the same type of results as in the case of non-viscous interacting HDE for $\Gamma<0$. Therefore, bulk viscosity may play the role of interaction for HDE and can explain acceleration as well as the phase transition of the universe. The evolution of DP for various values of the model parameters has been shown in Fig. 4. We notice that for some values of the parameters it is possible to get a suitable form of q which is positive early on, representing deceleration, and negative at later epochs, representing acceleration.

3.2 Statefinder diagnosis

Let us apply the statefinder diagnosis to the viscous HDE model also. We obtain the expression of the statefinder

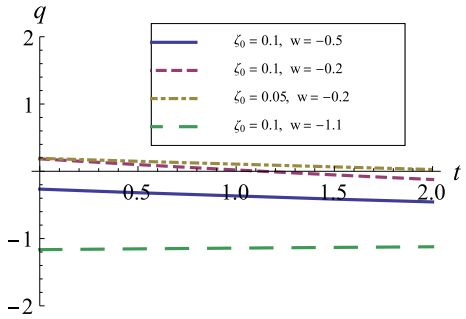

Fig. 4 The figure of deceleration parameter q against cosmic time t has been plotted for $M_p=1$, $c=1$ and $H_0=10$

parameter r as follows

$$r=e^{-\frac{3\zeta_0 t}{M_p^2 c^2}}\Big[\frac{9\zeta_0^2}{4M_p^4 c^4 H_0^2}e^{\frac{3\zeta_0 t_0}{M_p^2 c^2}}-\frac{9\zeta_0}{4M_p^2 c^2 H_0}e^{\frac{3\zeta_0 t_0}{2M_p^2 c^2}}$$
$$\times\Big((w-1)e^{\frac{3\zeta_0 t}{2M_p^2 c^2}}+2(1+w)e^{\frac{3\zeta_0 t_0}{M_p^2 c^2}}\Big)$$
$$+\Big(e^{\frac{3\zeta_0 t}{M_p^2 c^2}}+\frac{c^2(1+w)^2 M_p^2}{4\zeta_0}e^{\frac{3\zeta_0 t}{M_p^2 c^2}}$$
$$-9(1-w^2)e^{\frac{3\zeta_0(t+t_0)}{2M_p^2 c^2}}\Big)\Big]. \tag{33}$$

The expression of the statefinder parameter s can be obtained easily from the formula $s=\frac{r-1}{3(q-\frac{1}{2})}$ using the values of q and r from Eqs. (32) and (33), respectively. We have plotted the $r-s$ trajectories in Fig. 5a and the $r-q$ trajectories in Fig. 5b. In Fig. 5a, the trajectories behave like quintessence for $w>-1$; however, for $w<-1$ the trajectories have higher positive value of r and negative value of s, and approach the ΛCDM model at late times like the Chaplygin gas model. In Fig. 5b, we observe that all the trajectories show quintessence like behavior in the late time evolution. Moreover, the trajectories show almost quintessence behavior for smaller negative values of w. Thus, we observe that the viscous HDE model shows more similarity with the quintessence model than the interacting HDE model.

4 Conclusion

To resolve the mysteries of cosmology like the recent accelerated expansion and the cosmic coincidence problem, the concept of HDE in the framework of BD theory has been studied in the literature. In BD theory, a number of authors have obtained a time dependent deceleration parameter using various forms of DE and taking the assumption $\phi\propto a^n$ [17–19,24,25]. In the present paper, we demonstrate that the assumption $\phi\propto a^n$ naturally leads to a constant deceleration parameter, irrespective of the form of DE in BD theory.

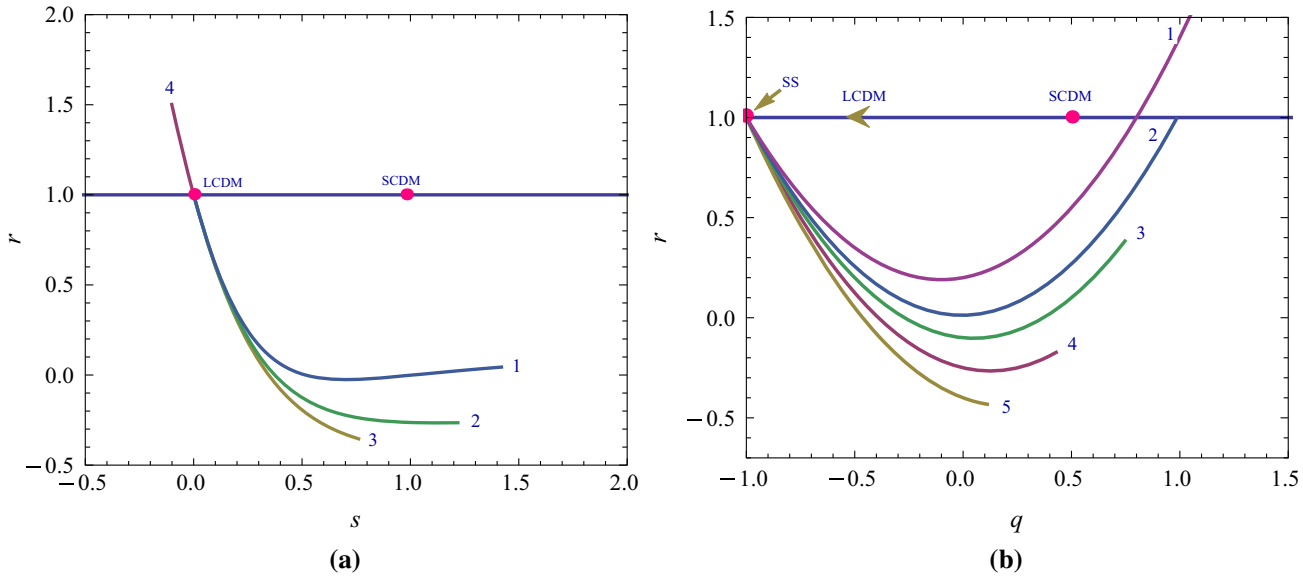

Fig. 5 The figure **a** plots the $r - s$ trajectories and the figure **b** plots the $r - q$ trajectories. We have taken $H_0 = 10$, $M_p = 1$, $c = 1$ and $t_0 = 1$ to plot the trajectories. In figure (**a**), the trajectory 1 is plotted for $w = -0.35$ and $\zeta = 1/5$, the trajectory 2 is plotted for $w = -0.5$ and $\zeta = 1/3$, the trajectory 3 is plotted for $w = -0.6$ and $\zeta = 1/3$ and the trajectory 4 is plotted for $w = -1.03$ and $\zeta = 1/3$. In figure (**b**), we have taken $\zeta = 1/2$ and the trajectories 1, 2, 3, 4 and 5 are plotted for $w = -0.2$, $w = -0.33$, $w = -0.4$, $w = -0.5$ and $w = -0.6$, respectively

Taking into account this problem with power law form, a logarithmic form was introduced in [26]. We have considered HDE with Hubble length as IR cut-off in the framework of BD theory, and we do not use any specific form of the BD scalar field. We obtain the value of the scale factor and corresponding value of ϕ for the HDE model. As the assumption $\phi \propto a^n$ in Brans–Dicke theory is not suitable to study the phase transition of the universe; therefore, we have found a suitable value of ϕ for HDE. Further, we discuss the possible evolution of the universe using the DP and apply statefinder diagnosis to compare our model with existing standard models.

First, we consider a non-viscous interacting HDE model and obtain the value of the scale factor. Further, the expression of the BD scalar field ϕ has been obtained with the help of values of scale factor and energy density of the HDE in the wave equation of the BD scalar field ϕ. We obtain the DP to discuss the evolution of the non-viscous interacting HDE model. Using the DP, we observe that if there is an energy transfer from HDE to DM, i.e. $\Gamma > 0$, then both quintessence and phantom HDE are viable candidates to describe the early time phase transition (inflation to deceleration) under suitable constraints on the parameters as given in Sect. 2. However, phantom HDE may accommodate the recent accelerated expansion for $\Gamma > 0$ but is not able to describe the recent phase transition. For $\Gamma < 0$, we observe the accelerated expansion throughout the evolution for both quintessence and phantom HDE. To accommodate the recent phase transition, the HDE must not cross the quintessence divider, i.e., $w > -\frac{1}{3}$. Our results show that we can obtain the recent accelerated expansion in the phantom region for $\Gamma > 0$ and $\Gamma < 0$, in the quintessence region for $\Gamma < 0$, but the recent phase transition is possible for the quintessence region only. As is well known, the phase transition is an integral part of cosmic history. Therefore, to achieve the recent phase transition HDE must not cross the quintessence divider. The statefinder diagnostic has been applied to compare our model with existing standard models. We observe that the trajectories for $\lambda < 0$ show more similarities with trajectories of the quintessence model than the trajectories for $\lambda > 0$. We also observe that as we increase the negative value of w, the trajectories deviate more and more form quintessence.

Further, we consider viscous HDE with constant bulk viscous coefficient $\zeta = \zeta_0$, and assume that there is no interaction between the HDE and DM. As in the case of interacting HDE for $\Gamma < 0$, we observe the recent phase transition for non-interacting bulk viscous HDE below the quintessence region, i.e, for $w > -1/3$. The justification for $w > -1/3$ is the bulk viscous nature of the HDE adding the extra negative pressure to the effective pressure. As w passes the boundary line of the quintessence region ($w \leq -1/3$), the effective negative pressure becomes sufficient to accelerate the universe throughout the evolution, which rules out the decelerated expansion of evolution. In the quintessence and phantom region, we have observed the accelerated expansion of the universe. Thus, We observe that non-interacting bulk vis-

cous HDE produces almost the same type of results as in the case of non-viscous interacting HDE. Therefore, bulk viscosity may play the role of interaction for HDE and can explain acceleration as well as the phase transition of the universe. Further, we apply the statefinder diagnosis to the viscous HDE model. In this model, we observe that the trajectories of both $r - s$ and $r - q$ show more similarity with the quintessence model than the interacting HDE model.

Acknowledgements The authors are thankful to the referee for his constructive suggestions to improve the manuscript. One of the authors, A. Beesham, acknowledges the National Research Foundation of South Africa for providing a research grant (Grant numbers: 118511).

Data Availability Statement This manuscript has no associated data or the data will not be deposited. [Authors' comment: There is no separate data to submit. All data generated or analysed during this study has been included in the article.]

Funded by SCOAP[3].

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DOI: http://ijmer.in.doi./2023/12.03.19
www.ijmer.in

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:3(1), March: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd March 2023
Publication Date:10th April 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

SOME COMMON FIXED POINT RESULTS IN METRIC SPACE

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Abstract : Fixed point theory is a powerful tool in mathematics. In this paper, we introduce a common fixed point results in new generalize b-metric-like spaces. This result extend and generalize many existing results in the literature.

Key-Words : Metric Space, b-metric space, common Fixed point.

1.Introduction :

The concept of b-metric space was introduced and studied by Czerwik [3], since then serval papers have been dealt with fixed point theory for single-valued and multivalued operators in b-metric spaces. Amini-Harandi [2] introduced the notion of metric-like space, which is an interesting generalization of partial metric space. Akkouchi[4], have studied Common fixed point theorem for expansive mappings under strict implicit conditions on b-metric spaces. Recently, Mohammed Ali Algamdi [1] introduced a new generalization of metric-like space and partial metric space is called a b-metric-like space and studied some fixed point theorem of b-metric-like space. These results improved some well-known results in the literature.

2.Preliminaries :

In this section, we recall some of the metric spaces and mappings as follows :

Definition 2.1 [1] A b-metric-like on a non empty set X is a function $D : X \times X \to [0, +\infty)$ such that for all $p, q, r \in X$ and a constant $K \geq, 1$ the following three conditions hold true :

(A1): if $D(p, q) = 0 \Rightarrow p = q$

(A2): $D(p; q) = D(q, p)$

(A3): $D(p, q) \leq K(D(p, r) + D(r, q))$

The pair (X, d) is called a b-metric-like space.

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DOI: http://ijmer.in.doi./2023/12.03.19
www.ijmer.in

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
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Publisher: Sucharitha Publication, India
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Example 2.2 [1] Let X = [0 +∞). Define the function D : $X^2 \to [0+\infty)$ by D(p; q) = $(p+q)^2$. Then (X, D) is a b-metric-like space with constant K=2. Clearly (X, D) is not a b-metric or metric-like space.

Indeed, for all p, q, r ∈ X.

$D(p, q) = (p+q)^2 \le (p+r+r+q)^2 = (p+r)^2 + (R+q)^2 + 2\,(p+r)\,(r+q)$

$\le 2\,[(p+r)^2 + (r+q)^2]$

$= 2\,(D(p\ r) + D(r\ q))$

and so (A3) holds. Clearly, (A1) and (A2) hold.

Definition 2.3. An element (a , b) ∈ X × X is called a coupled fixed point of T : X × X → X if a = T(a, b) and b = T (b, a).

Definition 2.4. An element (a, b) ∈ X ×X is called a coupled coincidence point of S, T : X ×X → X if S (a, b) = T (a, b) and S (b, a) = T (b, a).

Example 2.5 . Let X = **R** and S, T : X × X → X defined as

S(a, b) = a + b – ab + sin (a+b) and T (a, b) = a+b+cos (a+b)

for all a, b ∈ X. Then (0, π/4) and (π/4, 0) are coupled coincidence points of S and T

Definition 2.6. An element (a, b) ∈ X × X is called a of S, T : X × X → X if

a = S(a, b) = T (a, b) and b = S (b, a) = T (b, a).

Example 2.7. Let X = **R** and S, T : X × X → X defined as

S(a, b) = ab and T (a, b) = a + $(b–a)^2$

for all a, b ∈X. Then (0, 0) and (1, 1) are common coupled fixed points of S and T.

3. Main Result :

Theorem 3.1. Let (X, **D**) be a compact b-metric-like space and a constant K ≥ 1 and let the mapping S, T : X × X →X satisfy.

$$\mathbf{D\,(S(a,\,b),\,T(u,\,v))} \le \alpha\frac{D(a,u)+D(b,v)}{2} + \beta\frac{D(a,S(a,b))D(u,T(u,v))}{(1+D(a,u)+D(b,v))} + \gamma\frac{D(u,S(a,b))D(a,T(u,v))}{(1+D(a,u)+D(b,v))} \qquad 3.1$$

for all a, b, u, v ∈ X and α, β ≥ 0 with K α+β < 1 and α+λ<1. Then S and T have a unique common coupled fixed point in X.

Proof. Step 1 : Firstly, we show that a_n, b_n are Cauchy sequence in X.

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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
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DOI: http://ijmer.in.doi./2023/12.03.19
www.ijmer.in

Let a0, b0 $\in$ X be any arbitrary points. Define $a_{2k+1} = S(a_{2k}, b_{2k})$, $b_{2k+1} = S(b_{2k}, a_{2k})$ and $a_{2k+1} = T(a_{2k+1}, b_{2k+1})$, $b_{2k+2} = T(b_{2k+1}, a_{2k+1})$ for k = 0, 1, 2, 3,.....

$$D(a_{2k+1}, a_{2k+2}) = D(S(a_{2k}, b_{2k}), T(a_{2k+1}, b_{2k+1}))$$

$$\alpha \frac{D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1})}{2} + \beta \frac{D(a_{2k}, S(a_{2k}, b_{2k}))D(a_{2k+1}, T(a_{2k+1}, b_{2k+1}))}{(1 + D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1}))}$$

$$D(a_{2k+1}, a_{2k+2}) \leq \gamma \frac{D(a_{2k+1}, S(a_{2k}, b_{2k}))D(a_{2k}, T(a_{2k+1}, b_{2k+1}))}{(1 + D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1}))}$$

$$D(a_{2k+1}, a_{2k+2}) =$$

$$\alpha \frac{D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1})}{2} + \beta \frac{D(a_{2k}, a_{2k+1})\, D(a_{2k+1}, a_{2k+1})}{(1 + D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1}))} +$$

$$\gamma \frac{D(a_{2k+1}, a_{2k+1})D(a_{2k}, T(a_{2k}, a_{2k+2}))}{(1 + D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1}))}$$

$$D(a_{2k+1}, a_{2k+2}) \leq$$

$$\alpha \frac{D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1})}{2} + \beta \frac{D(a_{2k}, a_{2k+1})\, D(a_{2k+1}, a_{2k+2})}{(1 + D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1}))} +$$

$$\gamma (2D)(a_{22k+1}, a_{2k+2})$$

$$D(a_{2k+1}, a_{2k+2}) \leq$$
$$\alpha \frac{D(a_{2k}, a_{2k+1})}{2} + \alpha \frac{D(b_{2k}, b_{2k+1})}{2} + \beta D(a_{2k+1}, \alpha_{2k+2}) + \gamma (2D(a_{2k+1}, a_{2k+2}))$$

$$(1 - \beta - 2\gamma) D(a_{2k+1}, a_{2k+2}) \leq \alpha \frac{D(a_{2k}, a_{2k+1})}{2} + \alpha \frac{D(b_{2k}, b_{2k+1})}{2(1 - \beta - 2\gamma)}$$

$$D(a_{2k+1}, a_{2k+2}) \leq \alpha \frac{D(a_{2k}, a_{2k+1})}{2(1 - \beta - 2\gamma)} + \alpha \frac{D(b_{2k}, b_{2k+1})}{2(1 - \beta - 2\gamma)}$$

$$D(a_{2k+1}, a_{2k+2}) \leq \alpha \frac{D(a_{2k}, a_{2k+1})}{2(1 - \beta)} + \alpha \frac{D(b_{2k}, b_{2k+1})}{2(1 - \beta)} \qquad (3.2)$$

similarly

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DOI: http://ijmer.in.doi./2023/12.03.19
www.ijmer.in

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
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$$D(b_{2k+1}, b_{2k+2}) \leq \alpha \frac{D(b_{2k}, b_{2k+1})}{2(1-\beta)} + \alpha \frac{D(a_{2k}, a_{2k+1})}{2(1-\beta)}$$

Add (3.2) and (3.3)

$$[D(a_{2k+1}, a_{2k+2}) + D(b_{2k+1}, b_{2k+2})] \leq \frac{\alpha}{(1-\beta)} [D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1})]$$

$$= h [D(a_{2k}, a_{2k+1}) + D(b_{2k}, b_{2k+1})]$$

Where $0 < h = \frac{\alpha}{(1-\beta)} < 1$. Similarly

$$D(a_{2k+2}, a_{2k+3}) \leq \alpha \frac{D(a_{2k+1}, a_{2k+2})}{2(1-\beta)} + \alpha \frac{D(b_{2k+1}, b_{2k+2})}{2(1-\beta)}$$

similarly

$$D(b_{2k+2}, b_{2k+3}) \leq \alpha \frac{D(b_{2k+1}, b_{2k+2})}{2(1-\beta)} + \alpha \frac{D(a_{2k+1}, a_{2k+2})}{2(1-\beta)}$$

Adding above equation, we get

$$[D(a_{2k+2}, a_{2k+3}) + D(b_{2k+2}, b_{2k+3}) \leq$$

$$\frac{\alpha}{(1-\beta)} [D(a_{2k+1}, a_{2k+2}) + D(b_{2k+1}, b_{2k+2})]$$

$$= h [D(a_{2k+1}, a_{2k+2}) + D(b_{2k+1}, b_{2k+2})]$$

Continuing in this way,

$$(D(a_n, a_{n+1}) + D(b_n, b_{m+1})) \leq h(D(a_{n-1}, a_n) + D(b_{n-1}, b_n)) \leq - - - -$$

$$\leq h^n (D(a_0, a_1) + D(b_0, b_1))$$

Now, if

$$D(a_a, a_{n+1}) + D(b_n, b_{n+1}) = \delta_n, \text{then } \delta_n \leq h\delta_{n-1} \leq - - - \leq h^n \delta 0$$

For $m > n$, we have

$$(D(a_n, a_m) + D(b_n, b_m)) \leq K(D(a_n, a_{n+1}) + D(b_n, b_{n+1})) + \ldots$$

$$+ K^{m-n} (D(a_{m-1}, a_m) + D(b_{m-1}, b_m))$$

$$\leq Kh^n \delta_0 + K^2 h^{n+1} \delta 0 + \ldots + K^{m-n} h^{m-1} \delta_0$$

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DOI: http://ijmer.in.doi./2023/12.03.19
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$$< K h^n \left[1 + (Kh) + (Kh)^2 + \dots \right] \delta_o$$

$$= \frac{Kh^n}{1 - Kh} \delta_o \to 0 \text{ as } n \to \infty$$

This shows that $\{a_n\}$ and $\{b_n\}$ are Cauchy sequence in X. Since X is a complete b-metric-like space, there exists a, b $\in$X such that $a_n \to \infty$ and $b_n \to \infty$ as $n \to \infty$.

Step 2 : Now, We show that a = S (a, b) and b = S (b, a). We suppose on the contrary that a $\neq$ S (a, b) and b $\neq$ S (b, a) so that

$$D(a, S(a, b)) = \ell_1 > 0 \text{ and } D(b, S(b, a)) = \ell_2 > 0$$

Consider

$$\ell_1 = D(a, S(a, b)) \leq K\,[D(a, a_{2k+2}) + D(a_{2k+2}, S(a, b))]$$

$$\leq KD(a, a_{2k+2}) + KD(T(a_{2k+1}, b_{2k+1}) S(a, b))$$

$$KD(a, a_{2k+2}) + K\alpha \frac{D(a_{2k+1}, a) + D(b_{2k+1}, b)}{2} +$$

$$K\beta \frac{D(a, S(a, b)) D(a_{2k+1}, T(a_{2k+1}, b_{2k+1}))}{1 + D(a_{2k+1}, a) + D(b_{2k+1}, b)}$$

$$K\gamma \frac{D(a_{2k+1}, S(a, b)) D(a, a_{2k+2})}{1 + D(a_{2k+1}, a) + D(b_{2k+1}, b)}$$

by taking $k \to \infty$, we get, $\ell_1 \leq 0$, which is contradiction. Therefore,

D (a, S (a, b)) = 0. This implies a = S (a, b). Similarly, we can prove that b = S (b, a).

It follows similarly we can show that a = T (a, b) and b = T (b, a).

So we have proved that (a, b) is a common coupled fixed point of S and T.

Step 3 : We now show that S and T have a unique common coupled fixed point.

Let (a*, b*) $\in$ X $\times$ X be another common coupled fixed point of S and T. Then

$$D(a, a^*) = D(S(a, b), T(a^*, b^*))$$

$$\leq \alpha \frac{D(a, a^*) + D(b, b^*)}{2} + \beta \frac{D(a, S(a, b)) D(a^*, T(a^*, b^*))}{1 + D(a, a^*) + D(b, b^*)}$$

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DOI: http://ijmer.in.doi./2023/12.03.19
www.ijmer.in

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$$\gamma \frac{D(a^*, S(a,b))D(a, T(a^*,b^*))}{1+D(a,a^*)+D(b,b^*)}$$

$$= \alpha \frac{D(a,a^*)+D(b,b^*)}{2} + \beta \frac{D(a,a)D(a^*,a^*)}{1+D(a,a^*)+D(b,b^*)}$$

$$\gamma \frac{D(a^*,a)D(a,a^*)}{1+D(a,a^*)+D(b,b^*)}$$

$$D(a,a^*) \le \frac{\alpha}{2} D(a,a^*) + \frac{\alpha}{2} D(b,b^*) + 4\beta D(a,a^*) + \gamma D(a^*,a))$$

$$D(a,a^*) \le \frac{\alpha}{(2-\alpha\ 8\beta-2\gamma)} D(b,b^*) \le \frac{\alpha}{(2-\alpha-2\gamma)} D(b,b^*)$$

Similarly, we can easily prove that

$$D(b,b^*) \le \frac{\alpha}{(2-\alpha-2\gamma)} D(a,a^*)$$

Adding, we get

$$D(a,a^*)+D(b,b^*) \le \frac{\alpha}{(2-\alpha-2\gamma)} [D(a,a^*)+D(b,b^*)]$$

$$(2-2\alpha-2\gamma)[D(a,a^*)+D(b,b^*)] \le 0$$

$$D(a,a^*)+D(b,b^*) = 0$$

This implies, a = a* and b = b*.

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Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/01.01.20
www.ijmer.in

A COMPARATIVE STUDY OF GHOST DARK ENERGY MODELS

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Abstract In the present paper ghost dark energy models are studied. The results are compared with the help of cosmological parameters in different theories of gravity. The future scope of the research in existing theories of gravity is also discussed.
Keywords: Gravity, Ghost Dark Energy, cosmological parameters, existing theory.

1 Introduction

The present universe is going under an accelerated expansion is confirmed in 1998 by Perlmutter and Reiss [1, 2, 3]. There are several number of observations like Wilkinson Microwave Anisotropy Probe (WMAP) [4, 5], Sloan Digital Sky Survey (SDSS) [6, 7] and Planck data [8, 9] which also confirmed the accelerated expansion of the universe. A hypothetical form of energy so-called 'dark energy' (DE) with negative pressure is assumed to be responsible for the accelerating expansion of the Universe. Now the biggest challenge in the field of cosmology is that what is the factor responsible for this accelerated expansion of the universe. It is suggested by the cosmological observations that the Universe is composed of about 68% Dark Energy(DE), 27% of dust matter and rest other forms of matter. It is assumed that some unknown type of energy might exists in the cosmos to derive this accelerated expansion. This unknown type of fluid is known as DE. The strange behavior of DE is a challenge before cosmologist and physicist. A class of models is available in cosmology to discuss the phenomenon of DE. The most convincing model of DE is ΛCDM model. It is also popularly known as cosmological constant model. This model experiences two major problems known as fine-tuning and cosmic coincidence problems [10].

A lot of DE models are proposed in the literature like K-essence [13, 14], tachyon[15, 16], phantom [17, 18, 19], ghost condensate [20, 21], quintom [22, 23, 24], holographic dark energy [25, 26], agegraphic dark energy [27, 28]. In the last few years, the models of ghost dark energy(GDE) are proposed to discuss the evolution of the universe. The GDE originated from Veneziano ghosts in quantum chromodynamics (QCD) theory [29, 30, 31, 32]. It is assumed that the contribution of the ghost field to the vacuum energy can be treated as a possible candidate of DE. It does not need any new degree of freedom while other DE models explain the accelerated expansion either with the help of new degree(s) of freedom or by modifying gravity theories. The Veneziano ghost field was introduced to solve problems in QCD. The positive and negative norms of QCD ghost field cancel each other and leaves no trace in the physical subspace. It was contended that they have small role to vacuum energy in time-

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DOI: http://ijmer.in.doi./2023/01.01.20
www.ijmer.in

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
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dependent or curved space background [33]. A lot of DE models are available to show the evolution of the universe using GDE and Generalized Ghost Dark Energy(GGDE) [34, 35, 36].

2 Ghost dark energy

The ghost field does not have any participation in vacuum energy in Minkowaski space-time rather it has a non vanishing role to the vacuum energy in a dynamical background. This vacuum energy has magnitude of order $\Lambda^3_{QCD}H$, where Λ^3_{QCD} is QCD mass scale [33] and H is known as Hubble's parameter. Using the fact that $\Lambda QCD \sim 100M_eV$ and $H \sim 10^{-33}eV$ for the current time provide right order of magnitude $\rho_D \sim (3 \times 10^{-3}eV)^4$ for ghost energy density [33]. In this way GDE model provides a required amount of DE and solve fine-tuning problem associated with ΛCDM model. The energy density of GDE is given by [33, 37, 38]

$$\rho_d = g_1 H \tag{1}$$

where H is the Hubble's parameter and g_1 is a constant.
The author [39] has shown that the role of the Veneziano QCD ghost field to vacuum energy is not exactly of order H and the sub-leading term H^2 appears because of the fact that vacuum expectation value of the energy momentum tensor is conserved in isolation [40]. It was shown that vacuum energy of the ghost field can be written as $H + O(H^2)$. It has been expected that the second term H^2 plays a crucial role in early evolution of the Universe which may act as the early DE [41]. It has been observed that including the second term with original GDE density, it was in good accordance [41, 42] with the observational data as compared to usual GDE. In the generalized model, the energy density is defined as

$$\rho_g = g_1 H + g_2 H^2 \tag{2}$$

where g_1 and g_2 are constant. This form of model is known as the GGDE model.

3 Advancement in ghost dark energy models

Cosmological Constraints on Ghost Dark Energy in the Brans-Dicke Theory by Using Markov Chain Monte Carlo simulation(MCMC) Approach was studied by [43]. It has been shown that cosmological parameters$\Omega_b h^2$,$\Omega_c h^2$,$\Omega_D E$ and Ω_k matches with the result of ΛCDM model. It is also observed that the parameter ϵ decreases whereas the BD parameter ω increases. The best fit value of $\epsilon = \frac{1}{\omega}$ is in good accordance with results of other cosmological constraining works [44, 45, 46]. This value is also matches with the observations from solar system tests such as Cassini experiment.

Effects of anisotropy on interacting ghost dark energy in Brans-Dicke theories was studied by [35]. The author found the EoS parameter of the ghost DE and BD theory models in the case $b^2 \neq 0.12$ can not cross phantom divide line(PDL) while $b^2 = 0.12$ can PDL. Also it is observed that increasing of the anisotropy and the interaction parameter is increased

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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:1(1), January: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd January 2023
Publication Date:10th February 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/01.01.20
www.ijmer.in

the phantomic. Evolution of the interacting ghost DE density parameter in BD theory depends on the anisotropy density parameter $\Omega_\sigma 0$ and the coupling constant b^2. In this study it is shown that in an anisotropic Universe the present cosmic acceleration is followed by a decelerated expansion in an early matter dominant phase. The method of sound squared speed shows the stability of ghost dark energy in BD theory. It is shown that in anisotropic Universe with ghost DE of BD theory, the Hubble parameter are bigger than the ghost DE of FRW and ΛCDM model.

Ghost dark energy in f(R) model of gravity was studied by [47]. The model was studied for interacting and non interacting case for FLRW line element with three kinds of interaction for better results. The deceleration parameter and equation of state parameter shows accelerated expansion of the universe and equation of state parameter can cross phantom divide line $w_d = -1$ from quintessence to phantom model for interacting and non interacting cases. The adiabatic sound squared speed shows that the model is stable for both interacting and non interacting cases. The model is fitted with supernova observational data for non interacting case and it is observed that the present value of equation of state can cross phantom divide line.

Interacting Ghost Dark Energy in Brans-Dicke Theory was proposed by [48]. The model is discussed in the absence of interaction first and it was found that the universe enters in accelerated phase at late times. The model is also discussed for interacting case for both flat and non flat universe. It was found that for both the cases the universe enters in accelerated phase and can cross phantom divide line also. The authors [50] investigated generalized ghost dark energy in HoravaLifshitz cosmology. The equation of state of dark energy is found with for low redshifts. This model is discussed for non flat universe. The generalized second law of thermodynamics valid in case of non-phantom nature of generalized ghost dark energy

Stability of ghost dark energy in CBD Model of gravity is another approach proposed by [51]. The ghost dark energy model in chameleon Brans-Dicke theory in absence of interaction is studied for non flat FLRW metric. The cosmological parameters shows the positive accelerated expansion of the universe and equation of state parameter can cross the phantom divide line. Also the model is found to be stable for suitable choices of parameter. The model is also discussed for interacting case with three usual interaction terms. The model is found to be stable for interacting case as in non interacting case.

Generalized Ghost Dark Energy in DGP Model was proposed by [52]. The monotonically decreasing nature of Hubble's parameter H is achieved which satisfies the observational results. The generalized ghost dark energy parameter shows DE dominated era in late future. The Equation of State parameter can never cross phantom divide line. The DP with respect to scale factor is shown and observed that the signature of DP flipped at the rate $a \sim 0.5$ and shows phase transition from deceleration to acceleration phase which matches with today's observation. The prime analysis shows only thawing region. No freezing region is observed. The model is observed as stable model among existing model of gravity.

Ghost Dark Energy in the DGP Braneworld was another issue discussed by [53]. It is observed that the equation state parameter can not cross PDL and mimics cosmological constant in late time. The energy density parameter of GDE tends to zero at early time and approaches to one in late times. The universe also shows that there is a phase transition from decelerated to accelerated phase. But this model is found to be instable. The freezing

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DOI: http://ijmer.in.doi./2023/01.01.20
www.ijmer.in

region is also observed in the model.

4 Conclusion and future scope

It is observed from the present study that the ghost dark energy and generalized ghost dark energy in different theories of gravity able to explain the recent accelerated expansion of the universe. The model is found to be stable in most of the model. The ghost dark energy model in $f(R,T)$ gravity theory is still an area of research where we can discuss the evolution of the universe may be in more better way. The generalized ghost dark energy in Brans Dicke theory of gravity may also gives some better results. The statefinder pair can also be applied to distinguish the model among the existing models of gravity.

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TSME
Journal of Management

Volume 12, No. 1 & 2 (Jan.-June and July-Dec. 2022) ISSN 2249-6092

TSME JOURNAL OF MANAGEMENT

Bi-Annual (refereed) Journal Vol. 12, No. 1 & 2
(Jan.-June & July-Dec. 2022)

PUBLISHED BY

TSME Journal of Management is a Bi-Annual (Refereed) journal brought out by The Socie Management Education, India (TSME). Addressed to management practitioners, researcher academics, the Journal aims to engage rigorously with practices, concepts and ideas in the fi management, with an emphasis on providing managerial insights to a wide audience. The expressed in this publication are purely personal views of the authors and do not reflect the vi the TSME or its Journal of Management.

TSME Journal of Management

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Volume 12, No. 1 & 2 (Jan.-June & July-December 2022) ISSN 2249-6092

FINANCIAL AND PROFITABILITY PERFORMANCE OF STATE BANK OF INDIA: AN EMPIRICAL STUDY FOR EXAMINING IMPACT OF COVID 19 PANDEMIC

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Dr. Bhag Singh Bodla**

ABSTRACT

The banking sector is considered a vital segment of a modern economy. Therefore, the efficiency of banks is of vital importance. In order to ensure a healthy financial system and an efficient economy, banks must be carefully evaluated and analysed. A strong banking sector is necessary for boosting entrepreneurial activities, creating jobs, eradicating poverty, and increasing Gross Domestic Product (GDP) growth. The aim of this paper was to evaluate Financial and Profitability Performance of State Bank of India (SBI) before and after the entry of Covid-19 Pandemic. The SBI was chosen deliberately for the study because it is the oldest and biggest public sector bank in India. The reference period of the study ranges from the Financial Year 2018 to Financial Year 2022, thus it included COVID-19 Pandemic period and two years before it. To achieve the objective of this paper, the secondary data was applied which was collected from annual reports of SBI and from the website of money control. Several parameters were used in this study to measure profitability and financial performance of the bank under reference. Findings of the study brought out that the net interest income (NII) raised to Rs 1207 bn. in 2022 from Rs 749 bn. in year 2018, registering a CAGR of 13 per cent. However, the non-interest income of SBI was seen in reverse gear as it indicated a negative 2 per cent CAGR during this period. The impact of covid-19 pandemic was clearly visible as the growth of advances came down to 6 percent in 2020 and 5 per cent in 2021 from 13 percent in 2019. Covid-19 Pandemic also exerted alarming effect on valuation of SBI as its share price fell around 63 per cent in FY 2020 as compared to FY 2019. However, the price of SBI's share recovered very fast in 2021 when it surged 85 per cent.

Keywords: Banking, State Bank of India, Profitability, Non-performing assets, ROCE

INTRODUCTION

Banks are the financial intermediary that accepts deposits and channels those deposits into lending activities. They are the active players in financial market. The essential role of a bank is to connect those who have surplus funds with those who seek finances. Banks play very important roles in the economic development of nations as they, to a large extent, wield control over the supply of money in circulation and are the main stimuli of economic progress. Economic development is a dynamic and continuous process which is highly dependent upon the mobilisation of resources, investment, and the operational efficiency of the various segments of the economy. Therefore, a strong banking sector is vital for growth, creating jobs, generating wealth, eradicating poverty, entrepreneurial activity and increasing Gross Domestic Product (GDP) growth. After the post economic liberalization and globalization, there has been a significant impact on the banking industry. In order to ensure a healthy financial system and an efficient economy, banks' performance must be carefully evaluated and analysed.

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While banks help business organizations by rendering a wide range of products & services, the products and servi... are more or less identical from one bank to another, and there is little scope for differentiating between them. Therefore, it is necessary to measure the banks' individual performance to determine their contribution to busines... development (Dhanasekodi Rengasamy, Nov. 6, 2012). It is inevitable that banks continue to attract significan... attention from the public & scrutiny by financial regulators as there is a growing need to evaluate banks in a more efficient manner. Not only supervising institutions, regulators and bank management bodies, but also claims o... banks, are becoming increasingly concerned about the stability and sustainability of these financial institutions. Th... performance of banks should also be evaluated because of several other reasons such as determine their operational results and overall financial condition; measure their assets quality, management quality & efficiency, w... achievement of their objectives; as well as ascertain their earning quality, liquidity, and capital adequacy.

It is under above background, the present study titled- "Financial and Profitability Performance of State Bank o... India: A Study of last 20 Years" has been conducted. The paper is divided in to five sections besides introductio... These are: evolution and development of SBI, review of literature, research methodology, data analysis results an... discussion, and conclusion.

EVOLUTION AND DEVELOPMENTS OF SBI

Banking in India originated in the 18th century. The oldest commercial bank in India, SBI originated as the Bank o... Calcutta in 1806. After three years of its formation the bank was issued a royal charter and renamed as the Bank o... Bengal. Like the Bank of Bombay and the Bank of Madras, it was known as presidency bank and jointly owned b... the provincial government and private subscribers. On 27 January 1921 the presidency banks were merged to form... the Imperial Bank of India (IBI), which then became the largest commercial enterprise in the country. The Imperia... Bank of India became the State Bank of India on July 1, 1955 after Government of India took control of a maj... Reserve Bank of India (RBI) taking a 60 per cent stake in it. In 1959, the government passed the State Bank of Ind... (Subsidiary Banks) Act thus making the eight banks that had belonged to princely states subsidiaries of SBI. In 200... the government took over the stake held by the RBI. In April 2017, associates banks of SBI, including State Ban... of Bikaner and Jaipur (SBBJ), State Bank of Mysore (SBM), State Bank of Travancore (SBT), State Bank of Patia... (SBP), State Bank of Hyderabad (SBH) and Bharatiya Mahila Bank, merged with State Bank of India, making it... largest consolidation exercise in the banking history of India.

State Bank of India (SBI) is a state-owned commercial bank and financial services statutory body headquartered i... Mumbai. This bank now a day is a Fortune 500 company and an Indian Multinational company having 229 offic... in 31 foreign countries ("Fortune Global 500 list". Retrieved 16 January 2021 from Wikipedia).The rich heritage a... legacy of over 200 years, accredits SBI as the most trusted Bank by Indians through generations. SBI, enjoys a... 1/4th market share and serves over 45 crore customers through its vast network of over 22,000 branches, 63... ATMs/ADWMs, 71,968 BC outlets. The bank focuses on innovation and customer centricity. The core values of t... Bank include- Service, Transparency, Ethics, Politeness and Sustainability. The Bank has diversified business... through its various subsidiaries i.e. SBI General Insurance, SBI Life Insurance, SBI Mutual Fund, SBI Card, etc... has spread its presence globally and operates across time zones through Growing with times. (The Editors... Encyclopedia Britannica; Aug 22, 2022: https://www.britannica.com/topic/State-Bank-of-India).

Since nationalization, SBI has served the needs of Indian economic development through rural-developm... initiatives and microcredit programs and by financing major agricultural and industrial projects and raising loans... the government. SBI's loan products, including home, personal loans, car loans, debit and credit cards and ... cards, cater to all kinds of customers, claims the bank. The bank also deals in education loans, health insura... options, vehicle and home insurance; demat accounts, wealth management, precious metals and private banking. S... continues to redefine banking in India, as it aims to offer responsible and sustainable Banking solutions (Hind... Dhaun;- June 20, 2022)

Being one of the largest commercial and systemically important banks, SBI shares a greater responsibility and role as compared to other banks. The functions performed by SBI are divided into two categories- ordinary banking functions and central banking functions. These banking functions are further subdivided into many categories. SBI acts as an agent of RBI, where there are no branches of RBI to perform the following functions- maintenance of currency Government's Bank and Bank bankers. The SBI also acts as a clearinghouse for all commercial banks in places where RBI does not have its presence.

REVIEW OF LITERATURE

Bodla and Verma (2006) recommended that CAMEL rating of banks would help the Reserve Bank of India to identify the banks whose performance needs special supervisory attention. Under the above background, an attempt was made to use CAMEL model for examining the performance of public sector banks in India. The public sector is chosen logically as till now this sector enjoys more than 74% share in both total advances as well as deposits in India. More specifically, this study was aimed to evaluate the financial performance of leading Public Sector Banks using the CAMEL approach.

Sangwi and Nazir (2010) stated that sound financial health of a bank is the guarantee not only to its depositors but is equally significant for the shareholders, employees and whole economy as well. As a sequel to this maxim, efforts have been made from time to time, to measure the financial position of each bank and manage it efficiently and effectively. In this paper, an effort has been made to evaluate the financial performance of the two major banks operating in northern India . This evaluation has been done by using CAMEL Parameters, the latest model of financial analysis. Through this model, it is highlighted that the position of the banks under study is sound and satisfactory so far as their capital adequacy, asset quality, Management capability and liquidity is concerned.

Singh and Tandon (2012) conducted a study which was aimed to examine the financial performance of SBI and ICICI Bank, public sector and private sector respectively. The research was descriptive and analytical in nature. The data used for the study was entirely secondary in nature. The financial performance of SBI and ICICI Bank was evaluated on the basis of ratios such as credit deposit, net profit margin etc. The period of study taken was from the year 2007-08 to 2011-12. The study found that SBI is performing well and financially sound than ICICI Bank but in context of deposits and expenditure ICICI bank has better managing efficiency than SBI.

Kumar and Sharma (2013) examined the performance of top 10 banks in India having most noteworthy market capitalization with the assistance of camel model for the year 2006 to 2010. This study found that Kotak Mahindra Bank on the first rank and ICICI bank on second rank in so far as capital adequacy ratio is concerned. Both of these banks are proficient in overseeing and taking care of the liquidity. SBI had the highest level of NPA among all banks followed by ICICI bank.

Lakhtaria (2013) investigated the three public sector banks, namely Bank of Baroda, State Bank of India and Punjab National Bank utilizing camel model methodology. This study brought out that Bank of Baroda remained at the top followed by Punjab National Bank and State Bank of India.

Jaiswal and Jain (2016) analyzed the financial performance of SBI and ICICI banks. The State Bank of India, popularly known as SBI is one of the leading bank of public sector in India. SBI has 14 Local Head Offices and 57 Zonal Offices located at important cities throughout the country. ICICI bank is the second largest, leading bank of private sector in India The Bank has 2,533 branches and 6,800 ATMs in India. The study is descriptive and analytical in nature. The collected data was secondary in nature and collected from various reports issued by these banks through internet. The comparison of financial performance of these two banks was made on the basis of ratio analysis. The results indicated that the SBI is performing well and financially sound than ICICI Bank. Also the market position of SBI is better than ICICI in terms to earning per share, price ratio per share and dividend payout ratio, but on the

other hand ICICI bank is performing well in terms of NPA and provision for NPA in comparison of SBI bank.

Parvin and Rathra (2018) analyzed the performance of ICICI and SBI Bank utilizing the CAMEL model. The indicated that ICICI bank needs to improve its performance concerning capital adequacy ratio and assets quality while the SBI needs to concentrate on improving its performance with respect to productivity, assets quality and liquidity.

Amita and Bodla (2019) used CAMEL methodology for evaluating the performance of public sector banks in India in very recent period, i.e. 2014-15 to 2018-19. This study took a sample of top ten public sector banks selected on the basis of total assets and market capitalisation. These banks included Bank of Baroda, State Bank of India, Punjab National Bank, Union Bank of India, IDBI, UCO Bank, Syndicate Bank, Bank of India, Central Bank and Canara Bank. The data was collected from annual reports of these banks and various other secondary sources like www.moneycontrol.com. Various ratios were calculated measuring the aspects of CAMEL which includes capital adequacy, asset quality, management efficiency, earning quality and liquidity.

The coronavirus crisis has left some banks struggling to hang on to deposits, as funds migrate to the perceived safety of state-owned lenders. Besides other smaller private lenders, more prominent name among such banks is Indusind RBL Bank Ltd, and Yes Bank (Singh and Bodla). According to a report (March, 30, 2020) by Credit Suisse Group AG analysts led by Ashish Gupta, more than 25%, 35% and 45% of loan book is of most vulnerable sectors to the lockdown, such as small businesses and automobile finance in case of ICICI Bank, Axis Bank, and Indusind Bank Ltd respectively. Ability to withstand deposit shocks will be key for Private Banks for their survival in coming several months.

The analysis made in the paper authored by *Amita et al (2020)* describes the story of the way Yes Bank collapsed. The analysis has indicated that Yes Bank's loan grew from Rs 1,32,000 crore in FY 2017 to Rs 2,41,000 crore in FY 2019. That is an increase of 80% in just two years, when most banks were finding it difficult to lend. According to the authors understanding based on available reports of the experts, Yes Bank's unusually large loan disbursals were made to already stress corporate groups. These firms had enough clout to further game the system by using new loans to prevent old loans from being declared NPAs. In nutshell, loans given to undeserving firms with poor credit standing has remained the real cause of crisis faced by YES BANK. For this both the management of bank and supervisory body (i.e. RBI) seems culprits as they had powers to decide and regulate the operations of the bank.

PURPOSE OF THE STUDY AND RESEARCH METHODOLOGY

The pandemic posed an unprecedented challenge to banks in India and had an all-pervasive impact on its functioning. Fitch Ratings predicted increased risk for banks in the financial year FY2022 (rating, 2021). This study aims to examine the impact of the pandemic on various parameters of performance of SBI like Growth of Total assets and liabilities, Deposits, Credit, Capital Adequacy, Net Interest Income, NPA level, ROA, etc.

This paper has used descriptive research design. Descriptive research is a research method that describes the characteristics or features of the population or phenomenon that is being studied. The scope of this paper is limited to evaluation of the financial and profitability performance of the biggest public sector bank (i.e. SBI). The size is being measured in terms of the size of assets and business. The secondary data for the last five years, that is, from year 2017-18 to 2021-22 was used for the study. Data was taken from the annual reports of this bank and website named www.moneycontrol.com. To assess performance ratio analysis and statistical analysis of SBI's financial statements was made.

Banks take in deposits from consumers and businesses and pay interest on some of the accounts. In turn, banks use the deposits and either invest those funds in securities or lend to companies and to consumers. Since banks must

interest on their loans, their profits are derived from the spread between the rate they pay for the deposits and the rate they earn or receive from borrowers. Banks also earn interest income from investing their cash in short-term securities like Treasury bills. However, banks also earn revenue from fee income that they charge for their products and services that include wealth management advice, checking account fees, overdraft fees, ATM fees, interest and fees on credit cards.

In view of above discussion the parameters of performance taken for analysis include balance sheet items like deposits, advances, investments and the indicators like Capital adequacy, Asset quality, Management efficiency and Profitability. Year-on-year growth of select variables and the compound annual growth rates are calculated through the use of MS-Excel. The limitations of the study are: first, it is based on the secondary data and the limitation of using secondary data may affect the results; second, the secondary data was taken from the annual reports of the SBI and websites. It may be possible that the data shown in the annual reports and websites have errors which do not show the actual position of the banks.

RESULTS AND DISCUSSION

At the outset of analysis, the performance analysis of the SBI has been made on the basis of size of bank. According to Achcampong Prince et al (2014), the size of the bank has a positive impact on stock prices. The logical relationship is that the size of the bank in terms of the total assets controlled by the bank will be able to send a signal to investors and potential investors. Thus, the analysis of size of bank is meaningful. The size of the bank has been measured in terms of size of the deposits, advances, total business and the balance sheet size.

Table 1 indicates a compound annual growth rate of 10 per cent in the total assets and liabilities separately during 2018 – 2022. The balance sheet total increased from Rs 34548 billion to Rs 49876 billion in this duration of five years. The year-on-year growth of balance sheet after the Corona pandemic (i.e., during 2021 & 2022) remained significantly higher than before the start of the pandemic. This may be attributed to RBI's and India government initiatives to protect the economy from ill effects of the pandemic.

Table 1 further show that Cash & Bank Bal. with RBI has registered 28 percent and 21 percent growth rate during 2021 and 2022 respectively. The above phenomenon may be attributed to increased saving by people during pandemic. Balances with Banks and Call Money have increased by 85 per cent in 2020 and 54 per cent in year 2021. The investments grew by 29 per cent and 10 per cent during 2021 and 2022 respectively.

Loans and advances are the bread and butter for most banks and are usually the largest asset on the balance sheet. Investors monitor loan growth to determine whether a bank is increasing their loans and putting to use the bank's deposits to earn a favorable yield. SBI has Rs 19349 billion in loans in 2018 which went up to 27346 in 2022 and thus registered a CAGR of 9 per cent during the study period. However, the impact of covid-19 pandemic is clearly visible from table 1 as the growth of advances came down to 6 percent in 2020 and 5 per cent in 2021 from 13 percent in 2019. While the fixed assets of SBI indicated a negative (i.e., -1%) CAGR during last five years, the 'other assets' of the bank have registered a robust CAGR (11%) in the same period.

Deposits are the largest liability for the bank and include saving account, fixed deposits, and recurring deposit accounts. Although deposits fall under liabilities, they are critical to the bank's ability to lend. If a bank doesn't have enough deposits, slower loan growth might result, or the bank might have to take on debt to meet loan demand which would be far more costly to service than the interest paid on deposits.

A notable development in Indian banking system following the onset of the COVID-19 pandemic has been the rapid and sustained growth in aggregate bank deposits. Deposits have shown a sluggish growth from FY 2017-18. But banks have recorded the highest growth rate during the pandemic– FY 2020-21. (Naveen Chaudhary (2022). It is

obvious from table 1 that the deposits of SBI grew by 14 per cent in 2021 which is the highest growth during ... five years. Borrowings of this bank, on the other hand, declined by 33 percent in 2020 but increased by 22 per ... in 2021.

SBI's trend of deposits during pandemic resembles to that of US, where following the onset of the COVID ... pandemic in 2020, the deposits-to-GDP ratio jumped to over 75 percent and has remained near that level during ... pandemic period to date. (Quoted in Andrew Castro et al June 03, 2022).

Table 1: Year-on-Year and Compound Growth of Assets and Liabilities (Rs. in Bn.)

Particulars	FY 2018	FY 2019	YOY (%)	FY 2020	YOY (%)	FY 2021	YOY (%)	FY 2022	...
Capital & Liabilities									
Capital	9	9	0	9	0	9	0	9	...
Reserves & Surplus	2182	2200	1	2311	5	2530	9	2792	...
Deposits	27063	29114	8	32416	11	36813	14	40515	...
Borrowings	3621	4030	11	3147	-22	4173	33	4260	...
Other Liabilities & Provisions	1671	1456	-13	1631	12	1820	12	2299	...
TOTAL LIABILITIES	34548	36809	7	39514	7	45344	15	49876	...
Assets									
Cash & Bank Bal. with RBI	1504	1769	18	1667	-6	2132	28	2579	...
Balances with banks & call money	415	456	10	844	85	1298	54	1367	...
Investments	10610	9670	-9	10470	8	13517	29	14814	...
Advances	19349	21859	13	23253	6	24495	5	27340	...
Fixed Assets	400	392	-2	384	-2	384	0	377	...
Other Assets	2270	2663	17	2896	9	3518	21	3399	...
TOTAL ASSETS	34548	36809	7	39514	7	45344	15	49876	...

Primarily the function of a bank is managing the spread between the interest that it pays consumers and the ... it receives from their loans. In other words, when the interest that a bank earns from loans is greater than the ... it pays on deposits, it generates income from the interest rate spread. The size of this spread is a major determ... of the profit generated by a bank. Table 2 presents both absolute values and growth rates of interest income, ... expenses, operating income and expenses, staff expenses and overhead expenses of SBI during the period ... 2022. Interest income has increased from Rs 2205 bn. in 2018 to Rs 2755 bn. in 2021-22. The interest ... increased at a CAGR of 6 per cent during this period of five years. In contrast, the interest expenses ... CAGR of 2 per cent only in the corresponding period. This may be due to decrease in interest rate charged by ... because of lower repo and reverse repo rates during COVID-19 Pandemic.

Net interest income (NII) is an important indicator in evaluating banks because it reveals a bank's net profit ... interest-earning assets, such as loans or investment securities. The net interest income raised to Rs 1207 bn. in ... from Rs 749 bn. in year 2018, registering a CAGR of 13 per cent. However, the non-interest income of SBI was ... in reverse gear as it indicated a negative 2 per cent CAGR during this period. The staff expenses and overh... expenses of this bank have increase at a CAGR of 11 per cent and 8 per cent respectively during the study p... The net profit of SBI has grown at a phenomenal of above 40 per cent in each of the last four years.

Table 2: Growth of Income and Expenses of State Bank of India (2018-2022) (Rs. in Bn.)

Particulars	FY 2018	FY 2019	YOY (%)	FY 2020	YOY (%)	FY 2021	YOY (%)	FY 2022	YOY (%)	CAGR (%)
Int. Income	2,205	2,429	10	2,573	6	2,652	3	2,755	4	6
Int. Expenses	1,456	1,543	6	1,592	3	1,544	-3	1,548	0	2
Net Int. Income	749	885	18	981	11	1,107	13	1,207	9	13
Non-Interest Income (NII)	446	368	-18	452	23	435	-4	406	-7	-2
Total Op. Income	1,195	1,251	5	1,433	15	1,542	8	1,613	5	8
Staff Expenses	332	411	24	457	11	509	11	501	-2	11
Overhead Expenses	268	286	7	295	3	317	8	358	13	8
Total Op. Expenses	599	697	16	752	8	827	10	860	4	9
Operating Profit	595	554	-7	681	23	716	5	753	5	6
Net Profit	-65	9	113	145	1581	204	41	317*	55	232

* After accounting for provision of Rs. 7,418 crores on account of change in family pension rules

Capital adequacy is a financial performance that assesses a bank's ability to guarantee its activities with sufficient capital. The ratio to determine capital adequacy used in this study is the Capital Adequacy Ratio (CAR). Banks that have good capital adequacy will increase investor confidence to invest so that stock prices increase. The return-on-assets (ROA) and return-on-equity (ROE) ratios are important profitability ratios, indicating the rate of profit a company earns on its assets and equity capital respectively. Table 3 shows an upward trend in case of both of these profitability indicators in case of SBI from the period 2018 to 2022. Before onset of the covid in 2020, the position of the bank in terms of both of these indicators was bad which has improved a lot during and after it. To be more precise, ROE of the bank has turns out 13.92 per cent in year 2022 which is almost double to that of the year 2020. The above pattern is also confirmed by earning per share (EPS) which has increased to Rs 35.49 in 2022 from the loss of Rs 7.67 per share in 2018. However, the cost to income ratio of the bank remained range bound between 50 per cent and 55 per cent in this duration of five years.

Table 3: Profitability, Capital Adequacy, Management efficiency & Asset quality indicators of SBI during 2018-22

Major Performance Indicators	FY 2018	FY 2019	FY 2020	FY 2021	FY 2022
ROA (%)	-0.19	0.02	0.38	0.48	0.67
ROE (%)	-3.78	0.48	7.74	9.94	13.92
EPS (Rs.)	-7.67	0.97	16.23	22.87	35.49
Cost/ Income Ratio (%)	50.18	55.70	52.46	53.60	53.31
Capital Adequacy Ratio (Basel 3) (%)	12.60	12.72	13.06	13.74	13.83
Cost of Deposits (%)	5.30	5.10	4.94	4.20	3.83
Yield on Advances (%)	8.28	8.49	8.72	7.97	7.58
Yield on Resources Deployed (%)	7.31	7.35	7.19	6.32	6.07
Net Interest Margin (%)	2.50	2.78	2.97	3.04	3.12
Gross NPA Ratio (%)	10.91	7.53	6.15	4.98	3.97
Net NPA Ratio (%)	5.73	3.01	2.23	1.50	1.02
Op. Profit/Total Assets (%)	-0.17	-0.5	-0.77	-0.93	-1.48
Op. Expenses/Total Assets (%)	1.87	1.82	1.9	1.89	1.73

The capital adequacy ratio (CAR) is a measure of how much capital a bank has available, reported as a percentage

of a bank's risk-weighted credit exposures. The purpose is to establish that banks have enough capital on reserve to handle a certain amount of losses, before being at risk for becoming insolvent. The banks are required to maintain a capital adequacy ratio of 9 per cent as prescribed by RBI. As per table 3, the CAR of SBI has remained 13 per cent approximately during the reference period for this paper, meaning thereby the bank has succeeded to keep a sound capital adequacy ratio.

Non-performing Assets (NPA is another key indicator of a bank's performance which measures the asset quality. Reserve Bank of India defines NPA as any advance or loan that is overdue for more than 90 days. "An asset becomes non-performing when it ceases to generate income for the bank," said RBI in a circular form 2007. Gross non-performing assets of SBI has reduced to 3.97 per cent in 2022 as against to 10.91 per cent in year 2018. Similar reduction can be seen in so far as net on-performing assets are concerned as they came down to 1.02 per cent from 5.73 percent in the corresponding period.

Operating cost to asset ratio is an important efficiency ratio for the fact that we are able to track the changes in operating cost with respect to changes in asset and also an indicator of the bank's business mix. Table 3 shows that the operating expenses to total assets ratio has been hovering between 1.82 per cent and 1.91 per cent from 2018 to 2021, however it came down to 1.73 per cent in year 2022.

Table 4: Market related ratios of SBI

Market Related Ratios	FY 2018	FY 2019	FY 2020	FY 2021	FY 2022
Share Price in Rs (as on last day of the year) & Year-on-year growth (%)	249.9	320.75	196.85	364.3	493.55
		-28.35	(-38.63)	-85.06	-35.48
Price to Book Ratio (%)	1.26	1.6	0.9	1.51	1.83
Market Capitalization (Rs. In Cr) & Year-on-year growth (%)	2,23,046	2,86,257	1,75,681	3,25,123	4,40,474
		-28	(-39)	-85	-35
Earnings Per Share (Rs.)	-7.67	0.97	16.23	22.87	35.49
P/E Ratio (%)	-32.58	330.67	12.13	15.93	13.91

In the end, the performance of SBI was evaluated from shareholders' point of view by taking some market related parameters like growth in share price, price to book ratio, size and growth of market capitalization, earnings per share and P/E ratio. The results related to above parameters are given table 4. It is obvious from this table that Covid-19 Pandemic exerted alarming effect on valuation of share of SBI as the same declined to Rs 196.85 in the FY 2020 from Rs 320.75 (i.e., decline of 62.94%) a year ago. However, a V-shape recovery began in India's stock market as the Government of India started initiating policy measures to protect the economy and the people from ill effects of Corona virus. This recovery was also seen in case of share price of SBI which surged by 85 per cent and 35 per cent during FY 2021 and FY2022 respectively. EPS of this bank, in contrast, indicated a rising trend meaning thereby the bank was able to improve its profit during the Corona virus. Beside others, the good economic and policy initiatives of the government were the driving force behind the good profitability.

CONCLUSION

This study has offered the following main findings:

- The total assets and liabilities of the SBI increased at a CAGR of 10 per cent during 2018 – 2022. The year-on-year growth of the assets remained significantly higher after the onset of Corona pandemic (March 2020) than before the start of the pandemic. This may be attributed to the operational and policy measures initiated by RBI and Indian government to protect the economy from ill effects of the pandemic.

- SBI has Rs 19349 billion in loans in 2018 which went up to 27340 in 2022 and thus registered a CAGR of 9 per cent during the study period. However, the impact of covid-19 pandemic was clearly visible as the growth of advances came down to 6 percent in 2020 and 5 per cent in 2021 from 13 percent in 2019. In contrast to loans, the deposits received by SBI which had shown a sluggish growth in FY 2017-18 recorded the highest growth rate during the pandemic (FY 2020-21). The deposits of SBI grew by 14 per cent in 2021 which is the highest growth during last five years. The above trend conforms to that observed in his study by Naveen Chaudhary (2022).

- The interest income of the SBI has increased at a CAGR of 6 per cent during the study period. In contrast, the interest expenses indicated a CAGR of 2 per cent only in the corresponding period. This may be due to decrease in interest rate charged by banks because of lower repo and reverse repo rates. The net interest income (NII) increased at CAGR of 11 per cent. However the non-interest income of SBI was seen in reverse gear as it indicated a negative 2 per cent CAGR during this period.

- Gross non-performing assets of SBI has reduced to 3.97 per cent in 2022 as against to 10.91 per cent in year 2018. Similar reduction can be seen in so far as net on-performing assets are concerned as they came down to 1.02 per cent from 5.73 percent in the corresponding period.

- Covid-19 Pandemic exerted alarming effect on valuation of SBI as its share price fell around 63 per cent in FY 2020 as compared to FY 2019. However, a V-shape recovery began in India's stock market in May 2020 immediately after the Government of India started initiating policy measures to protect the economy and the people from ill effects of lock-down which was imposed to prevent the spread of Corona virus. This recovery was also seen in case of share price of SBI which surged by 85 per cent and 35 per cent during FY 2021 and FY2022 respectively.

From the above findings, it may be concluded that the Lockdown which was imposed to spread the corona virus resulted in a very severe effect on the loan book and value of shares of SBI as both of these declined considerably. Fortunately, the situation started coming under control as a result of timely corrective action taken by India's Government, Reserve Bank of India and the governing board of the bank. However, worry is not over till now because of inflationary trend throughout the world. RBI has taken several policy measures to control inflation. Based on the present state of India's economy and the continuous efforts made by the banking regulator let us hope the inflation would come down.

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CHARACTERIZATION AND APPLICATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES DERIVED FROM LEAF AND CALLUS OF *Viola canescens* WALL. ex ROXB.

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Abstract. Green production of nanoparticles using plant extracts is an intriguing field of study that has the potential to serve as an alternative to the hazardous chemical synthesis processes. In this study, silver nanoparticles (AgNPs) were prepared utilizing aqueous extracts of the mature leaves (PAgNPs) and seedling leaf derived callus (CAgNPs) of *Viola canescens* Wall. ex Roxb. Callus growth was obtained on Murashige and Skoog (MS) medium supplemented with 2,4-D (2.0 mg/L) and BAP (0.5 mg/L) was harvested and used for biogenic synthesis of AgNPs. The UV–visible spectra of leaf and callus mediated AgNPs revealed the SPR absorption band at 424.8 nm and 437 nm, respectively. The biochemical interaction and crystalline nature of the AgNPs were evaluated by Fourier transformation infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis. The surface morphology and composition of both the samples were confirmed by HRTEM and EDS analyses. The average particle size as calculated from HR-TEM histogram study of both CAgNPs and PAgNPs was found 9.15 nm and 13.9 nm, respectively. The synthesized AgNPs showed significant antibacterial activity against *Bacillus cereus, Bacillus subtilis* and *Escherichia coli*. The inhibition zone of both gram-negative and gram-positive bacteria reflected the broad spectrum antibacterial properties of AgNPs. Callus mediated AgNPs showed better antibacterial results with 30±0.90 mm inhibition zone. The protocol of antibacterial potential of silver nanoparticles generated from *V. canescens* plant and callus extracts is a very important aspect in technological point of view in having applications in the biomedical field and deserves to be recognized.

Keywords: antimicrobial activity; callus; FTIR; silver nanoparticles; TEM; *Viola canescens*; XRD.

INTRODUCTION

Viola canescens Wall. ex Roxb. (family: Violaceae) commonly known as Himalayan White Violet, is a prostrate, hairy perennial herb found mostly at an altitude of 2000 meters all the way through temperate Himalayan regions of Pakistan, India, Bhutan, and Nepal. It is used to medicate various conditions such as bronchitis, cold, cough, flu, cancer of the throat, respiratory tract problems, eczema, epilepsy, gastric acidity, pyrexia, dysentery, rheumatism etc [29, 31]. Qualitative testing of ethanolic and methanolic extract of this plant revealed the presence of different phytochemicals like alkaloids (violin), quercitrin, methyl salicylate, saponins, phenols, flavonoids, carbohydrates, tannins and triterpenes [9, 38]. Plant also exhibits antimicrobial and antispasmodic activity, antifungal activity, hepatoprotective activity and properties like antioxidant, analgesic, diaphoretic, carminative and aphrodisiac [25, 35, 36].

In recent past, interest in plant parts and plant based products of Ayurveda has led a sharp jam in bulk requirement and resulting in illegal and large scale extraction of medicinal and aromatic plants and this causes the reduction in plant diversity [44, 45]. Owing to its far-ranging use, this plant was over uprooted from wild threatening to its extinction. Various factors like extensive grazing, overexploitation, deforestation, effect of invasive plants, change in environmental conditions, and attack of pathogens are liable for making the conservation status of *V. canescens* as endangered in different regions [20, 29]. Thus, micropropagation is an efficient method for mass multiplication.

The modern biotechnological techniques have resulted in the establishment of economically beneficial green industries. Nanoscience deals with the structural and functional aspects of matter in atomic and molecular scale [24, 47]. In these dimensions, the characteristic physio-biochemical properties of material often change dramatically with high surface to volume ratio. It has dramatically revolutionized the interdisciplinary research by generating importance in various fields of material science, optics, mechanics, electronics, biotechnology, agriculture and the pharmaceuticals industry [1, 17].

Silver is a precious noble metal because of their usefulness in antimicrobial applications, biosensors, cosmetic fibres etc [10]. AgNPs preparation by green synthesis approaches have advantages over conventional methods involving physical and chemical synthesis by being environmental friendly, economical and need of fewer instruments [28]. The advantage of plant and plant derived extracts for green synthesis of AgNPs is that they can be easily available, easily cultured, safe and non-toxic. Moreover, plant extract contains a broad spectrum of metabolites such as enzymes, proteins, amino acids, polysaccharides, vitamins, organic compounds etc. resulting as natural reducing, capping and stabilizing agents to prepare nanoparticles without the use of any hazardous, toxic and expensive chemical substances [2]. Thus, AgNPs have emerged as a potential solution to fight against the diverse pathogenic microorganisms.

Various parts of the *Viola sp.* are used to develop antibacterial medications based on medicinal plant extracts. In nanomedicine, antimicrobial properties of AgNPs are among the most promising materials, now under investigation. Silver nanoparticles has the ability to interact with the cell wall of microorganisms, producing ROS (reactive oxygen species) that eventually kill the cell. As a result, we can hypothesize that combining both, i.e. *V. canescens* extract and AgNPs, can enhance their antibacterial properties.

To date, only a few investigations have been published on the antibacterial activity of callus mediated green synthesized AgNPs [22, 32, 43]. Khajuria et al. [23] also reported the callus mediated synthesis of ZnO nanoparticles (NPs) from *V. canescens* but the production of various other metallic NPs such as silver and, gold using various plant extracts of *V. canescens* are still required to develop an efficient and healthy green route for controlling the various endemic diseases with less adverse effect. So, the present study was undertaken with an objective to synthesize and characterize biogenic AgNPs from leaf and leaf derived callus of *V. canescens*, and to test them for antibacterial activity against *Escherichia coli*, *Bacillus cereus*, and *Bacillus subtilis*.

MATERIALS AND METHODS

Callus induction

Healthy seeds of *Viola canescens* were collected from Morni hills, Panchkula, Haryana, India. Selected seeds were surface sterilized using different concentration of mercuric cloride ($HgCl_2$) and germinated on Murashige and Skoog (MS) medium supplemented with 0.5 mg/L GA_3 [39]. The MS media containing 2,4-D (2.0 mg/L) and BAP (0.5 mg/L) was used for callus initiation from seedling leaf explants of *Viola canescens* (Fig. 1). The MS basal medium consisted of 3% sucrose, 0.8% agar. The pH of the medium was adjusted to 5.8. The cultures were incubated at 25±2 °C with 55–65% relative humidity under a 16-h light/8-h dark cycle at 27 μM $m^{-2}s^{-1}$ PAR light intensity from 6500 K colour temperature white fluorescent tubes. The best *in vitro* regenerated mature callus considered to be rich in primary and secondary metabolites was excised aseptically and used for biogenic synthesis of AgNPs.

Preparation of aqueous extract

Fresh biomass of callus and leaves were collected and dried in hot air oven at 40° C for 48 hr. Five grams of air dried biological material was immersed in 100 mL of deionized water and kept on magnetic stirrer at 60°C for 6 hr in a dark room before being centrifuged at 5500 r/min for 10 min. This aqueous solution was then filtered with the help of Whatman's Filter paper no. 1. The filtrate was stored at 4° C for further experiment.

Biosynthesis of AgNPs

The reaction mixture was prepared by adding 10 mL of the aqueous callus/leaf extract and 90 mL of 1 mM silver nitrate ($AgNO_3$) in a 250 mL flask and incubated in a dark place at room temperature for about 48 h. Visual examination was carried out by observing change in color of the solution. When the plant and callus extracts were heated and kept in the dark, their colors turn rusty brown and dark brown, indicating the production of AgNPs (via reduction reaction i.e., Ag^+ reduced to Ag^0 nanoparticles). Both plant leaf mediated silver nanoparticles (PAgNPs) and callus mediated silver nanoparticles (CAgNPs) were subjected to spectrophotometry analysis.

Ultraviolet-visible (UV-vis) spectra analysis

The synthesis and characterization of AgNPs of leaf and callus extracts were confirmed by using a double beam UV- Visible spectrophotometer (Model- 2202, Systronics, India). The spectra were recorded between 200 and 800 nm after 48 hrs for studying the optical property of AgNPs. After the biosynthesis, the reduced solution containing the AgNPs was separated by centrifugation at 12,000 rpm for 10 min at room temperature. Supernants were discarded and AgNPs were redispersed in distilled water and purified by repeated centrifugation for five times. The pellet obtained was air-dried in hot air oven to evaporate excess liquid; and was used for further characterization.

X-ray Diffraction spectroscopy

The silver nanoparticles derived from different extracts were examined by XRD measurements. The crystalline structure of synthesized AgNPs was investigated. The silver nanoparticles were centrifuged at 12,000 rpm for 20 minutes and the pellets were re-dispersed in ethanol and centrifuged again for 10 minutes to get rid of any unwanted entities. The centrifugation and re-dispersion process in ethanol was carried out three times for better removal of impurities from silver nanoparticles. After purification, the pellets were dried at 60°C in an oven. Afterward, the crystalline metallic Ag was examined by PANalytical X'Pert Pro –PW 3040/60 X-ray Diffractometer (45 kV,

Figure 1. *In vitro* regenerated mature callus from leaf explants of *V. canescens* on MS media aliquoted with 2,4-D (1.5 mg/L) + BAP (0.5 mg/L)

40 mA) with Cu-Kα radiation in scattering range m(2θ) of 30–80°. The particle size of synthesized NPs was calculated using Scherrer's equation, which is as follows: $D = \frac{K\lambda}{\beta \cos\theta}$

where:

D = mean crystal size;
K= 0.94 (Scherrer's constant);
λ = 1.5406 Å (X-ray wavelength);
β = X-ray diffraction broadening, in radians;
θ = Bragg's peak angle (2θ).

High Resolution Transmission Electron Microscopy (HR-TEM) and Energy Dispersive X-ray Spectroscopy (EDX)

The morphology, structure, and composition of silver nanoparticles using high resolution-transmission electron microscopy using HR-TEM (JEM-2100 Plus electron microscope, JEOL Ltd.), with 200 kV accelerating voltage and EDX at Sophisticated Analytical Instrumentation Facility, CIL and UCIM, Punjab University, India.

Dynamic light scattering (DLS) and Zeta Potential analysis

The particle mean size and size distribution of AgNPs were measured at 25°C using Particle Size Analyzer by dynamic light scattering (DLS). The dried AgNPs were dissolved in double distilled water and loaded in quartz curvet for analysis using a particle size analyzer. The standard solutions were first run to known the size distribution, the detectors record the energy scattered, absorbed at particular angle and scattering patterns. Then, the values of samples were compared with these standard values. For this, Microtrac Nanotrac Wave Particle Size, Zeta Potential analyzer was used to evaluate the biologically produced solutions.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)

AgNPs prepared with dried callus extract were subjected to FTIR spectroscopy by fourier transform infra-red spectrophotometer (Perkin Elmer, Model RZX) in the range of 4000-450 cm^{-1}. The solid powder sample of AgNPs was crushed, mixed with potassium bromide (KBr) in the ratio of 2:98 by weight and subjected to hydrolic pressure of about 1.5 bar for few seconds to make a disc. The spectrum was collected with eight scans co-added at 4 cm^{-1}. The abscissa range of the instrument is 4000 to 400 cm^{-1}. The detector was purged carefully using clean nitrogen gas to increase the signal level and reduce moisture. The sample discs were then introduced into the instrument and the spectrum was recorded.

Antibacterial Assay

The antibacterial activity of the synthesized AgNPs was determined by using the agar well diffusion method. Stock cultures of all the gram-positive (*Bacillus cereus* MTCC 430), *B. subtilis* MTCC 441) and gram-negative (*Escherichia coli* MTCC 1885) bacteria were maintained at 4°C and transferred to Mueller-Hinton broth and incubated for 24 h at 37°C for the preparation of fresh active cultures. The bacterial strains were obtained from the Department of Biotechnology, Kurukshetra University, Haryana (India). The inoculum density was maintained at 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL). Freshly prepared solidified Mueller-Hinton agar plates were inoculated by spreading 100 µl of bacterial inoculum and 6 mm diameter wells were made by using sterilized cork borer. A total of 100 µg of samples (leaf extract (PE) and silver nanoparticles) were sonicated in 100 µL of dimethyl sulfoxide (DMSO) and poured in the well. Plates were allowed to settle for 1 hr at room temperature for the diffusion of samples and then incubated for 24 hrs at 37°C [33]. DMSO and Ciprofloxacin were served as negative and positive control respectively.

RESULTS

Synthesis of AgNPs

The synthesis of AgNPs showed change in colour from light brown to dark brown colour in aqueous $AgNO_3$ solution due to excitation of surface plasmon vibrations phenomena in these nanoparticles thereby initially confirming the bioreduction of 1 mM $AgNO_3$ solution. When the combinations were heated and incubated at ambient temperature for 24 hr, the reduction process was complete; no additional color change was detected. Leaf extract had a stronger color intensity than callus extract. In control trails, no brown color was observed in the absence of extracts, thereby showing that change in color is associated with the presence of phyto-extracts.

UV-visible spectrum analysis

The silver metal has free electrons which produce surface plasmon resonance (SPR) absorption band because of the combined vibration of electrons of metal nanoparticles in resonance with light waves. The nanoparticles were ultrasonically dispersed in distilled water for absorbance measurements between 200 nm – 800 nm. The peak of nanoparticles produced by leaf and callus extracts, which were assigned to the SPR of AgNPs, was measured at wavelengths of 424.8 nm and 437 nm, respectively (Fig. 2a,b).

XRD analysis

The XRD pattern of the leaf and callus extracts derived AgNPs showed well defined diffraction peaks. The typical diffraction peaks of leaf extract mediated AgNPs lied at 2θ = 38.07, 43.96, 63.78 and 76.96, while callus mediated AgNPs peaks lied at 8.19, 44.28, 64.52 and 77.46. According to the results of the XRD spectrum, the produced silver nanoparticles were found to be crystalline in nature. The peaks were ascribed to silver face-centered cubic (fcc) and crystalline reflection planes (111), (200), (220) and (311) respectively (JCPDS no. 04-0783). XRD examination revealed the existence of high peaks, indicating the presence of active Ag content, as confirmed by indexing (Fig. 3a,b). The mean diameter of synthesized AgNPs using plant and callus was estimated as 13.67

nm and 10.39 nm, respectively, which corresponded to the nanoparticle size measured by HRTEM analysis (Table 1).

HR-TEM analysis of synthesized AgNPs

The HR-TEM provided additional information regarding the size and morphological characteristics of the silver nanoparticles *viz*, spherical in shape, well dispersed, and there was no evidence of aggregation (Fig. 4a-d). The mean particle size of CAgNPs and PAgNPs, as determined by HR-TEM image obtained, was found to be 9.15 nm and 13.9 nm, respectively. The particle size histograms of PAgNPs and CAgNPs indicated that the maximum NPs are present in the range between 4 nm to 18 nm and 6 nm to 9 nm, respectively (Fig. 4e-f). These results are consistent with the SPR vibrations geometry (Fig. 2).

Energy dispersive X-ray (EDX) analysis of synthesized AgNPs

The elemental composition of AgNPs was detected by EDX analysis [7]. The highest proportional of Ag signals revealed qualitative and quantitative determination of silver (Fig. 5). A few peaks of copper (Cu), carbon and other elements were also found, confirming the presence of *V. canescens* biomolecules on the surface of produced silver nanoparticles. Consequently, the copper peak was used to coat the sample.

DLS and Zeta Potential analysis

The particle size of AgNPs synthesized by leaf extract varied between 150-250 nm (Fig. 6). Plant leaf extract produced silver nanoparticles with mean size of 210 nm. The synthesized particles possess a charge of -39.86 mV. Polydispersity Index (PDI) for AgNPs was below 0.282. The size of AgNPs synthesized by using callus extract was found to vary between 130-200 nm. Mean size of CAgNPs was around 180 nm.

FTIR analysis

FTIR provides molecular fingerprint for the identification organic materials and helps us in the confirmation of numerous functional groups of biomolecules involved in the capping, production, and stability of AgNPs. FT-IR analysis of *V. canescens* leaf extract-AgNPs and callus extract-AgNPs, revealed various bands of absorption spanning from 3460 - 613 cm^{-1}, indicating the existence of functional groups. The absorption spectra of AgNPs produced from leaf extract (Fig. 7a) exhibits peaks at 3458 cm^{-1}, 2062 cm^{-1}, 1634 cm^{-1}, 1390 cm^{-1}, 1111cm^{-1} and 618 cm^{-1}. The peaks of CAgNPs (Fig. 7b) are visible at 3460 cm^{-1}, 2050 cm^{-1}, 1630 cm^{-1}, 1389 cm^{-1}, 1252 cm^{-1}, 1074 cm^{-1} and 613 cm^{-1}.

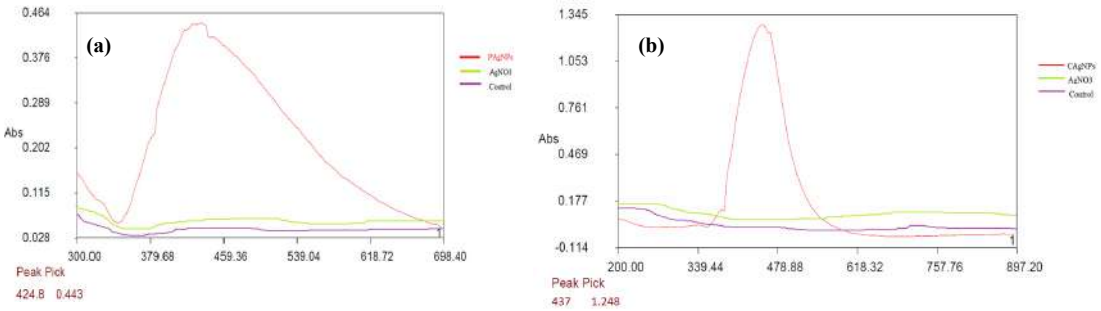

Figure 2. UV-Visible spectral analysis of AgNPs synthesized by using a) leaf extracts (PAgNPs), b) callus extracts (CAgNPs) of *V. canescens*.

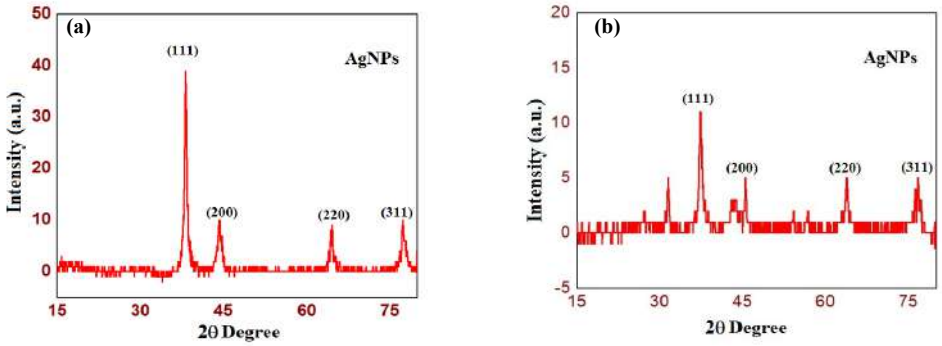

Figure 3. XRD analysis of green synthesized AgNPs using a) Leaf extract, and b) Callus extract.

Table 1. Size of biologically synthesized AgNPs calculated by Debye-Scherrer's equation

PAgNPs			CAgNPs		
Position (2θ)	**FWHM**	**Size (nm)**	**Position (2θ)**	**FWHM**	**Size (nm)**
38.07	0.576	14.56	38.19	0.665	12.64
43.96	1.152	7.42	44.28	1.052	8.15
63.78	0.48	19.49	64.52	0.800	11.74
76.96	0.768	13.21	77.46	1.127	9.03

*FWHM - Full Width at Half Maximum

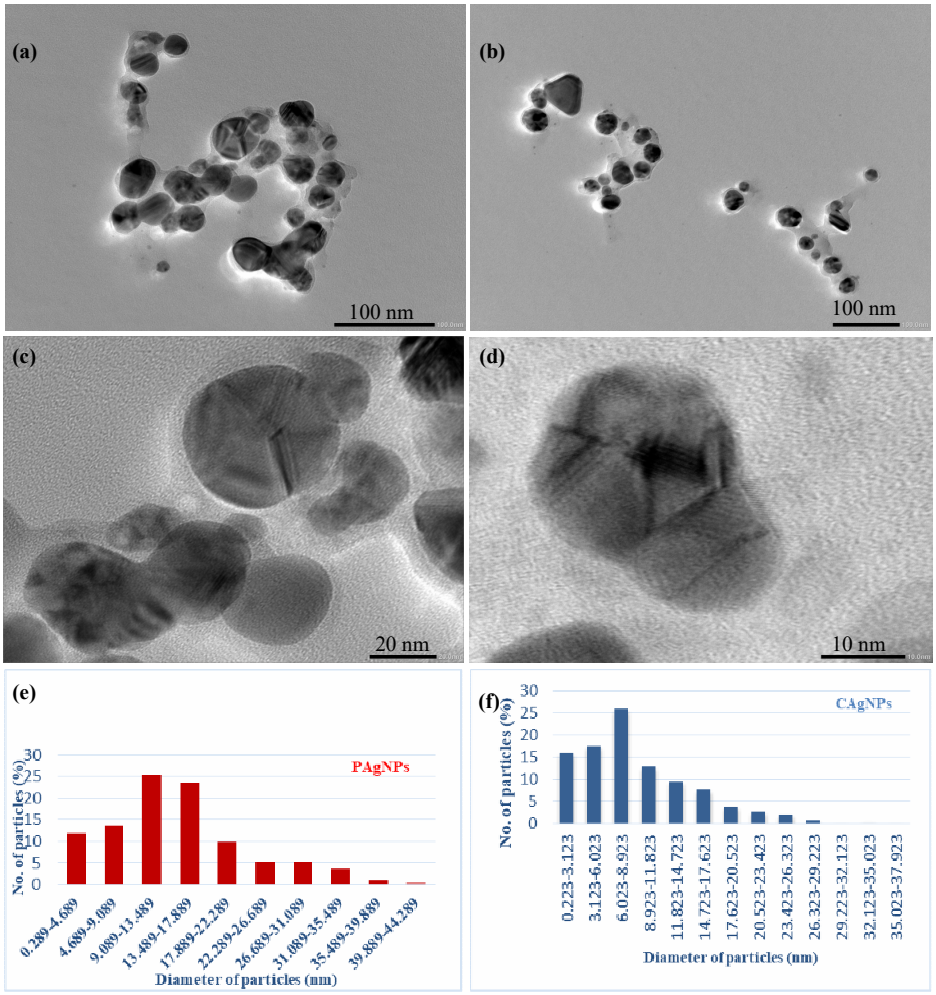

Figure 4. HR-TEM images showing different magnifications of AgNPs (a-d); and histogram of PAgNPs (e) and CAgNPs (f).

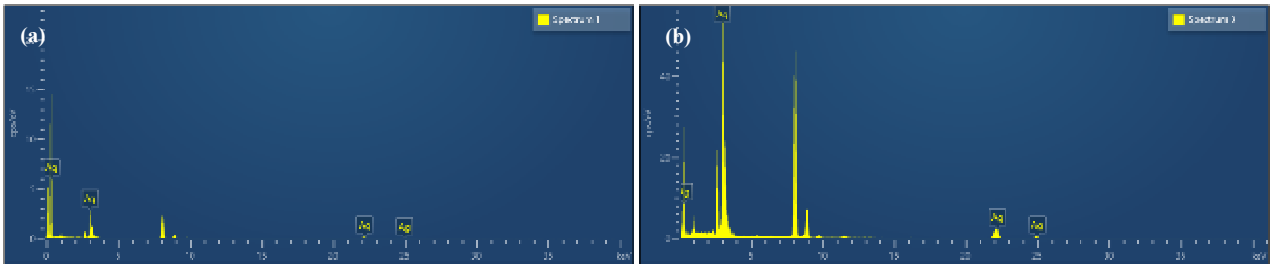

Figure 5. EDX spectroscopy of AgNPs synthesized using (a) leaf extract; (b) callus extract

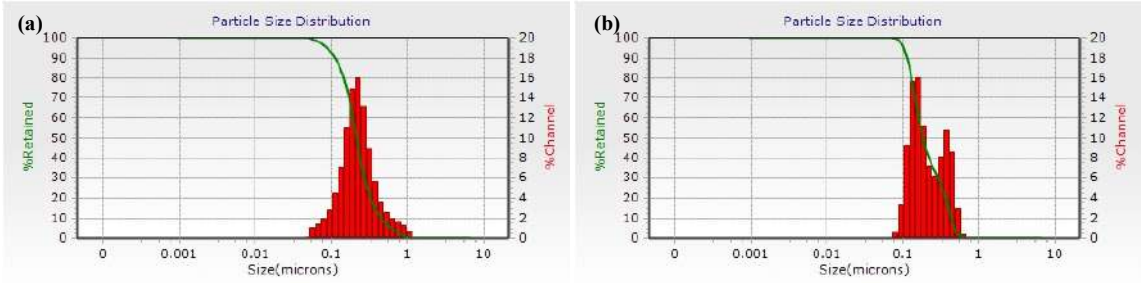

Figure 6. PSA analysis of biologically synthesized AgNPs by: (a) Leaf extracts (b) Callus extract

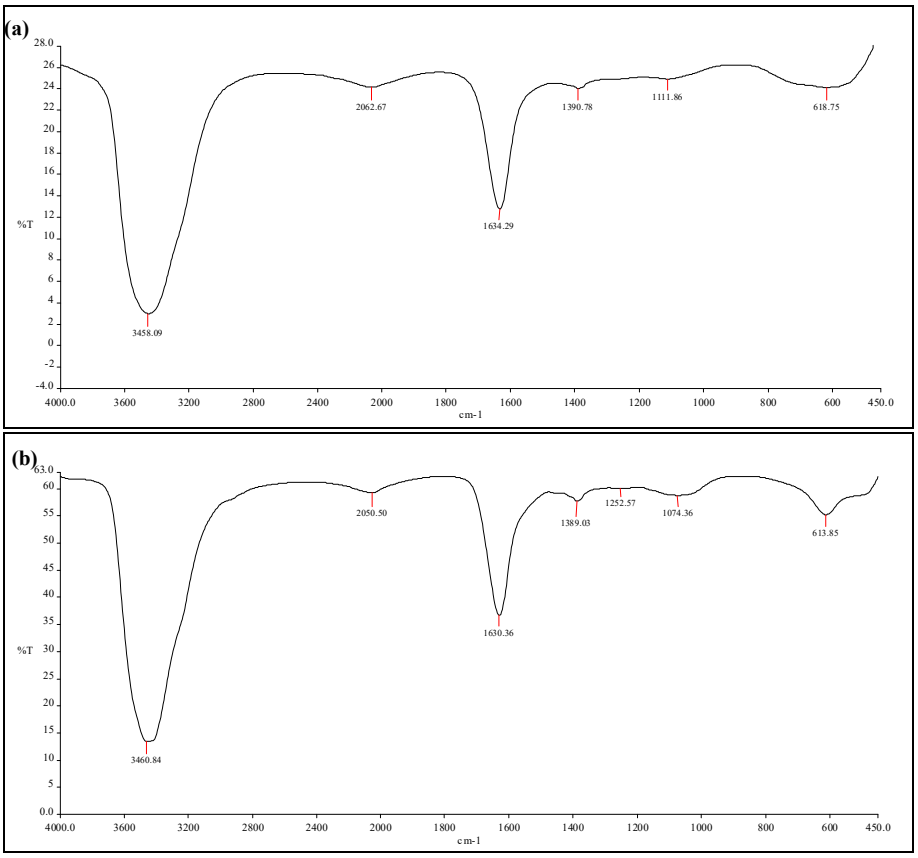

Figure 7. (a) FT-IR profile of Silver nanoparticles prepared from leaf extract (b) FT-IR profile of Silver nanoparticles prepared from callus extract.

Antimicrobial activity of AgNPs

After 24 hours of incubation, the leaf extracts, AgNPs and positive control (ciprofloxain) effectively inhibited bacterial growth over DMSO (Table 2). PAgNPs demonstrated strong antibacterial activity against gram-positive bacteria. Leaf extracts mediated AgNPs showed the highest ZOI of 27.7±1.00 mm towards *B. subtilis*, while a lower degree of ZOI was found gradually in *B. cereus* (27.3±0.45 mm) as gram-positive bacteria and *E. coli* (25±0.80 mm) as gram-negative bacteria (Table 2 and Fig. 8). In the case of callus generated silver nanoparticles, the greatest ZOI was reported in *B. cereus* (30±0.90 mm) followed by *B. subtilis* (27±0.76 mm) and *E. coli* (26.9±1.2 mm). In all the tested microorganisms, the ZOI of the callus mediated AgNPs found higher than that of leaf extract-mediated AgNPs.

Table 2. Antibacterial activity (zone of inhibition, mm) of AgNPs synthesized using leaf extract and callus extract of *V. canescens*

Microbial strains (zone of inhibition in mm)	Leaf extract (PE)	PAgNPs	CAgNPs	Control
E. coli	23±0.90	25±0.80	26.9±1.21	36.66±1.2
B. cereus	25.7±1.21	27.3±0.45	30±0.90	34±1.57
B. subtilis	24±0.57	27.7±1.00	27±0.76	37±0.82

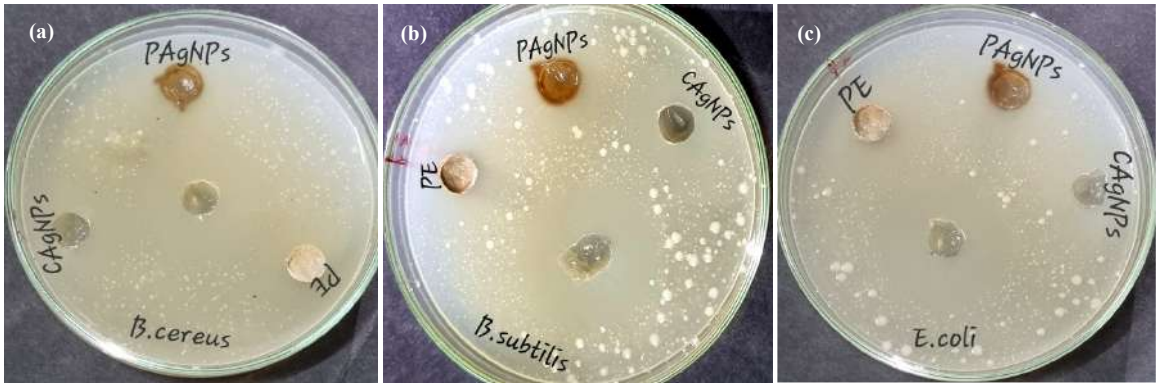

Figure 8. Antibacterial activity of PAgNPs and CAgNPs against: (a) *Bacillus cereus*, (b) *B. subtilis* and (c) *E. coli*.

DISCUSSION

Our findings substantiate the results reported by Cruz et al. [15]; Anjum & Abbasi, [4] and Groach et al. [19] indicating the completion of reduction reaction. The absorption band in the 400 to 450 nm region is typically for the silver nanoparticles [11, 19, 27]. According to XRD analysis, a sharp peak at 2θ of ~38.3° reveals the location (111) of silver, corresponding to face-centered cubic [6, 12]. The findings demonstrated that the AgNPs generated by plants and calli were made of high purity crystalline silver. The K value (constant) of 0.94 in the FCC and crystalline form of NPs, confirm the formation of silver NPs [16].

Similar observations regarding the size and morphological characteristics of the silver nanoparticles produced with leaf extracts of *Acacia nilotica* [40], flower extracts of Jasmine [5], and aqueous extracts of *Dracocephalum kotschyi* [13] respectively. The particles carry a charge of -33.75mV with a PDI below 0.276, demonstrating the stability of the green synthesized AgNPs [21]. The absorption bands at 3460 cm^{-1} & 3458 cm^{-1} in the FTIR spectra are due to OH stretch vibration of hydroxyl group and N–H stretch vibration of amines indicating the presence of the amine linkages of aniline. The band at 1634 cm^{-1} and 1630 cm^{-1} corresponds to C=O stretch bands of the carboxylic acid group [37]. The bands at 1252 cm^{-1} and 1074 cm^{-1} correspond to aryl -O stretch of aromatic ethers and C–N (amines) stretch vibration of proteins, respectively [22]. The bands at 618 cm^{-1} and 613 cm^{-1} correspond to S-S stretch vibration of disulphides bond [14].

The capping of several functional groups such as alkaloids, flavonoids, proteins, phenols, and glycosides was visible in the FT-IR spectra of plant and callus produced silver nanoparticles. Our results are in good agreement with other reports available on biosynthesis of AgNPs [19, 34, 46].

Silver nanoparticles may connect to the exterior of the plasma membrane interfering with components of the microbial electron transport system due to electrostatic attraction factors between the cell membranes of microbes of negative charge and positively charged NPs [3, 41]. The generation of silver metal free radicals also increases the oxidation forces responsible for the deterioration of membrane and nucleic acids causing cell death [26-42]. Similar reports on the inhibitory effects of green synthesized AgNPs on bacteria had been reported in *Azadirachta indica* [2]; *Crocus sativus* [8]; *Elaeagnus angustifolia* [30] and *Origanum majorana* [46]. Our study integrates nanotechnology and microbial biotechnology, leading to possible developments in the formulation of new types of bactericides [18].

In conclusion, silver nanoparticles were successfully produced from both plant leaves and *in vitro* developed callus of *V. canescens*. Green synthesis of AgNPs utilizing fresh leaves and callus from *V. canescens* has the potential to serve as an eco-friendly alternative to the hazardous chemical synthesis processes. The *in vitro* raised callus was proved to contain secondary metabolites resulting in antimicrobial activity. Thus, callus extract can be directly substituted in the extraction of certain useful drugs. Aside from that, *in vivo* studies are required to fully comprehend the function of *V. canecsens* mediated AgNPs and to assess their prospective uses in the biomedical area.

Acknowledgements. The authors are grateful to Kurukshetra University, Kurukshetra, for providing laboratory facilities to carry out this investigation. The first author (KR) is thankful Indian Council of Medical Research (ICMR), New Delhi for providing financial assistance in the form of Junior Research Fellowship. Thanks are also owed to Plant Tissue Culture Unit, Haryana Forest Department, Sonti, Kurukshetra, India for providing facilities for micropropagation work. We also acknowledge the Sophisticated Analytical Instrumentation Facility, CIL and UCIM, Punjab University, India for extending their FT-IR, HR-TEM and EDS facility in this work.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: July 25, 2022
Accepted: October 19, 2022
Published Online: October 27, 2022
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House

ACTIONS FOR *EX-SITU* CONSERVATION OF *Saussurea lappa* (DECNE.) CLARKE

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Abstract. *Saussurea lappa* (Decne.) Clarke (Asteraceae) is a well-known traditional medicinal herb in India and is enlisted in Appendix – I of CITES (Endangered Plant List). An efficient regeneration protocol through indirect organogenesis from leaf explants of *S. lappa* has been developed. Mature and cotyledonary leaf explants were inoculated on Murashige and Skoog (MS) culture medium containing 3% (w/v) sucrose and 0.8% (w/v) agar supplemented with various concentrations of auxins and cytokinins individually or in different combinations. Maximum callus induction (100%) with best visual growth was obtained on MS medium supplemented with 2,4-D (2.0 mg/L) and BAP (2.0 mg/L). The appearance of the callus varied depending upon the combinations and concentrations of plant growth regulators (PGRs) used. The highest shoot regeneration percentage (66.66%) with maximum number of shoots (4.9 shoots/explant) were obtained on MS medium containing BAP (3.0 mg/L) and NAA (0.10 mg/L). MS half strength media with NAA (0.5 mg/L) resulted in highest rooting percentage (85%) followed by IAA (0.5 mg/L). After acclimatization, plantlets grew normally under greenhouse and field conditions. This protocol could be used for rapid mass production, germplasm conservation and provides a basis for genetic improvement of *S. lappa.*

Keywords: *Saussurea lappa*; endangered; *in vitro*; indirect regeneration

INTRODUCTION

Saussurea lappa (Decne.) Clarke (family Asteraceae) commonly known as Kuth or Costus is an endemic perennial medicinal plant distributed in western Himalayas and neighbouring valleys at altitudes of 2500-3000 m [22]. It is a critically endangered medicinal plant species enlisted in Appendix – I of CITES (Endangered plant list) [14]. The roots of this plant contain an essential oil known as costus oil. The important constituents of the costus oil include saussurine, resinoids, inulin, essential oil and other alkaloids [31].

The species has found applications in pharmaceutical industry to treat more than forty-three diseases [37]. Diverse medicinal properties of *S. lappa* for example anti-inflammatory, suppress hepatitis B, anticancerous, antibacterial, antihepatotoxic, anti-arthritic, antiviral, antifungal, antiproliferative and antioxidant have attracted entrepreneurs to set eyes on this plant [7]. Its root extract is used as tonic useful in skin disease, epilepsy, paralysis, vermicide, diuretic, rheumatism, bronchitis, nervous disorders, brain stimulant, irregular menstruation, heart diseases, carminative, aphrodisiac, antihelminthic, treating deaf and ophthalmic condition [6, 22]. Because of its diverse medicinal properties, the species was overexploited by local population and pharmaceutical companies for the preparation of various valuable medicines. Large-scale uprooting from wild is the major factor threatening its existence. The conventional propagation of this plant is through root cuttings which is a commercial valuable part of the plant. The percentage of seed germination is very poor [11].

Due to excessive and destructive exploitation, it is getting fast depleted. The modern biotechnological techniques have come as a boon creating new dimensions in the field of agriculture for getting modern product with high yield and at faster rate [36]. The present investigation porpose an efficient protocol for indirect organogenesis of *S. lappa* by manipulating growth regulators on leaf explants. Also, the effect of subculture frequencies on *S. lappa* callus development is described.

MATERIALS AND METHODS

Plant material and sterilization. Healthy seeds of *S. lappa* were procured from Lahul valley of Himachal Pradesh (India). Healthy plants of *S. lappa* plants were collected from Forest Department, Jammu & Kashmir (India) and maintained in pots in the green house of Department of Botany, Kurukshetra University, Haryana (India). Surface sterilized seeds were inoculated on half strength MS medium [18] to raise seedlings for production of cotyledonary leaf [7]. Cotyledonary leafs excised from aseptically raised seedlings and mature leaf segments were used as explants. Explants were surface sterilized by washing with Teepol solution (5%) followed by washing under running tap water for fifteen minutes to remove the adhering dust particles. Thereafter, the explants were disinfected using 0.1 % (w/v) of mercuric chloride for 3 minutes followed by a brief rinse with 70% ethanol. These disinfected explants were then thoroughly washed with sterilized double distilled water to remove the traces of mercuric chloride.

Culture media and *in vitro* culture induction. The surfaces sterilized explants were trimmed into small pieces (0.8 X 1.0 cm) and inoculated on MS medium containing 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, India) supplemented with auxins (2,4-D, NAA) and cytokinin (BAP) in various concentrations of for callus induction and mass multiplication. The pH of the medium was adjusted to 5.8 prior and autoclaved at 1.5 kg cm^{-2} at 121°C for 20 min. The cultures were incubated under 16 hours

photoperiod with a photon flux density (PFD) of 40 µmol $m^{-2}s^{-1}$ at 25 ± 2°C and 70% humidity. After 4 weeks of inoculation, the efficiency of plant growth regulators (PGRs) was determined by recording the time required for callus induction, percent callus induction, colour and texture of callus. After 40 days of callus induction, both cotyledonary leaf and mature leaf explants callus were again subcultured (i.e. the 1st, 2nd & 3rd cycle) on their respective medium for callus multiplication and finally a mass of calli was harvested.

For shoot regeneration, the best *in vitro* regenerated callus from 3rd successive sub culture were excised aseptically and transferred to various concentrations (0.5- 3.0 mg/L) of BAP alone and in combination with IAA, NAA and 2,4-D.

Root development and ex-vitro acclimatization. In this study, we have used the *in vitro* rooting protocol established by us in our earlier research [7]. For root induction, the regenerated shoots (2.5-3.0 cm) were excised and cultured on either full or half strength MS media supplemented with (0.5- 2.0 mg/L) of IAA or NAA. The well rooted plantlets were gently washed under running tap water to remove the adhering agar with minimum injury. These plants were acclimatized and hardened in autoclaved sand: soil ratio (3:1). The plantlets were covered with glass jars to ensure humidity. They were supplied with half strength MS salt solution on alternate days. In third week, glass jars were removed for 3-4 hrs daily to expose the plants to the field conditions. After 4 weeks, these plants were transferred to bigger pots and were maintained under greenhouse for acclimatization.

Data analysis. All the experiments were repeated thrice with a minimum of twenty replicates per treatment. The statistical calculations were all carried out using SPSS (version11.5) statistical software. The difference between means was analyzed by one-way analysis of variance (ANOVA) and the differences contrasted using a Duncan's multiple range test at $P \leq 0.05$.

RESULTS

Callus Induction. The MS medium without PGRs did not show callus induction. The effects of different explants and the interactions between different plant growth regulators on callogenesis are presented in Tables 1 and 2. A wide range of variations in percent callus induction, growth and nature of callus were observed depending on concentrations and combinations of PGRs used.

It was noticed that callus proliferation usually started from the cut surface of the explant used and finally covered the whole explant. Significant differences in the percent callus induction and friability were observed among the different explants used (Table 1; Fig. 1a-b). Mature leaf explants showed great ability to induce callus, followed by cotyledonary leaf explants. In case of mature leaf explants, MS media supplemented with 2.0 mg/L of 2,4-D + 2.0 mg/L of BAP recorded the highest (86.66%) per cent callus induction in comparison among all the tested combinations (Table 1). Glassy white soft friable appearance of callus was observed in mature leaf explant on MS media supplemented with 2.0 mg/L of 2,4-D alone and 2.0 mg/L of 2,4-D + 2.0 mg/L of BAP, while creamish brown friable appearance of callus was observed on MS media supplemented with 2.0 mg/L of BAP (Fig. 1a). Among the various treatments tested with cotyledonary leaf explant, maximum percent callus response was observed on MS media supplemented with 2.0 mg/L of 2,4-D + 0.5 mg/L of BAP (Table 1). The different appearance of the callus was observed on all the treatments of cotyledonary leaf explant. The cotyledonary leaf callus on MS media supplemented with NAA (2.0 mg/L) recorded creamish white friable soft, BAP (0.5 mg/L) recorded whitish green compact, NAA (2.0 mg/L) + BAP (0.5 mg/L) recorded whitish green friable, 2,4-D (2.0 mg/L) recorded creamish white friable soft, 2,4-D (2.0 mg/L) + BAP (0.5 mg/L) recorded glassy white soft, while 2,4-D (2.0 mg/L) + BAP (0.5 mg/L) + NAA (2.0 mg/L) recorded glassy brown soft appearance of callus. Differentiation was not observed in any of the tested combinations of Table 1.

After 40 days of the 3rd subculture cycle, the best visual growth of callus with highest (96.66%) percent callus proliferation was recorded on MS media supplemented with 2.0 mg/L of 2,4-D + 2.0 mg/L of BAP from mature leaf callus (Table 2; Fig. 1c-d). This medium also produced shoot buds along with dark green friable callus. MS medium with 2.0 mg/L of BAP also produced creamish green brown friable appearance of callus with the appearance of shoot buds. In case of cotyledonary leaf callus, the per cent culture response between 67.77% and 71.11% was observed from medium with 2,4-D (2.0 mg/L) alone and with combination with BAP + NAA, which was comparatively lower in comparison to callus formation on the same medium from mature leaf callus. Therefore, was not tested for further shoot regeneration.

Shoot regeneration. The *in vitro* regenerated from the 3rd successive subculture of mature leaf segments on MS medium supplemented with 2.0 mg/L of 2,4-D + 2.0 mg/L of BAP were excised transferred to MS medium supplemented with various concentrations of BAP alone and with various combinations with NAA, 2,4-D and IAA for shoot induction (Table 3). MS medium devoid of any plant growth regulator did not show any response. MS medium supplemented with various concentrations (0.5 - 2.0 mg/L) of BAP resulted in the production of shoots with regeneration of green and compact appearance of callus (Fig. 1e). Among the various concentrations of BAP tested alone, MS medium supplemented with 2.0 mg/L of BAP recorded higher percent response with the production of 4 shoots per culture (Table 3). An increased visual growth of callus was observed with an increase in the

concentration of BAP in medium. However, among the various treatments used, the highest percent culture response (66.66%) with very good visual growth of callus was recorded on MS medium supplemented with 3.0 mg/L of BAP + 0.10 mg/L of NAA with the production of 4.9 number of shoots per culture followed by BAP (2.0 mg/L) + NAA (0.25 mg/L) (Fig. 1f). The combinations BAP (2.0 mg/L) with IAA (0.5 mg/L) and NAA failed to produce shoots.

Root development. The MS medium devoid of any plant growth regulator failed to develop any roots in excised shoots (Table 4). The half strength MS medium supplemented with various concentrations of IAA and NAA resulted in best results over full strength. Half strength MS medium containing IAA (0.5 mg/L) recorded an average number of 3.2 roots with 76% rooting. An increase in the concentration of PGRs along with strength of MS medium resulted into the production of more callus comparatively (Table 4).

Table 1. Effect of different plant growth regulators on different types of explants after 40 days of culture incubation

Explant + conc. of growth regulator in MS medium (mg/L)	Percent culture responding	Appearance of callus	Visual growth of callus*	Differentiation
Mature leaf explant + 2,4-D (2.0)	80.00^{d}	Glassy White, Soft, Friable	+ + + + +	-
Mature leaf explant + BAP (2.0)	75.55^{e}	Creamish brown, Friable	+ + + +	-
Mature leaf explant + 2,4-D (2.0) + BAP (2.0)	86.66^{ab}	Glassy White, Soft, Friable	+ + + + +	-
Cotyledonary leaf + NAA (2.0)	69.99^{e}	Creamish white Friable Soft	+ + + +	-
Cotyledonary leaf + BAP (0.5)	69.99^{e}	Whitish Green, Compact	+ + + +	-
Cotyledonary leaf + BAP (0.5) + NAA (2.0)	80.00^{c}	Whitish Green, Friable	+ + + + +	-
Cotyledonary leaf + 2,4-D (2.0)	79.99^{d}	Creamish White, Friable, Soft	+ + + +	-
Cotyledonary leaf + 2,4-D (2.0) + BAP (0.5)	84.44^{bc}	Glassy White, Soft	+ + + + +	-
Cotyledonary leaf + 2,4-D (2.0) + BAP (0.5) + NAA (2.0)	83.33^{bcd}	Glassy Brown, Soft	+ + + + +	-

*- No Callus, + Poor Callus (less than 50% coverage), ++ Less Callus (50% to 75% coverage), +++ Moderate Callus (more than 75% coverage), ++++ Good Callus (100% coverage), +++++ Very Good Callus (100% coverage + overlap)

Table 2. Effect of different plant growth regulators on 3rd sub culturing of callus after 40 days

Explant + conc. of growth regulator in MS medium (mg/L)	Per cent culture responding	Appearance of callus	Visual growth of callus*	Differentiation
Mature leaf explant + 2,4-D (2.0)	90.00^{b}	Glassy White, Soft	+ + + +	-
Mature leaf explant + BAP (2.0)	88.88^{c}	Creamish Green Brown, Friable	+ + +	Shoot buds
Mature leaf explant + 2,4-D (2.0) + BAP (2.0)	96.66^{a}	Dark Green, Friable	+ + + + +	Shoot buds
Cotyledonary leaf + NAA (2.0)	69.99^{ef}	Glassy Brown, Soft	+ + +	-
Cotyledonary leaf + BAP (0.5)	68.88^{fg}	Creamish Brown, Friable	+ +	-
Cotyledonary leaf + BAP (0.5) + NAA (2.0)	73.33^{d}	Glassy Brown, Soft	+ + +	-
Cotyledonary leaf + 2,4-D (2.0)	67.77^{fgh}	Glassy Brown, Soft	+ + +	-
Cotyledonary leaf + 2,4-D (2.0) + BAP (0.5)	72.22^{de}	Glassy Brown, Soft	+ + +	-
Cotyledonary leaf + 2,4-D (2.0) + BAP (0.5) + NAA (2.0)	71.11^{de}	Glassy Brown, Soft	+ +	-

*- No Callus, + Poor Callus (less than 50% coverage), ++ Less Callus (50% to 75% coverage), +++ Moderate Callus (more than 75% coverage), ++++ Good Callus (100% coverage), +++++ Very Good Callus (100% coverage + overlap)

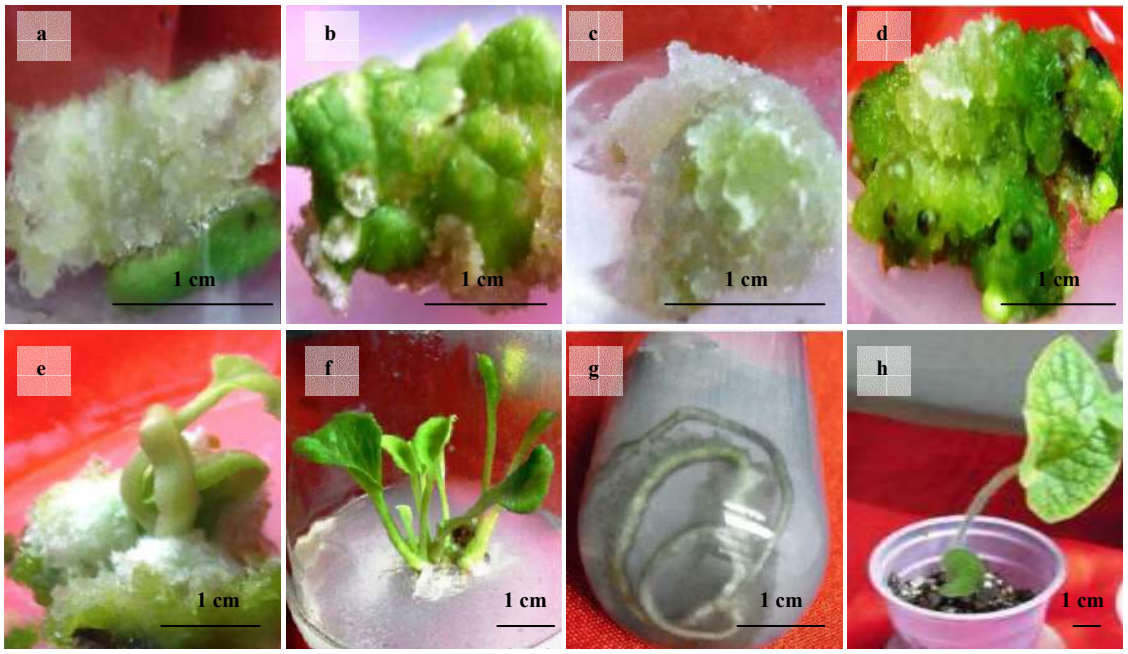

Figure 1. *In vitro* propagation of *S. lappa*: **(a)** Callus initiation from cotyledonary leaf explants on MS medium with 2,4-D (2.0 mg/L); **(b)** Callus initiation from mature leaf explants on MS medium with BAP (2.0 mg/L); **(c)** Callus proliferation from cotyledonary leaf explants after first subculture on MS medium + 2,4-D (2.0 mg/L) + BAP (0.5 mg/L); **(d)** Callus proliferation from mature leaf explants after third subculture on MS medium + BAP (2.0 mg/L) + 2,4-D (2.0 mg/L); **(e)** Callus induction along with shoot formation on MS medium with BAP (0.5 mg/L); **(f)** Indirect shoot regeneration from subcultured matured leaf callus on MS medium with BAP (3.0 mg/L) + NAA (0.10 mg/L); **(g)** Root initiation on full strength MS medium with IAA (2.0 mg/L); **(h)** Acclimatized plantlet.

Table 3. Effect of different plant growth regulators alone and in combination on shoot regeneration from callus derived from 3rd sub culture of mature leaf explant in medium containing 2,4-D (2.0 mg/L) + BAP (2.0 mg/L)

Medium and conc. of growth regulator in MS medium (mg/L)	Per cent culture response/ bud break	No. of days required for the bud break	Appearance of callus	Visual growth of callus*	No. of shoots per culture
MS Medium	-	-	-	-	--
MS+BAP (0.5)	40.00[f]	75[d]	Green, Compact	++	1[d]
MS+BAP (2.0)	56.66[d]	54[b]	Green, Compact	+++++	4[c]
MS +BAP (2.0) + NAA (0.5)	59.99[c]	-	Whitish Greenish, Friable	+++++	-
MS +BAP (2.0) + IAA (0.5)	11.11[g]	-	Whitish Greenish, Friable	+++++	-
MS +BAP (3.0) + 2,4-D (0.5)	84.44[a]	-	Whitish Greenish, Friable	+++++	-
MS + BAP (2.0) + NAA (0.25)	47.77[e]	61[c]	Greenish, Compact	++++	4.6[b]
MS + BAP (3.0) + NAA (0.10)	66.66[b]	51[a]	Greenish, Compact	++++	4.9[a]

*- No Callus, + Poor Callus (less than 50% coverage), ++ Less Callus (50% to 75% coverage), +++ Moderate Callus (more than 75% coverage), ++++ Good Callus (100% coverage), +++++ Very Good Callus (100% coverage + overlap)

Table 4. Effect of half strength MS medium and full strength MS medium with or without various concentrations of NAA and IAA in root regeneration from excised shoots after 40 days of culture

Medium	Plant growth Regulator	Rooting (%)	Number of roots	Root morphology
MS half strength	-	-	-	-
MS full strength	-	-	-	-
MS half strength	0.5 mg/L IAA	76[b]	3.2[b]	Long ,Thin
MS full strength	2.0 mg/L IAA	70[c]	1.9[d]	Long ,Thick
MS half strength	0.5 mg/L NAA	85[a]	6.4[a]	Long, thin and very less callus formation [7]
MS full strength	2.0 mg/L NAA	20[d]	2.0[cd]	No rooting and Profuse callus formation

- (no response)

Full strength MS medium supplemented IAA (2.0 mg/L) resulted in 70 percent rooting with an average production of 1.9 long and thick roots. (Fig. 1g). Among auxins, both NAA and IAA proved to be the good root inducer, but IAA also induced roots formation without callus production. However, the highest number of roots was observed on culture medium with NAA (0.5 mg/L).

After 4 weeks, these plantlets were transferred to under natural field conditions of photoperiod and temperature. More than 60% of *in vitro* raised plantlets were characterized by well-developed leaves with roots and showed no morphological abnormalities (Fig. 1h).

DISCUSSION

Plant tissue culture offers a fast alternative to conventional propagation technique and it is used as a complementary strategy for the conservation and utilization of genetic resources [7, 12, 15]. Indirect regeneration involves the formation of callus. Callus is an undifferentiated mass of cells, obtained by culturing explants on nutrient medium [19, 20]. The MS medium devoid of any PGRs did not report callus induction. It may be due to the insufficient level of endogenous growth hormones in explants to induce callusing and requires an exogenous supply of PGRs to trigger cell division [8]. Under *in vitro* conditions, the application of cytokinins causes a decrease in cell wall lignification, facilitating callus initiation and growth [13]. It was noticed that callus proliferation usually started from the cut surface of the explant used and finally covered the whole explant. It may be due to accumulation of auxins at the point of injury, which stimulated cell proliferation in the presence of growth regulators [1]. Dhital *et al*. [3] also reported that leaf explants produced earlier callusogenesis with higher frequency of callus induction in potato cultivars. Contrary, Shirin *et al.* [27] emphasized internodal segments, as a best explant source for *in vitro* callus induction. The variation in the apperance of callus with different PGRs have also been noticed by Ehsandar *et al.* [4] and Kumlay & Ercisli [13] on *Solanum tuberosum.* It could be due to differences in endogenous growth regulators in the explants [16].

Among the cytokinins, BAP is the most frequently used hormone, which interacts actively with 2,4-D or NAA for callus initiation and its maintenance [21]. The optimal concentration of the PGRs depend on plant genotype and type of explants used [17]. Faisal & Anis [5] obtained higher frequency of callus formation on MS medium containing 2,4-D in *Tylophora indica*. 2,4-D is very ideal auxin to initiate callus and somatic embryogenesis in different medicinal plant species, whereas IAA, IBA, NAA could also be used as stimulants in a lesser extent [9, 25].

Dark green and compact calli have good regeneration ability for shoot proliferation [10]. The auxin and cytokinin ratio acts as an important factor in determining organogenesis in micropropagation experiments [32]. Different endogenous level of cytokinins and auxins along with exogenous plant hormones that exist in cultured medium combine together to induce organogenesis. BAP was more efficient for shoot induction due to its ability to induce and produce natural hormones such as zeatin within the tissue through natural hormone system [26]. The vital role of BAP for *in vitro* shoot induction from different explants of medicinal plant species is well documented [2]. Lower concentration of NAA in combination with BAP also facilitates better morphogenesis and enhanced the rate of shoot buds differentiation in *Cassia* sp. [23, 24].

The proliferation of callus depends on the media composition and optimization of subculture period for maintaining its growth in order to obtain higher callus

biomass with regenerative potency [17]. It is recommended to subculture a callus at every 4–6 weeks [18].

Many researchers have reported the effectiveness of half strength MS medium for root induction [30]. Effective *in vitro* rooting on regenerated shoots grown on MS medium supplemented with NAA or IAA has been well documented in *Aegle marmelos* [34], *Stevia rebaudiana* [28] and *Puya berteroniana* [30]. Reports on acclimatization and hardening were reported for *Glycyrrhiza glabra* [35], *Stevia rebudiana* [29], *Spilanthes acmella* [33] and *Simmondsia chinensis* [12].

The success of any micropropagation protocol depends finally on the establishment of regenerated plantlets under *ex-vitro* field conditions with higher survival rate. Plants raised under *in vitro* conditions are maintained under controlled environmental factors such as: constant temperature, low light intensity, high humidity, supplementary sugar supply and growth regulators. Sudden changes in environment condition cause low survival or reduced growth rate [36].

In conclusion, this experimental study has resulted in an expeditious indirect regeneration protocol from mature leaf explants may be highly useful for raising quality planting material for mass multiplication and conservation of *S. lappa*. It could also facilitate phytochemical production, genetic transformation and pharmacological studies of this potential medicinal plant.

Acknowledgements. The authors are thankful to Kurukshetra University, Kurukshetra for providing laboratory facilities. One of the authors (RG) is thankful to the University for providing financial assistance in the form of URS.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: June 11, 2022
Accepted: October 20, 2022
Published Online: October 28, 2022
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House

RABINDRA BHARATI
JOURNAL OF PHILOSOPHY

UGC CARE Group I Journal
ISSN No: 09730087

CERTIFICATE OF PUBLICATION

This is to certify that the article entitled

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Published in

Department of Philosophy: Rabindra Bharati University

ISSN : 0973-0087

Vol. : XXIV, No:01, 2023 with IF: 6.986

UGC CARE Approved, Peer Reviewed and Referred Journal

Editor
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RABINDRA BHARATI JOURNAL OF PHILOSOPHY
ISSN : 0973-0087

TRAGIC FALL OF OKONKWO IN CHINUA ACHEBE'S *THINGS FALL APART*

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Abstract
Chinua Achebe's *Things Fall Apart*, has been one of the most popular, critically acclaimed, and discussed novels since its publication in 1958.Despite the apparently exhaustive critical attention paid to the novelcertain key aspects remain debatable. One such question deals with the reason forOkonkwo's downfall or fate.The paper intends to explore the possible reasons for Okonkwo's demise, his tragic fall.Throughout the novel Okonkwo lived as a courageous and invincible man. He would bow to none. He lived like a lion.Then what were the reasons that made this man embrace his shameful death. In choosing his own death he gave the message of not surrendering himself to anyone but his own fears and obstinacy.Okonkwo can be defeated by none other than Okonkwo. The paper suggests that a number of different sources of explanation appear to be plausible at various levels, but no argument could redeem his stature in the eyes of his family and society. The chief conclusion is that one who could have been the hero was turned into a villain by his hamartia. His hamartia was his hubris. He chose death over defeat. But his death does not make him a martyr, instead it doomed him to disgrace forever.

Keywords: Chinua Achebe, fate, downfall, *Things Fall Apart*, Okonkwo, demise, self-contradictory.

Introduction

Things Fall Apart is about the tragic fall of the protagonist, Okonkwo, and the Igbo culture. Okonkwo is a respected and influential leader within the Igbo community of Umuofia in eastern Nigeria.Very early in his life he determines to gain titles for himself and by his hardwork he becomes a powerful and wealthy man and gains two titles. He was a renowned wrestler, a fierce warrior, successful farmer of yams (a "manly" crop), hardworking, courageous and an impulsive man.He has three wives and many children who live in huts on his compound. He has a barn full of yams, a shrine for his ancestors, and his own hut, called an *obi*.

At an early age, Okonkwo has won a great fame in spite of his poor family background. He rises to a high position by dint of hard work and sincerity of earnest efforts. In those days, a man's position is adjudged in the society by his achievements and not by years of age."Age was respected among his people, but achievement was revered." (Achebe 12) In his thirties he enjoys the status with the aged people who can achieve high position only during their old age. "As the elders said, if a child washed his hands he could eat with the kings."(12) Even the elders holding high ranks and position in Ibo society holds him in high esteem. They invite him on the eve of the marriages of their sons and daughters. He attends important meetings of the rulers of the village.

Okonkwo's biggest fear is weakness, a trait he associates with his father and with women. Okonkwo despises and resents his father's gentle and idle ways. He resolves to overcome the shame that he feels as a result of his father's weaknesses by being what he considers to be "manly"; therefore, he dominates his wives and children by being insensitive and controlling. The protagonist of 'Things Fall Apart', Okonkwo is also considered a tragic hero. A tragic hero holds a position of power and prestige, chooses his course of action, possesses a tragic flaw, and gains awareness of circumstances that lead to his fall. Okonkwo's tragic flaw is his fear of weakness and failure.

Over the years, Okonkwo becomes an extremely volatile man; he is apt to explode at the slightest provocation. He violates the Week of Peace when he beats his youngest wife, Ojiugo, because she went to braid her hair at a friend's house and forgot to prepare the afternoon meal and feed her children. Later, he severely beats and shoots a gun at his second wife, Ekwefi, because she took leaves from his banana plant to wrap food for the Feast of the New Yam.

He is quick to anger, especially when dealing with men who are weak, lazy debtors like his father. Even though he feels inward affection at times, he never portrays affection toward anyone. Instead, he isolates himself by exhibiting anger through violent, stubborn, irrational behaviour. Okonkwo demands that his family work long hours despite their age or limited physical stamina, and he nags and beats his wives and son, Nwoye, who Okonkwo believes is womanly like his father, Unoka. When Nwoye and Ikemefuna, help him in planting the farm, he continuously finds fault with their work. He hopes that criticism will drive his son to be a great man and farmer.

Because Okonkwo is a leader of his community, he is asked to care for a young boy named Ikemefuna, who is given to the village as a peace offering by neighboringMbaino to avoid war with Umuofia. Ikemefuna befriends Okonkwo's son, Nwoye, and Okonkwo becomes inwardly fond of the boy.After the coming of the locusts, OgbuefiEzeudu, the oldest man in the village, relays to Okonkwo a message from the Oracle. The Oracle says that Ikemefuna must be killed as part of the retribution for the Umuofian woman killed three years earlier in Mbaino. He tells Okonkwo not to partake in the murder because the boy calls him “father”, but Okonkwo doesn't listen. He feels that not participating would be a sign of weakness. Consequently, Okonkwo kills Ikemefuna with his machete. Nwoye realizes that his father has murdered Ikemefuna and begins to distance himself from his father and the clansmen.

His life turns upside down when during the funeral of Ezeudu, Okonkwo's gun goes off, and Ezeudu's sixteen-year-old son is killed accidentally.Because the accidental killing of a clansman is a crime against the earth goddess, Okonkwo and his family must be exiled from Umuofia for seven years. The family moves to Okonkwo's mother's native village, Mbanta. After they depart Umuofia, a group of village men destroy Okonkwo's compound and kill his animals to cleanse the village of Okonkwo's sin. Obierika stores Okonkwo's yams in his barn and wonders about the old traditions of the Igbo culture.

Okonkwo is welcomed to Mbanta by his maternal uncle, Uchendu, a village elder. He gives Okonkwo a plot of land on which to farm and build a compound for his family. But Okonkwo is depressed, and he blames his chi (or personal spirit) for his failure to achieve lasting greatness.
Six missionaries, including one white man, arrive in Mbanta. The white man speaks to the people about Christianity. Okonkwo believes that the man speaks nonsense, but his son, Nwoye, is captivated and becomes a convert of Christianity.

After Okonkwo's exile is over, his family arranges to return to Umuofia. Before leaving Mbanta, they prepare a huge feast for Okonkwo's mother's kinsmen in appreciation of their gratitude during Okonkwo's seven years of exile.

When Okonkwo returns to Umuofia, he discovers that the village has changed during his absence. Many men have renounced their titles and have converted to Christianity. The white men have built a prison; they have established a government court of law, where people are tried for breaking the white man's laws; and they also employ natives of Umuofia. Okonkwo wonders why the Umuofians have not incited violence to rid the village of the white man's church and oppressive government.

Some members of the Igbo clan like the changes in Umuofia. Mr. Brown, the white missionary, respects the Igbo traditions. He makes an effort to learn about the Igbo culture and becomes friendly with some of the clan leaders. He also encourages Igbo people of all ages to get an education. Mr. Brown tells Okonkwo that Nwoye, who has taken the name Isaac, is attending a teaching college. Nevertheless, Okonkwo is unhappy about the changes in Umuofia.Okonkwo is opposed to the new ways. He feels that the changes are destroying the Igbo culture, changes that require compromise and accommodation — two qualities that Okonkwo finds intolerable. Too proud and inflexible, he clings to traditional beliefs and mourns the loss of the past.After Mr. Brown becomes ill and is forced to return to his homeland, Reverend James Smith becomes the new head of the Christian church. But Reverend Smith is nothing like Mr. Brown; he is intolerant of clan customs and is very strict.

Violence arises after Enoch, an overzealous convert to Christianity, unmasks an egwugwu. In retaliation, the egwugwu burn Enoch's compound and then destroy the Christian church because the missionaries have caused the Igbo people many problems.When the District Commissioner returns to Umuofia, he learns about the destruction of the church and asks six leaders of the village, including Okonkwo, to meet with him. The men are jailed until they pay a fine of two hundred and fifty bags of cowries. The people of Umuofia collect the money and pay the fine, and the men are set free.

Later when Umuofia is at a meeting, the District Commissioner's messengers arrive to order the meeting dispersed. The angry Okonkwo confronts them, draws his sword and beheads their leader. When none of the other clansmen attempt to stop the messengers who escape, Okonkwo realizes that they will never go to war and that Umuofia will surrender. Everything has fallen apart for Okonkwo; he commits suicide by hanging himself.

Conclusion

This situation clearly shows Okonkwo's tragic flaw due to his inability to adapt to the changes of his culture, stubbornly seeking to stick to the old ways he once knows. Okonkwo has taken everything about the clan in a personal way and has acted accordingly. He has clung to a tiny desperate hope that the clan shall redeem itself by following his example to act like brave warriors. But that small hope is disappointed. In the end he loses all faith in the world around him and does not care to live in it anymore. When the District Commissioner comes with a posse of men to arrest Okonkwo, Obierika says to him:

"That man was one of the greatest men in Umuofia. You drove
him to kill himself; and now he will be buried like a dog …" (Achebe, 188)

It is clear that as a great man in Umuofia, Okonkwo cannot face the reality. Even to escape from this reality, Okonkwo does something against the earth goddess. His suicide is a message to his community that he could not adapt or survive in this newculture. Thus, Okonkwo hangs himself. The suicide then is a symbolic act, signifying the break between Okonkwo and his people. A man once truly integrated into his society has lost his place among his people because his culture fall apart around him.

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Research Article

Biogenesis of Bacterial and Fungal Endophytic Mediated Silver Nanoparticles

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Submission Date: 19-02-2023; Revision Date: 12-03-2023; Accepted Date: 15-03-2023.

ABSTRACT

Aim: Nanoparticles play an important role to develop materials that are light, effective and ecofriendly with diverse applications. In this study, Bacterial and Fungal endophytes were screened for the synthesis of AgNPs. **Materials and Methods:** Endophytes for biogenesis of nanoparticles was isolated from various explants. Selected bacterial and fungal endophytes were identified by 18s rRNA and 16s rRNA sequencing. Endophyte mediated silver nanoparticles were characterized using different techniques like UV-visible spectroscopy, XRD, HRTEM, DLS, EDX and FTIR. **Results:** Optimization of AgNP synthesis by endophytic bacterial isolate *Bacillus cereus* showed optimum 5 mM silver nitrate concentration, reaction mixture temperature 37-40°C, reaction time 30 min and PVP as stable surfactant. Characterization carried out using advanced analytical instrumentation showed sharp peak at 424 nm by UV-visible spectroscopy. According to XRD and HR-TEM data analyzed it was observed that average size of AgNP was 12.4 and 12.9 nm respectively. DLS and zeta potential showed nanoparticles are dispersed evenly, FTIR analysis confirmed coating with primary and secondary amino acids which is responsible or stability of nanoparticles. EDX confirmed elemental composition of silver nanoparticles. **Conclusion:** Synthesized nanoparticles are uniform in size, ecofriendly, good bio compatibility, easy processing and stable. We can further study potential biological activity like plant growth promotion, nano pesticide encapsulations, antifungal, anti-bacterial, antioxidant and cytotoxicity of these synthesised AgNPs.

Keywords: Silver Nanoparticles, XRD, HRTEM, DLS, FTIR.

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INTRODUCTION

Due to the very small size, excellent magnetic properties and ability to change surface characteristics, high surface area to volume ratio nanoparticles possesses various physical, chemical and biological properties.[1] Silver nanoparticle possess diverse application in the field of optical, electronic, electrical, paint, textile, cosmetics, food industries, medical and environmental.[2] The common techniques used for the synthesis of nanoparticles includes chemical reduction, physical techniques and green synthesis. All these techniques are expensive, time consuming, poor compatibility and use the hazardous chemicals. Nanoparticles synthesized by physical and chemical methods are unstable and possess toxic chemicals present over their exterior limits various applications.[3] Plants are good sources of microbial diversity that secrete bioactive compounds, which can be used for the synthesis of nanoparticles.[4] Biogenesis approach is fast, economically feasible, simple and nanoparticles synthesized are biocompatible.[5,6]

Several reports are available for the biogenesis of silver nanoparticles by plant, fungi and bacteria.[7] High amount of biomolecules are produced by both bacteria and fungi hence prevent the agglomeration, which helps in stabilization of nanoparticles.[8] Endophytic microbes are recognized as most valuable group of microbes in terms of diversity and remedial potential.[9] These microorganisms nurture in the intercellular

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DOI: 10.5530/ajbls.2023.12.18

spaces of host plants without causing any damage.[10] Endophytes are able to synthesize same chemical compounds as synthesized by the host plant, make a possible adaptation to the host's microenvironment.[11]In this study we have isolated endophytes from various remedial plants for biogenesis of silver nanoparticles. Process for biogenesis of silver nanoparticles was optimized under different conditions. Morphological and structural characteristics were identified using UV-visible spectroscopy, XRD, DLS, HR-TEM, EDX and FTIR.

MATERIALS AND METHODS

Isolation and Characterization of endophytes

The endophytes were isolated from leaves, barks and root sections of various medicinal plants.[12] Plants, *Withania somnifera* (Ashwagandha), *Aloebarbadensis miller* (Aloe vera), *Tinospora cordifolia* (Giloi), *Bryophyllum* (Patherchatt), *Anthocephalus cadamba* (Cadamb), *Phyllanthus emblica* (Amla) and *Melia azadirachta* (Neem) were taken from the Kurukshetra University. Endophytic mediated biogenesis of silver nanoparticles and cleaning for further study was performed.[13,14] Endophytic bacterial strain VXB10 was identification by using 16s rRNA sequencing form CSIR-IMTech, Chandigarh. The 16s RNA sequence attained from CSIR-IMTech were subjected to BLAST analysis. Similarity study on genetic level was performed by using nBLAST. Endophytic fungal strain VXF2 was identified by using18s rRNA from Centre for Agriculture and Bioscience International, Bangalore.

Optimization study for biogenesis of silver nanoparticles

Concentration of Silver nitrate

Study for biosynthesis of AgNP with different concentration of silver nitrate and supernatant of VXB10 endophytic strain. Th esilver nitrate concentration varied from 1mM to 10mM which was mixed with supernatant in ratios (1:1). Then resulting solution was incubated for 30 min under bright condition for biogenesis of silver nanoparticles. Observations were made using UV-visible spectrophotometry.

Temperature and time

Opetimization study for biogenesis of silver nanoparticles under different temperature varying from 20 to 60°C. The silver nitrate with supernatant was incubated for 30 min at 20, 37, 40, 50 and 60°C. The effect of reaction time was checked for the biosynthesis of AgNPs by keeping reaction solution in direct sunlight at different time (2, 5, 10, 15, 20, 30, 40 and 50 min). The effect of incubation temperature and time was observed by UV-visible spectrum analysis.

Surfactant and stability

Surfactant acts as capping agent for nanoparticles, which increases stability and availability of nanoparticles. To study the effect of surfactants 0.1% of Polyvinyl Pyroidone (PVP), Ethylenediaminetetraacetic Acid (EDTA), Sodium Dodecyl Sulfate (SDS), Trisodium citrate and glyconic acid were added to reaction solution. The stability study of biologically synthesized AgNP was checked for 30 days by using UV-Visible Spectrophotometer.[15]

Characterization of synthesized AgNPs

Initially biologically synthesized AgNP were characterized using UV-visible spectrophotometry followed by advance instrumentation techniques like XRD, DLS, EDX, FTIR and HR-TEM.[13] XRD was done by comparing spectrum with standards in the Joint Committee of Powder Diffraction Standards issue 2010. Hydrophobic particle size, zeta potential and polydispersity index were predicted by DLS. Energy Dispersive X-Ray Spectroscopy was used to confirm elemental composition of AgNPs. The size, shape and arrangement of nanoparticles were confirmed by HR-TEM. Biomolecules and proteins on surface of AgNP were studied by using FT-IR spectrophotometer between 4000 - 400 cm^{-1}.

RESULTS

Biogenesis of AgNP by endophytes

A total of 79 endophytes (30 fungi and 49 bacterial) were isolated from the various part of remedial plants. The endophytes were coded as VXF1- VXF30 for fungi and VXB1-VXB49 for bacteria. The 10 bacterial endophytes and 8 fungal endophytes were showing a change in colour after reaction of silver nitrate with culture filtrate. The silver nanoparticles synthesis by both bacterial and fungal endophytes were confirmed by UV-visible spectroscopy as shown in Figure 1. The peak between 400 to 450 nm absorbance confirms the synthesis of nanoparticles.

The fungal endophyte VXF2 was showing peak at 432 nm with absorbance value 1.158. The fungal isolates (VXF9, VXF13, VXF18, VXF20, VXF25, VXF26 and VXF30) showed broad peaks with low absorbance value. The bacterial endophyte VXB10 was showing peak at 424 nm with absorbance value 2.763. The bacterial endophytes (VXB5, VXB24, VXB29, VXB34,

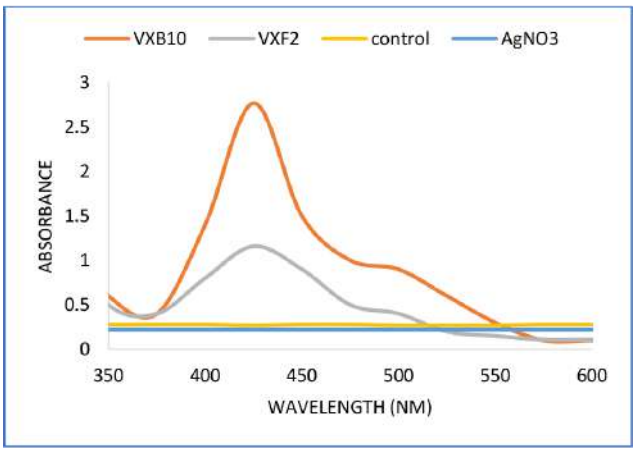

Figure 1: UV-vis analysis of endophytes VXF2 and VXB10.

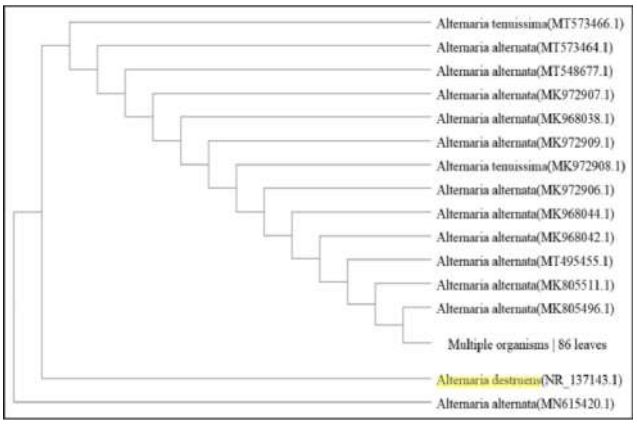

Figure 2: Phylogenetic tree constructed by neighbor joining method for endophytic fungi VXF2.

VXB35, VXB38, VXB39 and VXB40) showed broad peaks with low absorbance. Absorbance is co-related with the quantity of nanoparticles synthesized. The lower absorbance between 400-450 nm indicates the poor synthesis of nanoparticles.

To obtain the fungal biomass for the synthesis of nanoparticles is highly time consuming as compare to bacterial biomass. The synthesis of nanoparticles was slow when fungal biomass was used as compared to the use of bacterial supernatant. This is the reason only bacterial isolate VXB10 was used for the further study.

Identification of selected microbial isolates

The fungal isolate was identified by with fungus database from EBI and BLAST algorithm as shown in Figure 2. Fungal endophyte VXF2 was showing 100% similarity to *Alternaria destruens* (ATCC 204363). The 16s rRNA sequence of bacterial isolate VXB10. On the basis of BLAST analysis, VXB10 showed was showing 100% identity to *Bacillus cereus*. The phylogenetic tree

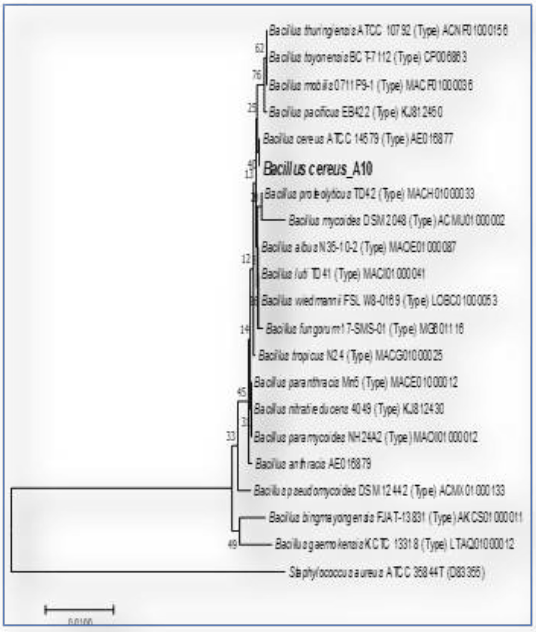

Figure 3: Phylogenetic tree constructed by neighbor joining method for endophytic bacterial isolate VXB10.

of bacterial endophyte VXB10 was constructed by Neighbor-Joining method was shown in Figure 3.

Optimization of silver nanoparticles biosynthesis

Silver nitrate concentration

The increasing concentration of silver nitrate resulted in higher synthesis of AgNP. A sharp peak around 420 nm was observed in case of 5 mM and 6 mM silver nitrate concentration as shown in Figure 4(a). The continuous rise in the absorbance peak intensities was observed with increasing concentration of silver nitrate (5 mM). It was observed that SPR peak after 5 mM concentration of silver nitrate became broadwhich depicts large size and agglomeration of nanoparticles. The UV-visi spectroscopy absorbance at 10 mM silver nitrate concentration was very less. The maximum absorbance was observed at 5 mM silver nitrate. All further experiments were carried out by mixing 5 mM silver nitrate with equal proportion of supernatant.

Optimization of temperature

The change in temperature effect the synthesis of nanoparticles. At 20 and 30°C change in colour of reaction solution was very slow. The colour of reaction solution was dark and prominent at 40, 50 and 60°C. The change in colour was recorded by UV-visible spectrophotometer as shown in Figure 4(b). The absorbance was high at 40°C followed by no significant

change at 50°C and 60°C. Therefore, further study was conducted at 40°C.

Time point for Biogenesis of silver nanoparticles

The change in colour was negligible up to 10 min. The prominent change in colour was seen after 15 min from pale yellow to dark brown. The significant increase in colour was not observed after 30 min of keeping the reaction solution. Spectrophotometrically, absorbance value increase consistently up to 30 min whereas, no significant increase in absorbance was recorded after 30 min as shown in Figure 4(c).

Surfactants optimization

In UV-vis analysis, maximum surface plasmon resonance absorbance value was recorded with 0.1% PVP as compare to 0.1% EDTA and 0.1% SDS followed by minimum absorbance with 0.1% Trisodium citrate and 0.1% Glyconic acid as shown in Figure 4(d). The results inferred that 0.1% PVP was better surfactant for stabilizing nanoparticles and reduce agglomeration. The reaction mixture containing PVP was stored 4°C to check the stability of nanoparticles at different time interval (1, 15 and 30 days). The stability of nanoparticle remain same as no change in the absorbance was at different time interval as shown in Figure 4(e). The stable position of SPR absorbance peak indicates the stability of nanoparticles. Hence, PVP was as most stable surfactant at low temperature.

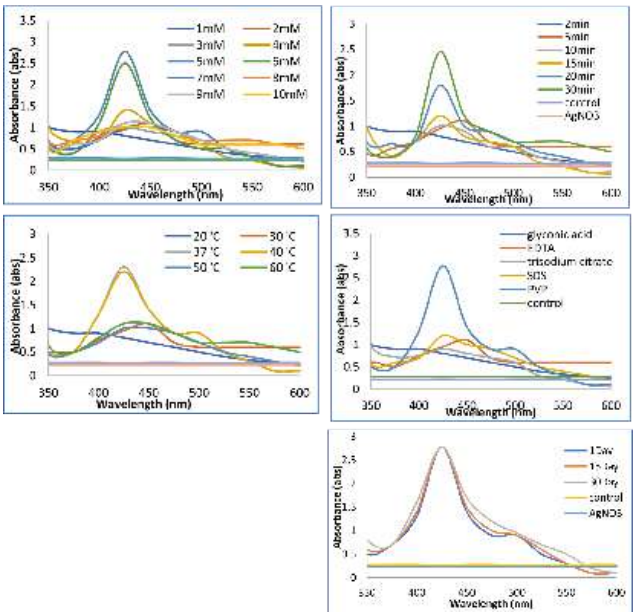

Figure 4: Silver nanoparticles biosynthesis at different a) concentrations of silver nitrate b) temperatures (20, 30, 37, 40, 50 and 60°C) c) time interval d) surfactants e) stability after 1, 15 and 30 days.

Characterization of synthesised silver nanoparticles

The cell free supernatant of VXB10 bacterial endophyte was mixed individually with silver nitrate (5 mM) in equal volume with PVP as capping agent, the reaction mixture was incubated for 30 min. The synthesized nanoparticles were showing maximum absorbance at 424 nm as shown in Figure 5(a). In XRD peak analysis four diffractions patterns were observed at 38.18°, 44.39°, 64.55° and 77.45° consistent to (111) (200) (220) and (311) face cantered cubic (fcc) planes for metallic silver as shown in Figure 5(b). The XRD spectrum analysed using Origin software for endophytic mediated nanoparticles was consistent with diffraction standard database JCPDS 04-0783. Usually high intensity is observed for fcc (111), which was similar to the data recorded in synthesized silver nanoparticles as shown in Table 1.

Hydrodynamic size of silver nanoparticles was estimated by using DLS. DLS analysis of synthesized silvern nanoparticle was done to know the particle size, zeta potential and polydispersity index. Size of biologically synthesized silver was in range of 172-449 nm with average size of 226 nm as shown in Figure 5(c). The Polydispersity Index (PdI) of synthesized silver nanoparticles was below 0.474 with net surface charge of -35.75 mV which infers to the stability of nanoparticles.

EDX analysis revealed the strong signal in domain of silver which confirms the elemental composition of silver nanoparticles as shown in Figure 5(d). EDX spectrum showed presence of silver (62.7%), copper (25.96%) and carbon (11.33%) respectively. The crystals of metallic silver usually show optical absorption at 3 keV due to SPR of biologically synthesized silver nanoparticles. The copper was observed due to copper gride used in sample support during analysis.

The HR-TEM images of biologically synthesized nanoparticles are shown in Figure 6. HR-TEM images when analysed using ImageJ software showed that nanoparticles were spherical and range from 2.5-30 nm with average size of 12.9 nm. These results are consistent with the shape as determined by the XRD analysis. The FTIR spectrum of biologically synthesized silver nanoparticles showed strong peaks at 3417.3, 2919.3, 2851.1, 1740.3, 1618.3, 1465.9, 1381.2, 1221, 1077.1, 827.3, 630 and 588.7 cm^{-1} as shown in Figure 7.

Table 1: Biologically synthesized AgNPs size calculated by Debye-Scherrer equation.

VXB10					
Position	**FWHM**	**Size (nm)**	**d-space**	**Miller indices (h,k,l)**	**Avg. Size (nm)**
38.18512	0.69274	12.1354788	2.354972	(1,1,1)	12.40
44.39455	0.87896	9.76173938	2.038927	(2,0,0)	
64.5515	0.66504	14.1286238	1.442524	(2,2,0)	
77.45878	0.74856	13.6041936	1.231214	(3,1,1)	

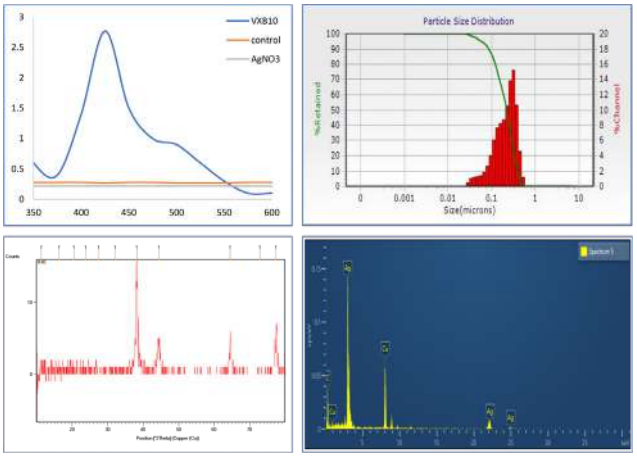

Figure 5: Characterization of nanoparticles synthesized by bacterial isolate a) UV-visible spectroscopy b) XRD analysis c) DLS d) EDX.

The FTIR peaks corresponding to different functional groups are shown in Table 2.

DISCUSSION

The isolated bacterial endophytes VXB10 was identified by using 16s RNA sequencing as *Bacillus cereus* and fungal endophytes VXF2 identified by using 18s RNA sequencing as *Alternaria destruens.* Both endophytes VXF2 and VXB10 were showing silvernanopartical synthesis form silver nitrate. The silver nanoparticles synthesised by endophytes *Cryptosporiopsis ericae, Aspergillus tamarii* and *Aspergillus versicolor.*[16-18] The bacterial isolate was easy to grow and showed faster reduction of silver nitrate as compared to fungi.[19] In fungal isolates due to high pigmentation the reduction of silver nitrate was taking more time as compared to bacterial isolates.[20] In this study bacterial endophytes were used for silvernanopartical synthesis. The silver nanoparticles show strong absorption at 424 nm in the visible range due to SPR. High absorbance value shows quantitative analysis of AgNPs. XRD peak data was used for estimating size silver nanoparticles by using Debye-Scherer's formula. The average size calculated for silver nanoparticles was 12.4 nm, which was similar to

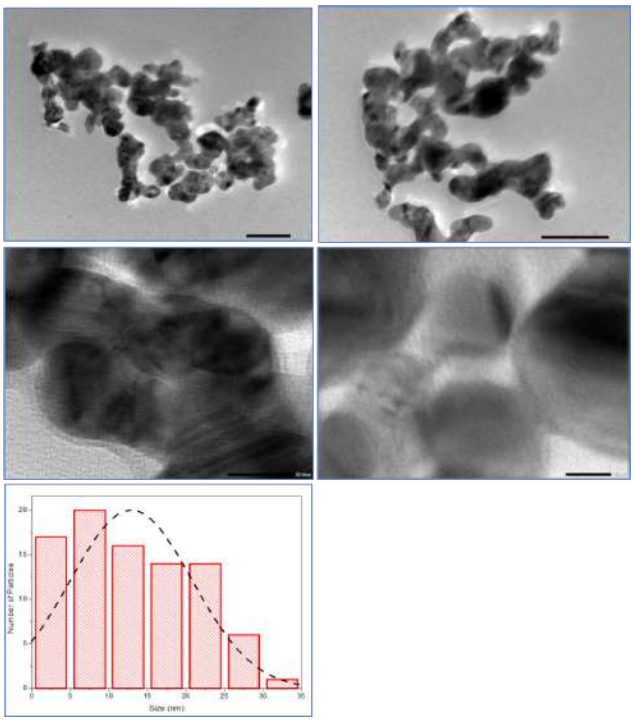

Figure 6: HR-TEM analysis of silver nanoparticles synthesized by bacterial isolate.

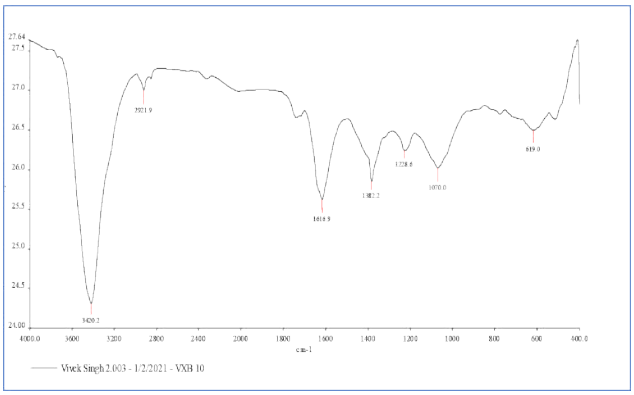

Figure 7: FTIR analysis of silver nanoparticles synthesized by bacterial isolate.

the nanoparticles size 12.9 nm calculated by HR-TEM image analysis. XRD spectrum broad peaks indicate the role of bacterial extract in particle formation and crystal nuclei growth.[21] Biogenesis of silver nanoparticles

Table 2: Analysis of FTIR spectrum of silver nanoparticles synthesized by bacterial endophyte VXB10.

Peak Value cm^{-1}	Corresponding functional group
3417.3	N-H primary and secondary amino acids
3564 and 3584	O-H stretch
1465.9	C-C aromatic stretch
2929.3 and 2851.1	-CH hydrocarbons
1740.3	Carbonyl compound
1618.3	C=O amide stretch
1065	-CH3 amino acid
2886	N-CH3 bend
1381.2	C-O stretch
1221	free carboxylate group
827.3	C-H linkage
630 and 588.7	Disulphide

from rizobial-isolate *Arthroderma fulvum* and discovered particles which were spherical with average diameter 15.5 ± 2.5 nm, with high uniformity and minor diameter distribution.[22] Energy Dispersive X-ray gives qualitative as well as quantitative status of elements involved in the formation of silver nanoparticles. The occurrence of primary silver was confirmed by EDX study, which confirms the silver element in reaction mixture was due to reduction of silver ions from silver nitrate.[23]In addition to size of metallic nanoparticles by XRD and HR-TEM, DLS also measures the size of stabilisers absorbed on the surface. As a result, DLS measurements of size are larger than TEM and XRD analysis measurements. PdI value ~0.4 indicated that the nanoparticles have highly polydisperse and moderately disperse distribution.[24] Zeta potential showed negative surface charge which explains the long-term stability, dispersity and high colloidal nature of nanoparticles. The surface charge could avoid agglomeration of nanoparticles and thus provide stable particles.[25] The interaction of biomolecules present in cell free extract with AgNPs was evaluated by using FTIR measurement of dried AgNPs. FTIR analysis showed presence of primary and secondary amines, alcohol or phenol O-H, saturated hydrocarbons, carbonyl compound and amino acids respirable of reduction of silver nitrate and coating nanoparticles for stability and better biocompatibility.[26] According to a previous statement, proteins can connect to nanoparticles via free amine groups or cysteine residues and by the electrostatic attraction of negatively charged carboxylate groups. This could lead to the stability of the silver nanoparticles by proteins.[27]The microbial strain used, temperature, capping agent and pH play an important role in synthesis of nanoparticles with specific size, shape and functional group.[28] The optimized condition for silvernanopartical synthesis were 5 mM silver nitrate, bacterial supernatant in ratio 1:1, light exposure for 30 min, reaction temperature of 40°C and PVP as most suitable surfactant. Nanoparticles could be stored to more than 30 days at 4°C for more than 30 days. The optimum condition for the synthesise of nanoparticles by *Escherichia coli* were pH 5-6, temperature 30-37°C.[29]

CONCLUSION

In this study fungal and bacterial isolates were used for the synthesis of silver nanoparticle. Bacterial endophytes have advantages over fungal endophytes for biosynthesis of silver nanoparticles due to their faster growth rates, easier culturing, and potential for genetic modification. Study has shown that bacterial endophytes produce smaller, more uniformly sized nanoparticles and can reduce silver ions more efficiently than fungal isolates. The bacterial endophyte was identified as *Bacillus cereus*. The synthesized silver nanoparticles were evenly distributed, small size, stable and crystalline in nature. We can further study potential biological activity (antifungal, anti-bacterial, antioxidant and cytotoxicity) of these synthesised AgNPs.

ACKNOWLEDGEMENT

The first author acknowledges World Bank TEQIP-III project for providing assistantship to carry out research work. We acknowledge GJU, Hissar and CIL/SAIF facility, Punjab University, Chandigarh and CCS, HAU, Hissar for providing with high end instruments.

Statements and Declarations

The first author acknowledges World Bank TEQIP-III project for providing assistantship to carry out research work. We acknowledge CIL/SAIF facility, Punjab University, Chandigarh for providing with high end instruments.

Author Contribution

Material preparation, data collection and analysis were performed by Vivek Singh and Deepak Kumar Malik. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AgNP: Silver nanoparticles; **HR-TEM:** High-resolution transmission electron microscopy; **XRD:** X-Ray Diffraction; **DLS:** dynamic light scattering; **EDX:** Energy Dispersive X-Ray Spectroscopy; **FTIR:** Fourier Transform Infrared; **PVP:** Polyvinyl Pyroidone **EDTA:** Ethylenediaminetetraacetic Acid; **SDS:** Sodium Dodecyl Sulfate.

SUMMARY

Bacterial endophyte VXB10 identified as Bacillus cereus (italics) can be used for synthesis of silver nanoparticles with uniform size, ecofriendly, good bio compatibility, easy processing and stable. We can further explore potential biological activity like plant growth promotion, nano pesticide encapsulations, antifungal, anti-bacterial, antioxidant and cytotoxicity.

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Cite this article: Singh V, Singhmar R, Rathi M, Bhatia D, Malik DK. Biogenesis of Bacterial and Fungal Endophytic Mediated Silver Nanoparticles. Asian J Biol Life Sci. 2023;12(1):130-7.

Indian Journal of Experimental Biology
Vol. 61, May 2023, pp. 364-372
DOI: DOI: 10.56042/ijeb.v61i05.858

Antimicrobial potential of polyvinyl pyrollidone stabilized silver nanoparticles synthesized by *Sphingobacterium multivorum*

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Received 14 October 2022; *Revised 16 March 2023*

Biological synthesis of nanoparticles is emerging as a promising solution to tackle issues associated with conventional synthesis methods. Silver nanoparticles (AgNPs), owing to their unique physiochemical and antimicrobial properties, attract more attention. In this study, we have made an attempt to develop ecofriendly and stable AgNPs with antimicrobial potential. AgNPs were synthesized using Gram negative *Sphingobacterium multivorum* supernatant and characterized by UV-Visible spectrophotometric analysis, X-Ray Diffraction analysis, Transmission electron microscopy, Fourier transform infrared and Dynamic Light scattering. Biosynthesized AgNPs exhibited broad spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria. Minimum inhibitory concentration was detected 19.5 µg for *E. coli* and *Staphylococcus aureus*. AgNPs have exhibited significant synergistic effect with all the selected antibiotics. The results demonstrated a rapid, economic and ecofriendly method for the synthesis of stable AgNPs and further evaluation of the antimicrobial activity against Gram-positive and Gram-negative bacteria.

Keywords: AgNPs, Antimicrobial, Dynamic light scattering (DLS), Polydispersity index (PDI)

Nanotechnology, a multidisciplinary field, is emerging exponentially form last few decades owing to its enormous application potential. Advances in nanotechnology have revolutionized many sectors like electronics, defence, cosmetics, agriculture, food, health and medicine[1-6]. Nanobiotechnology, an amalgamation of nanotechnology with biotechnology includes design and synthesis of materials or devices of nanometer range[7]. Ultrafine particles due to their nano scale size possess novel physiochemical and biological properties. The physiochemical properties of metal nanoparticles are based on the size, shape, composition, crystallinity and morphology. Anti-microbial resistance is a global threat growing at an alarming rate. There is a dire need to seek solution for this problem. Inefficacy of conventional antibiotics against drug resistance has pushed search for other novel options. Among metal nanoparticles, silver nanoparticles (AgNPs) gain more attention owing to their unique physiochemical and antimicrobial properties[8]. AgNPs have revolutionized different sectors like electronics, optics, catalysis and Raman scattering, pharmaceuticals and medicine[9,10]. Silver has been used since several years owing to their antimicrobial properties. Silver based products are employed in topical ointments, surgical bandages for wound healing and stents coating to prevent microbial infections[11,12].

A large number of physical and chemical processes have been exploited for synthesis of metal nanoparticles. However, these processes require high temperature/ pressure which is extremely harmful for environment. Selection of environment friendly and cost-effective process for the engenderment of nanoparticles is one of the major challenges in the newly emerging field of nanobiotechnology. Biologically fabricated AgNPs have significantly contributed to different areas like biosensor technology, biomedical, drug delivery, diagnostics, etc.[12-14]. Green synthesis methods are gaining more attention over conventional synthesis methods as they do not require toxic chemicals for their synthesis. Green synthesis processes are based on plant, bacteria and fungi mediated synthesis of nanoparticles. Microbes based synthesis is a rapid, economic, environmentally friendly and easy to scale up as compared to other methods[15-17]. Bacteria mediated synthesis may occur either extracellularly or intracellularly. Extracellular synthesis methods are preferred over intracellular methods due to easy downstream recovery and purification steps. In the

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present study, we have made an attempt to develop a rapid and environment-friendly method for synthesis of stable AgNPs and also evaluated their antimicrobial activity against Gram-positive and Gram-negative bacteria.

Materials and Methods

Isolation and characterization of bacteria from soil

The AgNPs synthesizing bacteria was isolated from metal contaminated soil by serial dilution method[17]. The isolated colonies were further screened for their ability to tolerate silver nitrate. The selected bacterial culture was identified on the basis of 16S rRNA sequencing performed at Institute of Microbial Technology, Chandigarh (India).

Extracellular synthesis of silver nanoparticles

The synthesis of AgNPs was carried out by extracellular method[17] and polyvinyl pyrollidone (0.1% PVP) was used as a stabilizing agent. The isolated bacterial culture DNP 5 was inoculated in basal salt media (BSM) for 48 h at 30°C under shaking conditions of 220 rpm. After incubation, the broth culture was centrifuged at 8000 rpm for 20 min. The supernatant was used for the synthesis of AgNPs. The synthesis of AgNPs was observed at regular intervals visually on the basis of colour change of culture supernatant from a transparent to brown appearance. The brown colour of culture supernatant was considered as a sign of the synthesis of AgNPs.

Characterization of silver nanoparticles

The produced nanoparticles were characterized by UV–Vis spectroscopy and X-Ray Diffraction analysis. The morphological examination of AgNPs was done by Transmission electron microscopy (Hitachi (H-7500) at CIL, Panjab University, Chandigarh. The particle size distribution and zeta potential of synthesized AgNPs was evaluated using dynamic light scattering (DLS) (Microtrac Nanotrac Wave Particle Size and Zeta Potential analyzer. Fourier transform infrared (FTIR) spectrum was recorded using FT-IR spectrophotometer (Horizon ABB) in the range of 4000-400 cm^{-1} (scan speed of 16 cm/s) and elemental composition was confirmed by energy dispersive X-ray spectroscopy (EDX).

Antimicrobial studies

Antimicrobial potential of biofabricated AgNPs was analyzed against 10 different test pathogens (seven bacteria and three fungi). The test microorganisms were *Escherichia coli*, *Bacillus subtilis*, *Psuedomonas aerugenosa*, *P. fluorescens*, *Staphylococcus aureus*, *Streptococcus mutans*, *S. pyrogenes*, *Fusaium graminearum*, *Candida albicans* and *C. glabrata.* The test cultures were procured from MTCC, IMTECH, Chandigarh. Antibacterial activity of synthesized AgNPs was determined by agar well diffusion assay with some modifications[17,18]. Further, the interaction between test pathogen *P. aeruginosa* and synthesised AgNPs was confirmed by TEM analysis.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of AgNPs for different test pathogens (*E. coli*, *P. aerugenosa*, *S. aureus*, *B. subtiliis* and *C. albicans*) was measured by agar well diffusion assay method with some modifications[17,18]. Different concentrations (19.5, 39, 78.1, 156.2, 312.5, 625 and 1250 µg) of synthesized AgNPs suspended in deionized water were used. The smallest concentration of AgNPs that inhibits the test pathogens was recorded as MIC.

Synergistic effect of AgNPs with antibiotic

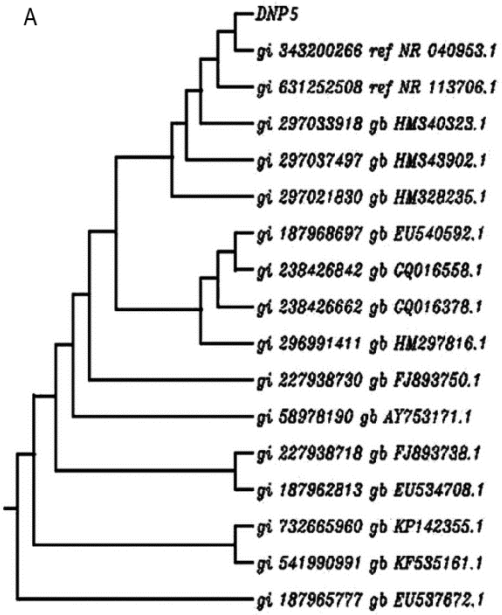
The collective effect of AgNPs and antibiotic was assessed by agar well diffusion assay as discussed earlier. Five different classes of antibiotics i.e. (1) Norfloxacin 10 µg (Quinolone), (2) 15 µg (Macrolide), (3) Rifampicin 5 µg (Rifamycin), (4) Kanamycin 30 µg (Aminoglycoside) and (5) Amoxyclav 30 µg (β Lacatm) were selected to measure synergistic effect of AgNPs. Antibiotic discs were soaked with 30 µL biologically synthesized AgNPs (0.1 mg/mL). The diameter of inhibition zone was measured as mean ± SD of the triplicate experiment[19].

Stability study

The synthesized nanoparticles were stored at 4°C for 40 days. The stability of synthesized nanoparticles was confirmed by UV-Visible Spectrophotometer analysis. Further, these stored nanoparticles were incubated in autoclave at 121°C for 40 min and then analyzed for antimicrobial activity.

Results

In the present study, we carried out bacterial supernatant mediated extracellular biogenesis of silver nanoparticles (AgNPs). The soil sample from metal contaminated site was used for isolation of bacteria capable to synthesize silver nanoparticles. The silver nanoparticles producing bacterial strains were screened on the basis of their growth on nutrient agar containing 1 mM silver nitrate ($AgNO_3$) at 30°C for 48 h. All the bacterial isolates were checked for



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Select: All None Selected:0
Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Sphingobacterium multivorum strain DNP 5 16S ribosomal RNA gene, partial sequence	2503	2503	100%	0.0	100%	KX011877.1
Sphingobacterium multivorum strain IAM 14316 16S ribosomal RNA gene, partial sequence	2503	2503	100%	0.0	100%	NR_040953.1
Uncultured bacterium clone ncd1047h04c1 16S ribosomal RNA gene, partial sequence	2492	2492	99%	0.0	99%	HM340323.1
Uncultured bacterium clone nbt120d01 16S ribosomal RNA gene, partial sequence	2492	2492	100%	0.0	99%	EU540592.1
Bacteroidetes endosymbiont of Metaseiulus occidentalis clone pAJ234 16S ribosomal RNA gene, partial sequence	2488	2488	100%	0.0	99%	AY753171.1
Uncultured bacterium clone ncd1052c09c1 16S ribosomal RNA gene, partial sequence	2486	2486	99%	0.0	99%	HM343902.1
Uncultured bacterium clone nbw774g02c1 16S ribosomal RNA gene, partial sequence	2486	2486	99%	0.0	99%	GQ016558.1
Uncultured bacterium clone C4_9 16S ribosomal RNA gene, partial sequence	2484	2484	99%	0.0	99%	KP142355.1
Sphingobacterium multivorum strain BAB-2422 16S ribosomal RNA gene, partial sequence	2483	2483	100%	0.0	99%	KF535161.1
Sphingobacterium multivorum strain NBRC 14947 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	NR_113706.1
Uncultured bacterium clone ncd490f01c1 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	HM328235.1
Uncultured bacterium clone ncd854c12c1 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	HM297816.1
Uncultured bacterium clone nbw772f12c1 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	GQ016378.1
Uncultured bacterium clone nbt24f07 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	FJ893750.1
Uncultured bacterium clone nbt24d02 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	FJ893738.1
Uncultured bacterium clone nbt75f11 16S ribosomal RNA gene, partial sequence	2481	2481	99%	0.0	99%	EU537672.1
Uncultured bacterium clone nbt182h08 16S ribosomal RNA gene, partial sequence	2481	2481	100%	0.0	99%	EU534708.1
Sphingobacterium sp. B2(2012) 16S ribosomal RNA gene, partial sequence	2477	2477	100%	0.0	99%	JX941542.1
Uncultured bacterium clone ncd490f09c1 16S ribosomal RNA gene, partial sequence	2475	2475	99%	0.0	99%	HM328259.1

Fig. 1 — (A) Graphic summary of BLAST analysis of 16 S rDNA sequence of DNP 5; (B) Phylogenetic tree constructed by UPGMA method; and (C) Description of BLAST analysis

extracellular biosynthesis of AgNPs and out of all bacterial isolates, DNP5 was selected for biosynthesis of AgNPs. On the basis of BLAST analysis, the 16S rDNA sequence of DNP5 exhibited 100 % identity to already reported sequence of *Sphingobacterium multivorum*. 16S rDNA sequence was submitted to NCBI (accession number KX011877) (Fig. 1).

Extracellular synthesis of silver nanoparticles was primarily confirmed by visual monitoring of colour change (transparent to dark brown). The test flask (supernatant, $AgNO_3$ (2 mM) and PVP exhibited light yellow colour within 2 min of incubation. The colour change was completed within 20 min of incubation (Fig. 2). However, control flasks containing only supernatant and silver nitrate remained colourless.

Further, biosynthesized AgNPs were characterized by UV–Visible spectroscopy analysis and spectrum exhibited peak at 418 nm (Fig. 3A). In the present study, one SPR symmetric shape peak was observed in UV-Visible spectral analysis of synthesized AgNPs. Hence, it could be inferred that the synthesized AgNPs are monodispersed and of spherical shape. On the basis of TEM analysis, silver nanoparticles were in the range of 17-30 nm and the average size of biosynthesized AgNPs was 22.28±0.2 nm (Fig. 3 B & C).

The crystal structure of biologically synthesized silver nanoparticles was determined by X-ray

Fig. 2 — Extracellular synthesis of silver nanoparticles

diffraction (XRD) analysis. The XRD pattern of synthesized AgNPs exhibited four peaks at 38.14, 44.35, 64.84 and 77. 53 which belong to 111, 200, 220, and 311 face centered cubic (fcc) planes respectively for metallic silver (Fig. 3D). Average size of biosynthesized silver nanoparticles was determined as 39.38 nm using Debye-Scherer's equation. The calculated size of nanoparticles was same as the size estimated by TEM analysis. The XRD spectrum for synthesized AgNPs was in agreement with JCPDS 04-0783 diffraction standard, which verified the crystalline nature of synthesized Ag-NPs.

The DLS analysis confirmed that silver nanoparticles synthesized using DNP 5 (*Sphingo-*

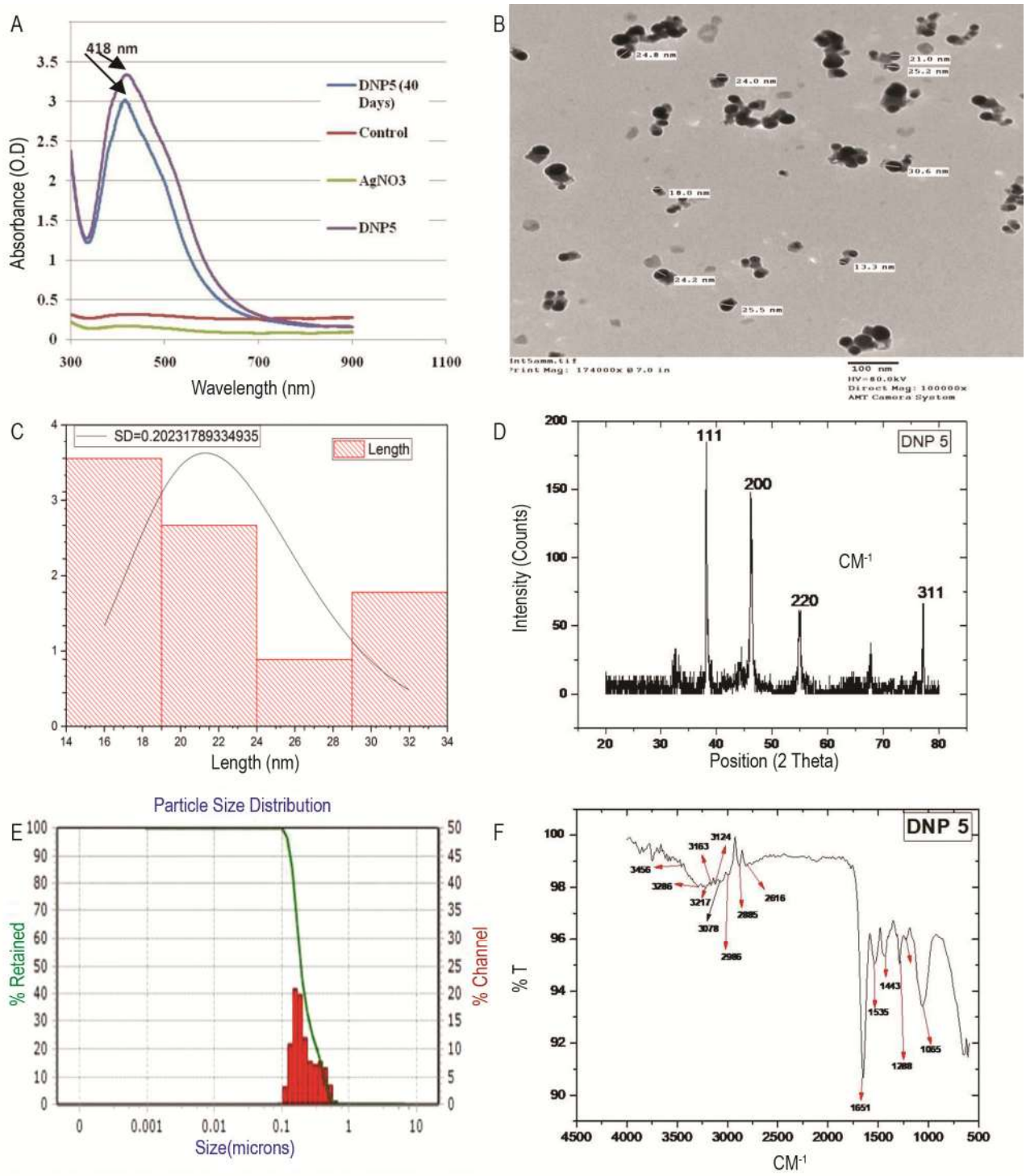

Fig. 3 — (A) UV-Visible spectral analysis; (B) TEM analysis; (C) XRD analysis; (D) DLS analysis; and (E) FTIR analysis of silver nanoparticles synthesized by using culture supernatant of DNP 5

bacterium multivorum) were in the range of 160-230 nm (Fig. 3E). Average size of DNP 5 synthesized silver nanoparticles was approximately 200 nm. The particles bear a charge of -41.55 mV. The polydispersity index (PDI) for biosynthesized AgNPs was less than 0.276.

The silver nanoparticles synthesized using DNP 5 (*Sphingobacterium multivorum*) exhibited peaks at 3456 cm^{-1}, 3124 cm^{-1}, 2986 cm^{-1}, 1774 cm^{-1}, 1651 cm^{-1}, 1535 cm^{-1}, 1288 cm^{-1}, 1227 cm^{-1} and 1065 cm^{-1} in FTIR spectrum (Fig. 3F). The peaks at 1651

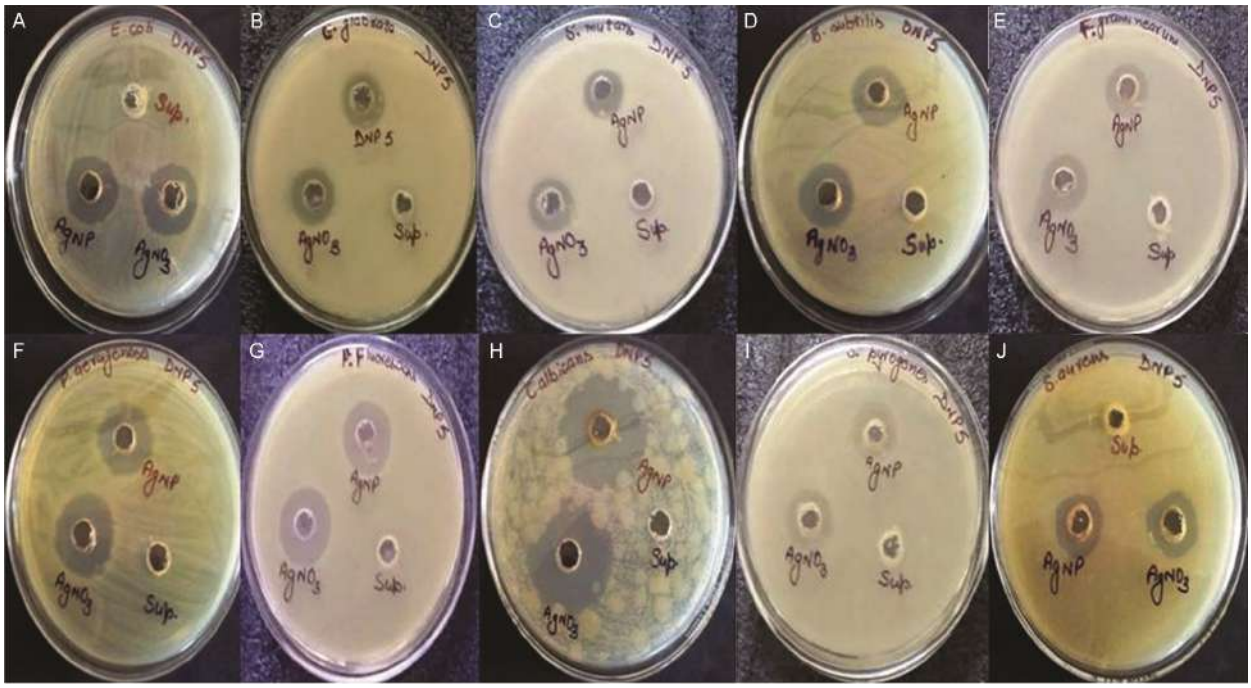

Fig. 4 — Antimicrobial activity of AgNPs synthesized by bacterial DNP 5 against test pathogens (A) *Escherichia coli*; (B) *Candida glabrata*,; (C) *Streptococcus* mutans; (D) *Bacillus subtilis*,; (E) *Fusarium graminearum* (F) *Pseudomonas aerugenosa*; (G) *Pseudomonas fluorescens*; (H) *Candida albicans*; (I) *Streptococcus pyrogenes*; and (J) *Staphylococcus aureus*

cm^{-1} corresponds to stretching of C=O amide I bands of peptide linkage and 1288 cm^{-1} corresponds to bending of NH and CN stretching of peptide. The peak at 1065 cm^{-1} indicated $–CH_3$ bending in amino acid and the peak at 1443 cm^{-1} was related to C=N vibration and 1535 cm^{-1} confirmed change in NO asymmetric stretch due to nitro compound. The peak at 2885 cm^{-1} and 3163 cm^{-1} was observed due to -CH vibration. The peak at 2616 cm^{-1} and higher energy region peaks at 3217, 3456 cm^{-1} correspond to alcohol or phenol O-H stretching. The peak at 3078 cm^{-1} and 3286 cm^{-1} indicated –NH stretching.

The energy dispersive X-ray analysis (EDX) is used to get the qualitative and quantitative information about elements. EDX exhibited strong signal in silver region and substantiated the formation of silver nanoparticles. EDX analysis demonstrated silver (66.2 %), chlorine (18.92%) and oxygen (14.8%) as elemental constituents of fabricated nanoparticles. On the basis of these characterizations, this could be concluded that synthesized particles are of silver and in nano range.

Further, the stability of synthesized AgNPs solutions was determined by UV-Vis spectra at intervals of 1, 15 and 40 days after storage at 4ºC. There was no change in the peak position even after 40 days (Fig. 3A). The constant position of absorbance peak confirmed that nanoparticles did not aggregate. Further, autoclaved nanoparticles also showed the same antimicrobial activity as shown by freshly synthesized silver nanoparticles. This can be inferred that nanoparticles colloidal solution could be stored for 40 days and were stable even at high temperature.

The antimicrobial activity of biosynthesized AgNPs against different test pathogens is shown in Fig. 4. The presence of inhibition zone signified the antimicrobial action of synthesized AgNPs. The inhibitory effect was present against all the test pathogens. The AgNPs synthesized by DNP 5 showed a maximum effect against *C. albicans* with an inhibition zone of 27.16±0.28 mm followed by *S. aureus* with a zone of inhibition of 24.66±0.57 mm. The minimum effect of AgNPs was observed against *S. mutans* (15.66±0.57 mm) and *Fusarium graminearum* (16.66±0.57). The Antimicrobial activity of biofabricated AgNPs was notably higher than $AgNO_3$ against all test pathogens Fig. 5.

In the present study, TEM analysis has also confirmed the attachment and penetration of silver nanoparticles in *Pseudomonas aerugenosa* (Fig. 6). The MIC values of silver nanoparticles for different strains were measured (Fig. 7). The MIC of silver

nanoparticles was measured as 19.5 µg for *E. coli* and *S. aureus*, 39 µg for *P. aerugenosa* and *C. tropicalis* and 78 µg for *B. subtilis*. At concentrations less than MIC, inhibition zone was not observed. The diameter of zone increased with silver concentration confirming that the antimicrobial activity is directly proportional to silver nanoparticles concentration.

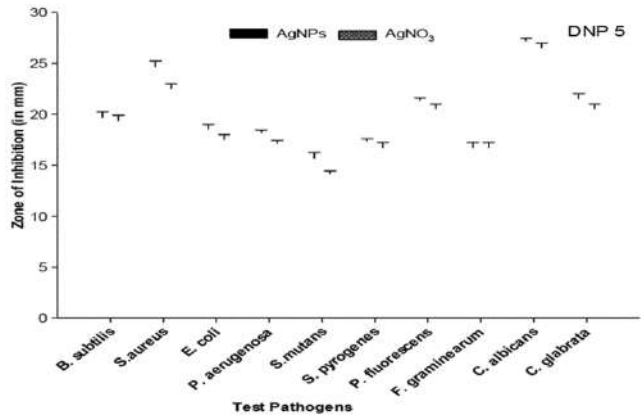

Fig. 5 — Inhibition zone (diameter in mm) of AgNPs synthesized by DNP 5

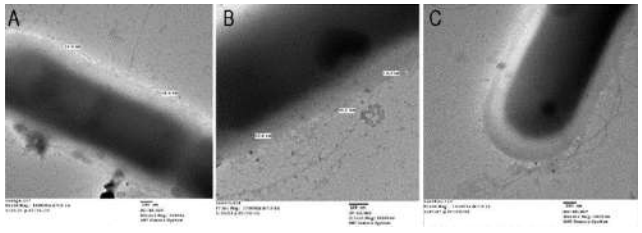

Fig. 6 — TEM analysis of interaction of AgNPs with *P. Aerugenosa*

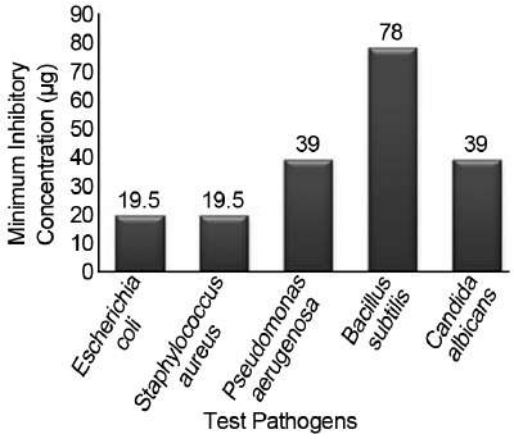

Fig. 7 — Minimum inhibitory concentration (MIC) of biologically synthesized AgNPs against different test pathogens

The synergistic effect of AgNPs with norfloxacin and kanamycin was observed against all selected pathogens except *S. aureus* shown in Table 1. The highest effect was against *P. aerugenosa* for both the antibiotics. *P. aerugenosa* was not sensitive to kanamycin but addition of AgNPs rendered them sensitive. The increase in antimicrobial activity of rifampicin and erythromycin after AgNPs addition was against all pathogens. However, in case of amoxyclav synergistic effect was only against *S. aureus* and *P. aerugenosa*.

The antimicrobial effect of antibiotic combined with AgNPs was statistically different from antibiotic alone. The measured synergistic effect of AgNPs was significant with all the classes of antibiotics.

Discussion

Green synthesis process is rapid, easy to perform, economic and also avoids the use of toxic chemicals and thus has overcome the limitations associated with physical/chemical synthesis methods[19,20]. Biological synthesis methods being ecofriendly are seeking more attention than other methods. Different biological materials like plants, bacteria and fungi have been exploited for the synthesis of nanoparticles[10,11,17,19-24]. Various studies have already exploited different bacterial strains for the synthesis of stable silver nanoparticles (AgNPs)[17,19,22,24].

In the present study, the selected bacterial strain DNP5 identified as *Sphingobacterium multivorum* showed ability to synthesize AgNPs by extracellular mechanisms. Extracellular synthesis method is preferred over intracellular synthesis due to easy scale up and purification which leads to reduced production cost. The screening of microbial isolate for silver nanoparticle synthesis is generally carried out on the basis of colour change[17]. The change in colour was due to the surface plasmon resonance (SPR) of silver nanoparticles formed in the medium[12,24]. The metal type, size and morphology of nanoparticles and

Table 1 — Synergistic effect of silver nanoparticles with antibiotics

Test pathogens	Nx	Nx^+	Rif	Rif^+	E	E^+	K	K^+	Amx	Amx^+
Escherichia coli	27	29	32	33	21	23	24	25	40	40
Staphylococcus aureus	30	30	15	17	25	27	21	21	10	13
Pseudomonas aerugenosa	20	23	13	15	11	13	0	14	0	11
Bacillus subtilis	33	36	21	23	28	30	23	24	30	30
Candida tropicalis	31	33	-	10	12	14	21	23	-	-

dielectric properties of the medium may affect SPR of silver nanoparticles[15,24-27].

Biosynthesized AgNPs were further characterized by UV–Vis spectroscopy, which measures the absorption spectra of AgNPs. The presence of characteristic peak at 418 nm confirmed the synthesis of AgNPs. The UV-Vis absorption peak around 420 nm is due to surface plasmon resonance (SPR) of AgNPs[28]. A single peak represents the spherical shape nanoparticles and two or more peak belongs to the anisotropic molecules[29]. The XRD spectrum of synthesized nanoparticles was in agreement with diffraction standard JCPDS 04-0783, which confirms the presence of elemental silver.

TEM images of biosynthesized AgNPs confirmed the spherical shape and monodispersity of nanoparticles. On the basis of TEM analysis, nanoparticles are in the range of 17-30 nm and average size of synthesized AgNPs is 22 nm.

The dynamic light scattering (DLS) calculate hydrodynamic size of the AgNPs and includes the size of stabilizers absorbed. Therefore, the size measured by DLS is larger than measured by other techniques. Stable AgNPs bear a minimum of ± 30 mV and the polydispersity index (PDI) below 0.3[30]. This inferred the stability of biologically synthesized AgNPs. The result of present study was consistent with the results of already reported studies. The mechanism of synthesis of nanoparticles is not clear; some studies have reported the role of nitrate reductase in reduction of silver nitrate to silver[31]. Different peaks observed in FTIR spectrum indicated the role of biological molecules viz. proteins or enzymes (present in bacterial supernatant) in the synthesis and stability of AgNPs. Stable absorption peak of synthesized AgNPs in UV-Visible spectrophotometric analysis confirmed the stability of nanoparticles. Antimicrobial activity of nanoparticles remained same even after incubating them at high temperature. Previous studies have already reported that PVP coated AgNPs are considered as the most stable nanoparticles in OECD recommended media (chloride present)[32].

Various studies have already documented the antimicrobial activity of AgNPs against micro-organisms[19,33-37]. In the present study, the biosynthesized AgNPs exhibited considerable antimicrobial activity against both bacterial strains and fungi strains. The precise mode of antimicrobial activity is not clear yet, but previous data reported that AgNPs due to small size can easily enter into bacterial cell, harm cell membrane and functions of cell, enhance the free radicals production and finally may leads to cell death[38]. The present study on the basis of TEM analysis also confirmed the attachment and penetration of AgNPs to bacterial membrane.

The Gram-negative bacteria possess external lipopolysaccharides and an inner thin peptidoglycan layer[39]. It is also proposed that the negative charges on lipopolysaccharides may attract positively charged AgNPs[40]. However, in the present study, synthesized nanoparticles are negatively charged. These AgNPs may inhibit the Gram-negative bacteria by metal reduction[41-43]. Different studies have reported different MIC values for the same pathogens[44-47]. Various factors, such as size and shape of nanoparticles, strains of pathogens, pathogen source and stabilization method can affect MIC value[48]. The antimicrobial effect of AgNPs is inversely proportional to their size. Nanoparticles, due to large surface to volume ratio, can easily make contact with pathogens that impart them high biocidal activity. Biosynthesized nanoparticles exhibited synergistic antimicrobial effect in combination with different classes of antibiotic. Various studies have supported that the addition of AgNPs can enhance the antimicrobial effect of antibiotics[49-51]. Different Level of activity increment is reported with different classes of antibioitics[52]. Combination of nanoparticles and antibiotic may decrease the required concentration of antibiotic and may help to fight against resistant microbial infections[53].

Conclusion

In the present study, we developed a rapid, economic and ecofriendly method for the synthesis of stable silver nanoparticles (AgNPs). Size and crystal nature of synthesized particles was in nano range as confirmed by TEM and XRD analysis, respectively. Synthesized AgNPs exhibited broad spectrum antimicrobial activity against different test pathogens and showed synergistic effect with selected classes of antibiotics. Therefore, biosynthesized nanoparticles possess great application potential in the medical field and can be explored to fight against alarming issue of antimicrobial resistance. However, a further mechanistic insight into the antimicrobial action of AgNPs and *in vivo* studies related to toxicity of biofabricated AgNPs need to be conducted before commercial applications.

Conflict of interest

Authors declare no competing interests.

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Integration among Selected Asian Stock Markets

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Abstract: The purpose of present study is to investigate the integration among selected Asian stock markets (India, Japan, H.K., China, South Korea, S. Lanka, Pakistan, Philippines, Taiwan and Indonesia). Daily closing prices of representative indices of selected stock exchanges of Asia for the period from 1 Jan. 2001 to 28th Feb. 2023 have been used for the purpose of analysis. Statistical tools namely descriptive statistics and correlation analysis and econometric techniques such as Augmented Dickey Fuller test, Granger Causality test and Johansen's cointegration test have been used. The results of granger causality test found that both bidirectional and unidirectional causality occurs in most of the cases and also found long run equilibrium relationship as well as short run relationship among the selected stock markets of Asia. It concludes that investors cannot get abnormal gains by portfolio diversification in Asian stock markets under the study.

Keywords: Stock market integration, portfolio diversification and Granger causality.

INTRODUCTION

Financial market reforms in international market during last decades break up the boundaries of world market for international investors. The globalization of the world stock markets is the most significant development that has occupied during the last years. Various factors such as liberalization of financial policies, increased transparency, deregulation of stock markets and change in political and economic environment of international market increased confidence of investors in stock market to invest. Because of the revolution of IT with high speed internet, the information is available to one at any place at any time at low cost. Capital markets are dependent on information and the information revolution has transformed these markets world over. Investors can now keep track of the movements of capital market and they react to the flow of information from around the world. These dealing sometime create huge waves of panic actions and reactions affecting global markets one by one. Investors, Govt. and institutions are concerned about the visible linking of geographically separated markets. The topic of stock market integration is an emerging issue in financial research now a days and it is also important for economist, researchers, investors, government and policy makers. The benefits of portfolio diversification may be reduced if the stock markets are found to be integrated with each other. If stock markets move together then investing in various markets would not generate any long term gain to portfolio diversification. So it is important to know for both investors and academicians the existence of integration among stock markets. The fundamental objective of diversification of portfolio is to minimize systematic risk. For an investor, diversification of portfolio in the international market is justifiable if and only if the gains from it exceed those from diversification in the domestic market. Stock market integration not only have implications for portfolio diversification, but also have importance for macroeconomic policies that influence trade and fiscal balances of countries and the financial policies of different agents within the capital improving economy. The issue is important for policy makers for the following reasons too: if stock markets are found to be closely linked then there is a danger that shocks in one market may spill over to other markets. The benefits due to stock market integration as it enables capital accumulation, skill and technology transfer through foreign direct invests. The present study is a humble attempt to analyze the integration among selected Asian stock markets to reap abnormal gains by the portfolio diversification in Asia. If integration exits, the strategy of diversifying one's portfolio may no longer apply.

REVIEW OF EMPIRICAL EVIDENCE

The integration among stock markets is a subject that has attracted worldwide concern. This section of paper presents a detailed review of the studies concentrating on Asian economies. These studies are reviewed for the purpose of the current study and are included herein. A study by Bahng (2003) found positive correlation between Indian stock market and other Asia's emerging markets. Indian stock market move towards the integration with other Asian markets. Nath and Verma (2003) showed no long run relationship between the stock markets of South Asia (India, Taiwan and Singapore). Narayan, Smyth and Nandha (2004) found a long run relation between the stock prices of four countries (Bangla-

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desh, India, Pakistan and Sri Lanka). There was unidirectional causality from stock prices in Pakistan to India, Sri Lanka to India and from Pakistan to Sri Lanka. The impulse response function showed that Bangladesh was the most exogenous of the four markets. Azad found long run relationship between the three markets (China, Japan and South Korea). Raju and Khanpuri (2009) revealed that all Asian stock markets (China, India, Thailand, Malaysia, Indonesia and South Korea) share positive but low correlations. They also found that international investors can get maximum benefits by investing in China, India and S.K. Gupta and Aggarwal (2011) found very weak correlation between the Indian stock market and H.K., Indonesia, Malaysia and Japan. They also found that Indian stock market offer diversification benefits to international investors looking for investment in the Asia Pacific Region. Sharma and Bodla (2011) found the existence of opportunity for diversification among South Asian countries (India, Pakistan and S. Lanka). Saha and Bhunia (2012) found the short run relationship among Asian stock markets and also found the existence of portfolio diversification opportunity for international investors in South Asian countries. Roa (2014) investigated the interrelationship among Indian stock market and selected stock prices of the Asia pacific region (India, Australia, Hong Kong, Indonesia, Japan, Malaysia, South Korea, Singapore and Taiwan) by taking monthly closing prices for the period from April 2004 to March 2014. The author used econometric tools and found both bidirectional and unidirectional causality among the selected indices and also the presence of long run equilibrium relationship between the SENSEX and other selected market indices. Babu, Hariharan and Srinivasan (2016) investigated the integration of Asian stock markets (Australian, India, China, Hong Kong and Japan) by using daily data from April 2009 to March 2014 and found long run relationship among the selected indices. Also found unidirectional causal relationship between Australia, Hong Kong and China. Between Indian, H.K., witnessed unidirectional causation with China. Patel R.J. (2017) found that BSE does not have a significant correlation with rest of the markets. Long term association and co integration was also found between related 14 stock exchanges. BSE was integrated with other markets in short run as well as in long run. Saji T.G. (2022) reinvestigated stock market linkages in Asian region (Japan, Singapore, South Korea, India and China) using monthly data over the period 1999-2019. By using VECM the author found weak price convergence among Asian markets and suggest several opportunities for global investors to optimize returns through portfolio diversification across leading stock markets of the region (Asia). On the basis of review of literature, we see that different methods of analysis have been used by the researchers about integration of stock markets of world. Correlation analysis, ADF test, Granger Causality test, Johansen's cointegration test have been used to analyze the data about stock market integration. Only one or at the most two methods were used in different researchers. *Bodla and Turan (2004), Chang et al (2006), Raju and Khanpuri (2009), Sharma and Gupta (2011)* and *Tripathi et al (2013)* used correlation analysis in their studies. *Yang et al. (2003), Hoque (2007), Nath and Verma (2003), Ibrahim (2005), Cheign and Glascock (2005) and Palamalai (2013)* were used cointegration test for data analysis. Granger Causality test has been used by many researchers in their studies to find the cause and effect relationship among different stock markets such as *Wong et al (2004), Tripathi and Sethi (2010), Iqbal et al (2011), Roa (2014)* and *Seth* and *Sharma (2015)*. So, we also use these econometric methods in our study.

The current study is the attempt to further investigate the long run, short run and causal relationship among the Asian stock markets for the period from Jan. 2001 to Feb. 2023. We will try to find out the existence of enough opportunities for diversification among the stock exchanges of Asia. Specifically, the aim of the present study is to achieve the following objectives:

1. To examine the correlation among the selected stock markets of Asia.
2. To study the long run equilibrium relationship among the selected stock markets.
3. To study the short run relationship among the selected Asian stock markets.
4. To investigate the cause and effect relationship among selected stock indices.

RESEARCH METHODOLOGY

Database

There were many studies on integration among international stock markets but very few studies are there on Asian stock market integration. The present study will fill this research gap. The proposed research is a study of the integration among Asian stock markets namely India, Japan, Hong Kong, China, South Korea, Sri Lanka, Pakistan, Philippines, Taiwan and Indonesia. The study used one stock exchange from each of the ten countries as a representative of the respective country. Bombay stock exchange, Colombo stock exchange, Karachi stock exchange, Shanghai stock exchange, Tokyo stock exchange, Hong Kong stock exchange, Korea stock exchange, Taiwan stock exchange, Indonesia stock exchange and Philippines stock exchange are selected as the benchmark stock exchange of India, Sri Lanka, Pakistan, China, Japan, Hong Kong, South Korea, Taiwan, Indonesia and Philippines respectively. BSE SENSEX index, All Share Price Index, KSE 100 index, SSE Composite index, NIKKIE 225, Hang Seng Index, Korea Composite Stock Price Index, Taiwan Capitalization Weighted Stock index, IDX Composite index and PSEi index are used as the representative index of above mentioned stock exchanges. This study was based on secondary data on the daily closing prices of the selected indices for the period started from 1 Jan. 2001 to 28th Feb. 2023 collected from www.yahoofinance.com and also verified from websites of different stock markets of the selected countries. E-Views and SPSS software were used to apply different econometric tools and statistical methods. Out of the time for which the data about stock indices were taken, it is observed that on few days, one or two of the exchanges were open while other(s) was (were) closed. The data for all the days on which any of the stock exchanges under reference were open, has been taken. As a result, there were missing values in the data of some of the stock exchanges for some days. These missing values have been

filled-up by taking average of the two nearest cases[1]. The total number of observations were 5781.

METHODOLOGY

Tools and techniques

Natural log of selected series gave the daily return of the given indices under the study. The formula of calculating the natural log of indices/closing prices was given as follows:

$$R_t = \ln (P_t / P_{t-1}) \quad (1)$$

Where:

R_t = Return on day 't',

P_t =Index closing value on day 't'

P_{t-1} = Index closing value on day 't-1'

ln= Natural log

Descriptive statistics was used to get an insight in to the data. The descriptive statistics included mean percent return (over the entire reference period), maximum and minimum return, and standard deviation, skewness, kurtosis and Jarque bera statastics. Karl Pearson's coefficient of correlation was used to see the correlation among selected stock markets. The modern portfolio theory propounded by Markowitz (1952), said that the benefits of diversification of portfolio can be reaped when the return on assets in which funds are invested have low correlation. It two stock markets have low correlation; making investment in them can lead the reduction of the systematic risk of the portfolio. If the correlation coefficient was more than 0.05, the correlation was significant otherwise not. The formula of correlation coefficient was as follows:

$$r = \frac{\sum(x-\bar{x})(y-\bar{y})}{N\sigma_x\sigma_y}. \quad (2)$$

Econometric analysis can be performed on stationary series. In order to check the stationary nature of all series, the augmented dickey fuller test was performed under the unit root test. The ADF test was used at level and at first difference on closing prices. In order to make the series stationary, we took log of the selected series and arrive at the daily returns of selected series. A process was said to be stationary if it's mean and variance remain unchanged over time. In other words, a time series was said to be stationary if it's probability distribution remains unchanged as time proceeds. To test the unit root problem, the most widely used test was ADF. The general form of ADF test can be written at level and first difference were as follows:

$$\Delta Y_t = \alpha + \beta t + \delta Y_{t-1} + \sum_{i=1}^{k} \gamma i \Delta Y_{t-i} + \mu_t \quad (3)$$

$$\Delta\Delta Y_t = \alpha + \beta t + \delta \Delta Y_{t-1} + \sum_{i=1}^{k} \gamma i \, \Delta\Delta Y_{t-i} + \mu_t \quad (4)$$

Hence, if the hypothesis, $\delta = 0$ is rejected for the above equations then it can be concluded that the time series did not have a unit root and is integrated of order zero I(0) i.e. it has stationary properties.

Johansen's cointegration test was applied on closing prices of the selected indices (which are not stationary in nature) to see the long run relationship among selected indices. The Johansen (1988) (1991, 1995) procedure tests the presence of long run relationship between the variables and to perform the cointegration analysis. If the two or more series were found to be co-integrating, then they were said to have common stochastic trend. They tend to move together in the long run. EViews supports VAR-based cointegration tests using the methodology developed in Johansen (1991, 1995) performed using a Group object or an estimated Var object. Consider a VAR of order :

$$Y_t = A_1 y_{t-1} + \ldots + A_p y_{t-p} + B x_t + \varepsilon_t \quad (5)$$

where y_t is a k-vector of non-stationary I(1) variables, x_t is a d-vector of deterministic variables, and ε_t is a vector of innovations. If there was not the existence of any long run comovement among the indices, then VAR model has been used. [2]If the cointegration exists, Vector Error Correction Model (VECM) is appropriate for further econometric analysis to discover the short run relationships. If the variables were cointegrated than VECM would be used for further analysis (Dhanraj, Gopalaswamy and Suresh, 2013, Aggarwal and Khurana, 2018). At the stationary log series, we applied granger causality test to see whether any indices granger causes other indices or not or to see the cause and effect relationship between the selected indices. The Granger (1969) approach to the question of whether x causes y is to saw how much of the current y can be explained by past values of y and then to see whether adding lagged values of x can improve the explanation. y is said to be Granger-caused by x if x helps in the prediction of y , or equivalently if the coefficients on the lagged x 's was statistically significant. It is pertinent to note that two-way causation is frequently the case; x Granger causes y and y Granger causes x. It was important to note that the statement "x Granger causes y" did not imply that y is the effect or the result of x. Granger causality measures precedence and information content but did not by itself indicate causality in the more common use of the term. In Granger's Causality, there were bi variate regressions of the under-mentioned form:

$$Y_t = \alpha_0 + \alpha_1 Y_{t-1} + \ldots + \alpha Y_{t-1} + X_{t-1} + \ldots + \beta X_{t-1} + \varepsilon_t \quad (6)$$

$$X_t = \alpha_0 + \alpha_1 X_{t-1} + \ldots + \alpha X_{t-1} + {}_1 Y_{t-1} + \ldots + \beta Y_{t-1} + \mu_t \quad (7)$$

for all possible pairs of (X, Y) series in the group. Where ε_t and μ_t are two white noise random disturbance terms. In equation (6), the study took lags ranging from 1 to l. In Granger's model, one can pick a lag length, l that corresponds to reasonable beliefs about the longest time over which one of the variables could help to predict the other. The reported F-statistics were the Wald statistics for the joint hypothesis:

$$\beta 1 = \beta 2 = \beta 3 = \beta t = 0 \quad (8)$$

[1] Sharma G. D. and Bodla B.S., *"Interlinkages Among Stock Markets of South Asia",* Asia Pacific Journal of Business Administration, 2011, Vol. 3, No. 2, pp. 136.

[2] Vardhan H., Sinha P. and Vij M., *"Behavior of Indian Sectoral Stock Price Indices in the Post Sub Prime Crises Period",* Journal of Advances in Management Research, 2015, Vol. 12, No.1, pp. 15-29 (17).

Table 1. Descriptive Statistics.

	India	Japan	H.K.	China	S.K.	Sri Lanka	Pakistan	Philippines	Taiwan	Indonesia
Mean daily % return	0.046	0.01	0.004	0.007	0.02	0.052	**0.056**	0.025	0.02	0.48
Median daily % return	0.07	0.03	0.01	0	0.05	0.008	0.065	0.008	0.03	0.06
Maximum daily % return	15.99	9.56	13.4	9.4	11.28	13.3	8.25	16.17	12.19	7.6
Minimum daily % return	-14.1	-12.1	-13.5	-9.25	-12.8	-13.9	-7.7	-13.08	-12.29	-10.9
Std. Dev. daily % return	1.34	1.39	1.40	**1.447**	1.3	1.11	1.23	1.23	1.22	1.22
Skewness	-0.42	-0.49	0.007	-0.35	-0.49	-0.49	-0.39	-0.28	-0.23	-0.65
Kurtosis	14.25	9.24	11.43	8.56	10.5	26.73	6.7	14.96	9.59	10.3

Source: Data Processed through E-Views Software.

Table 2. Correlation Analysis.

	India	Japan	H.K.	China	S.K.	Sri Lanka	Pakistan	Philippines	Taiwan	Indonesia
India	1									
Japan	0.34	1								
H.K.	0.48	**0.55**	1							
China	0.19	0.24	0.42	1						
S.K.	0.41	**0.58**	**0.60**	0.23	1					
Sri Lanka	0.06	0.07	0.06	0.02	0.05	1				
Pakistan	0.12	0.09	0.1	0.07	0.11	0.02	1			
Philippines	0.27	0.33	0.34	0.15	0.34	0.06	0.1	1		
Taiwan	0.34	0.47	**0.52**	0.22	**0.6**	0.07	0.10	0.33	1	
Indonesia	0.41	0.36	0.46	0.19	0.43	0.05	0.1	0.39	0.4	1

Source: Data Processed through E-Views Software.

The null hypothesis was that x does not Granger-cause y in the first regression and that y did not Granger-cause x in the second regression.

Results of the Study

The results of descriptive statistics (applied on return series) are as follows:

The descriptive statistics shows that highest mean return is 0.07% of Pakistan. The std. dev. of China is highest (1.44%) which show the highest risk factor. All the series are negatively skewed except Hong Kong and all series are leptokurtic in nature.

Table **2** shows the results of correlation analysis (applied on return series) and found significant correlation of Japan with South Korea and Hong Kong. Hong Kong stock market was found highly correlated with South Korea and Taiwan. There are also a significant positive correlation between South Korea and Taiwan.

Table **3** shows the results of ADF test (applied on closing prices). It shows that the series are non stationary in nature at level but at first difference, the t-statistics is more than the test critical value (irrespective of sign) in all the cases, which confirms that all the series are stationary in nature at first difference.

The optimum Lag test is very important for further analysis and the next step is to select the optimum lag length before we conduct the Johansen co integration test.

A total of five criteria are taken in to account in the study. LR sequential modified LR test statistic, final prediction error, Akaike information criterion, Schwarz information criterion and Hannan Quinn information criterion are used, -

Table 3. Augmented Dickey Fuller Test.

	At Level			At First Difference		
	Intercept	Trend	None	Intercept	Trend	None
India	0.75	-1.83	2.44	-31.51	-31.51	-31.41

Japan	0.65	-2.44	0.55	-52.37	-52.38	-52.36
H.K.	-1.99	-2.59	-0.31	-78.05	-78.05	-78.06
China	-2	-2.53	-0.33	-34.83	-34.83	-34.83
S.K.	-1.7	-2.85	0.57	-51.58	-51.58	-51.58
S. Lanka	-1.1	-2.89	0.51	-17.59	-17.59	-17.55
Pakistan	-0.79	-2.04	0.89	-68.3	-68.29	-68.26
Philippines	-1.19	-1.8	0.44	-75.61	-75.6	-75.6
Taiwan	-0.51	-2.25	1.14	-74.72	-74.72	-74.7
Indonesia	-0.64	-3.11	1.44	-74.5	-74.49	-74.45

Source: Data Processed through E-Views Software

		Intercept	trend	None
Level of significance	1%	-3.43	-3.95	-2.56
	5%	-2.862	-3.41	-1.94
	10%	-2.567	-3.127	-1.61

Table 4. VAR Lag Order Selection Criteria.

Lag	LogL	LR	FPE	AIC	SC	HQ
0	-491771	NA	4.51E+61	170.3432	170.3547	170.3472
1	-336440	310069.5	2.01E+38	116.5743	116.7012	116.6185
2	-335962	952.8386	1.76E+38	116.4433	116.6856*	116.5276*
3	-335863	196.1741	1.76E+38	116.4438	116.8014	116.5682
4	-335743	239.4241	1.75E+38	116.4367	116.9097	116.6012
5	-335625	232.9546	1.74e+38*	116.4306*	117.019	116.6353
6	-335539	170.6868	1.75E+38	116.4354	117.1391	116.6802
7	-335440	196.2209	1.75E+38	116.4356	117.2547	116.7206
8	-335351	175.5765*	1.75E+38	116.4394	117.3738	116.7645

* indicates lag order selected by the criterion

LR: sequential modified LR test statistic (each test at 5% level)

FPE: Final prediction error

AIC: Akaike information criterion

SC: Schwarz information criterion

HQ: Hannan-Quinn information criterion

Table 5. Johansen's Cointegration Test.
Unrestricted Cointegration Rank Test.

Hypothesized No. of CE(s)	Eigenvalue	Trace Statistic	0.05 Critical value	Prob.**	Max-Eigen Statistic	0.05 Critical Value	Prob.**
None *	0.0139	261.38	239.23	0.0033	81.22	64.5	0.0006
At most 1	0.008	180.16	197.37	0.25	47.23	58.43	0.399
At most 2	0.0061	132.92	159.52	0.53	35.68	52.36	0.75
At most 3	0.0046	97.24	125.61	0.68	26.47	46.23	0.92
At most 4	0.0044	70.49	95.75	0.70	25.47	40.07	0.73
At most 5	0.00279	45.01	69.81	0.83	76.18	33.87	0.94
At most 6	0.0023	28.83	47.85	0.77	13.79	27.58	0.83

At most 7	0.0016	15.03	29.79	0.77	9.56	21.1	0.78
At most 8	0.0009	5.47	15.49	0.75	5.35	14.26	0.69
At most 9	0.00001	0.11	3.84	0.73	0.11	3.84	0.73

Source: Data Processed through E-Views Software.

Trace statistics and Max-eigenvalue test indicates 1 cointegrating eqn(s) at the 0.05 level.

* denotes rejection of the hypothesis at the 0.05 level.

**MacKinnon-Haug-Michelis (1999) p-values.

which are shown that in the table **4**. The FPE and AIC tests show the lag length 5, which is very high. So, if we take such a high lag length, there is more risk. Therefore, lag length 2 as recommended by SC and HQ would be adopted in the present study. We will further proceed for Johansen's Cointegration test now.

Table **5** shows the results of cointegration test (applied on closing prices) and indicate that there is only one cointegrating equation at trace statistics and maximum eigenvalue statistics which prove the existence of long run equilibrium relationship between Asian stock markets. Now, we will proceed for VECM for further analysis.

Table **6** shows the VECM results. In the Indian stock equation, 1 period lagged changes in Hong Kong, Chinese, South Korean, Philippines and Taiwan stock prices are significant. In the Japanese and Hong stock equation, at 1 period lagged changes in all the countries stock prices are significant except Sri Lanka, Pakistan and Indonesian stock prices. In the Chinese stock equation, 1 period lagged changes in Indian, H.K., South Korean, Philippines and Taiwan stock prices are significant. In the South Korean stock equation, 1period lagged changes in Indian, H.K., Chinese, Sri Lanka and Taiwan stock prices are significant. In the Pakistan stock equation, 1 period lagged changes in Chinese stock prices are significant. In the Philippines stock equation, 1 period lagged changes in H.K, S.K., Taiwan and Indonesian stock prices are significant. In the Taiwan stock equation, 1 period lagged changes in Indian, Chinese, Philippines and Indonesian stock prices are significant. In the Indonesia stock prices, 1 period lagged changes in Indian, S.K., Philippines and Taiwan stock prices are significant. So, we can conclude that there is a short run relationship among the stock indices of Asia.

Now, we proceed for granger causality test for further analysis. We accept the null hypothesis for the cases with probability value above 0.05, we reject the ones with lesser than 0.05. The table **7** shows the results of granger causality test (applied on returns) as follows:

Table 6. (Vector Error Correction Model).

Error Correction:	D(Ind)	D(Japan)	D(HK)	D(China)	D(SK)	D(SL)	D(Pak)	D(Phil.)	D(TW.)	D(Indo.)
CointEq1	-0.32	0.29	0.43	0.19	0.39	0.02	0.01	0.12	-0.11	0.07
	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
	[-17.3]*	[15.2]*	[23.2]*	[9.2]*	[22.4]*	[1.46]	[0.36]	[7.10]*	[-6.60]*	[4.14]*
D(India(-1))	-0.30	-0.05	-0.15	-0.06	-0.14	0.00	0.02	0.01	0.13	0.05
	-0.02	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	-0.01
	[-19.1]*	[-3.26]*	[-9.01]*	[-3.51]*	[-9.05]*	[0.26]	[1.53]	[0.59]	[8.75]*	[3.11]*
D(Japan(-1))	-0.02	-0.54	0.05	0.00	0.02	0.01	0.02	0.02	-0.02	-0.02
	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
	[-1.50]	[-38.2]*	[3.79]*	[0.18]	[1.31]	[1.12]	[1.26]	[1.85]	[-1.29]	[-1.61]
D(H.K.(-1))	-0.14	0.15	-0.33	0.13	0.17	0.01	0.01	0.09	0.01	0.02
	-0.02	-0.02	-0.02	-0.02	-0.02	-0.01	-0.02	-0.02	-0.02	-0.02
	[-8.50]*	[8.2]*	[-19.2]*	[6.67]*	[10.3]*	[0.54]	[0.38]	[5.87]*	[0.53]	[1.21]
D(China(-1))	-0.04	-0.03	-0.05	-0.51	-0.02	0.00	-0.02	0.01	-0.03	-0.01
	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
	[-3.13]*	[-2.47]*	[-4.33]*	[-39.8]*	[-2.20]*	[-0.48]	[-2.20]*	[1.06]	[-2.70]*	[-0.50]
D(SK(-1))	-0.09	0.17	0.24	0.08	-0.32	-0.01	0.02	0.09	-0.03	0.05

	-0.02	-0.02	-0.02	-0.02	-0.02	-0.01	-0.02	-0.02	-0.02	-0.02
	[-5.10]*	[9.12]*	[13.1]*	[4.06]*	[-18.8]*	[-0.37]	[1.21]	[5.74]*	[-1.57]	[3.09]*
D(SL(-1))	-0.02	0.02	0.02	0.01	0.03	-0.43	0.00	-0.01	0.00	-0.02
	-0.01	-0.01	-0.01	-0.02	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
	[-1.32]	[1.36]	[1.59]	[0.84]	[1.95]*	[-35.6]*	[0.25]	[-0.76]	[0.09]	[-1.22]
D(Pak(-1))	-0.02	0.01	0.00	0.01	0.01	0.00	-0.47	-0.01	0.02	-0.01
	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
	[-1.7]	[0.87]	[0.15]	[0.57]	[0.41]	[0.30]	[-40.1]*	[-0.61]	[1.9]*	[-1.1]
D(Phill.(-1))	-0.09	-0.04	-0.04	-0.03	-0.02	0.01	0.00	-0.50	-0.03	-0.05
	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
	[-6.32]*	[-2.97]*	[-2.81]*	[-1.99]*	[-1.58]	[0.68]	[-0.03]	[-40.1]*	[-2.2]*	[-3.7]*
D(Taiwan(-1))	0.10	-0.17	-0.29	-0.12	-0.22	-0.01	-0.03	-0.11	-0.53	-0.07
	-0.02	-0.02	-0.02	-0.02	-0.02	-0.01	-0.02	-0.02	-0.02	-0.02
	[5.5]*	[-9.2]*	[-16.2]*	[-6.31]*	[-13.3]*	[-0.87]	[-1.78]	[-6.64]*	[-33.1]*	[-4.66]*
D(Indonesia(-1))	-0.01	0.02	0.00	0.02	0.02	0.00	0.00	0.05	0.04	-0.46
	-0.02	-0.02	-0.02	-0.02	-0.01	-0.01	-0.01	-0.01	-0.01	-0.01
	[-0.59]	[0.98]	[0.13]	[1.14]	[1.41]	[0.13]	[-0.1]	[3.49]*	[2.74]*	[-33.0]*
C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	[-0.01]	[0.004]	[0.01]	[-0.006]	[-0.003]	[-0.005]	[-0.02]	[0.003]	[0.007]	[0.007]

Table 7. Pair wise Granger Causality Test (Lag 2).

Null Hypothesis	F-stat	Prob.	Direction of Causality
Japan does not granger cause India	4.75	**0.008**	Japan granger cause India
India does not granger cause Japan	65.7	**6.00E-29**	India granger cause Japan
H.K. does not granger cause India	5.24	**0.005**	H.K. granger cause India
India does not granger cause H.K.	32.5	**9.00E-15**	India granger cause H.K.
China does not granger cause India	3.3	**0.033**	China granger cause India
India does not granger cause China	8.44	**0.0002**	India granger cause China
S.K. does not granger cause India	9.51	0.14	------
India does not granger cause S.K.	7.25	**0.0007**	India granger cause S.K.
S. Lanka does not granger cause India	1.9	0.14	------
India does not granger cause S. Lanka	7.25	**0.0007**	India granger cause S. Lanka
Pakistan does not granger cause India	0.65	0.51	--------
India does not granger cause Pakistan	13.76	**1.00E-06**	India granger cause Pakistan
Philippines does not granger cause India	3.85	**0.02**	Philippines granger cause India
India does not granger cause Philippines	106.1	**5.00E-46**	India granger cause Philippines
Taiwan does not granger cause India	5.34	**0.004**	Taiwan granger cause India

India does not granger cause Taiwan	76.6	**1.00E-33**	India granger cause Taiwan
Indonesia does not granger cause India	1.06	0.34	----------
India does not granger cause Indonesia	33.2	**4.00E-15**	India granger cause Indonesia
H.K. does not granger cause Japan	19.8	**2.00E-09**	H.K. granger cause Japan
Japan does not granger cause H.K.	0.92	0.39	----------
China does not granger cause Japan	1.21	0.29	------
Japan does not granger cause China	0.66	0.51	--------
S.K. does not granger cause Japan	13.2	**2.00E-06**	S.K. granger cause Japan
Japan does not granger cause S.K.	1.71	0.18	------
S. Lanka does not granger cause Japan	1.69	0.18	-------
Japan does not granger cause S. Lanka	2.53	0.07	-------
Pakistan does not granger cause Japan	2.55	0.07	------
Japan does not granger cause Pakistan	8.39	**0.0002**	Japan granger cause Pakistan
Philippines does not granger cause Japan	0.64	0.52	-----
Japan does not granger cause Philippines	30.45	**7.00E-14**	Japan granger cause Philippines
Taiwan does not granger cause Japan	2.29	0.1	-------
Japan does not granger cause Taiwan	16.14	**1.00E-07**	Japan granger cause Taiwan
Indonesia does not granger cause Japan	10.87	**2.00E-05**	Indonesia granger cause Japan
Japan does not granger cause Indonesia	4.83	**0.008**	Japan granger cause Indonesia
China does not granger cause H.K.	11.11	**2.00E-05**	China granger cause H.K.
H.K. does not granger cause China	2.09	0.12	--------
S.K. does not granger cause H.K.	4.42	**0.012**	S.K. granger cause H.K.
H.K. does not granger cause S.K.	6.6	**0.001**	H.K. granger cause S.K.
S. Lanka does not granger cause H.K.	0.88	0.41	-------
H.K. does not granger cause S. Lanka	3.73	**0.02**	H.K. granger cause S. Lanka
Pakistan does not granger cause H.K.	0.64	0.52	------.
H.K. does not granger cause Pakistan	7.23	**0.0007**	H.K. granger cause Pakistan
Philippines does not granger cause H.K.	0.97	0.37	-------
H.K. does not granger cause Philippines	73.11	**4.00E-32**	H.K. granger cause Philippines
Taiwan does not granger cause H.K.	3.31	**0.03**	Taiwan granger cause H.K.
H.K. does not granger cause Taiwan	52.67	**2.00E-23**	H.K. granger cause Taiwan
Indonesia does not granger cause H.K.	1.61	0.19	-------
H.K. does not granger cause Indonesia	3.11	**0.04**	H.K. granger cause Indonesia
S.K. does not granger cause China	0.85	0.42	------
China does not granger cause S.K.	2.78	0.06	------
S. Lanka does not granger cause China	2.09	0.12	------
China does not granger cause S. Lanka	.0007	0.99	------
Pakistan does not granger cause China	1.03	0.35	-----
China does not granger cause Pakistan	3.15	**0.04**	China granger cause Pakistan

Philippines does not granger cause China	1.6	0.2	------
China does not granger cause Philippines	11.8	**7.00E-06**	China granger cause Philippines
Taiwan does not granger cause China	2.5	0.08	-----
China does not granger cause Taiwan	0.17	0.84	------
Indonesia does not granger cause China	4.34	**0.01**	Indonesia granger cause China
China does not granger cause Indonesia	0.28	0.75	----
S. Lanka does not granger cause S.K.	1.45	0.23	------
S.K. does not granger cause S. Lanka	9.86	**5.00E-05**	S.K. granger cause S. Lanka
Pakistan does not granger cause S.K.	1.75	0.17	------
S.K. does not granger cause Pakistan	12.6	**3.00E-06**	S.K. granger cause Pakistan
Philippines does not granger cause S.K.	2.76	0.06	------
S.K. does not granger cause Philippines	65.1	**1.00E-28**	S.K. granger cause Philippines
Taiwan does not granger cause S.K.	1.62	0.19	-----
S.K. does not granger cause Taiwan	35.3	**6.00E-16**	S.K. granger cause Taiwan
Indonesia does not granger cause S.K.	6.48	**0.0015**	Indonesia granger cause S.K.
S.K does not granger cause Indonesia	9.5	**7.00E-05**	S.K. granger cause Indonesia
Pakistan does not granger cause S. Lanka	0.28	0.75	-----
S. Lanka does not granger cause Pakistan	1.98	0.13	-----
Philippines does not granger cause S. L.	1.5	0.2	-----.
S. Lanka does not granger cause Philip.	1.5	0.2	-------
Taiwan does not granger cause S. Lanka	3.05	**0.04**	Taiwan granger cause S. Lanka
S. Lanka does not granger cause Taiwan	1.2	0.28	----
Indonesia does not granger cause S.L.	5.17	**0.005**	Indonesia granger cause S. Lanka
S. L. does not granger cause Indonesia	0.53	0.58	------
Philippines does not granger cause Pak.	3.77	**0.02**	Philippines granger cause Pakistan
Pak. does not granger cause Philippines	1.67	0.18	-----
Taiwan does not granger cause Pakistan	10.4	**3.00E-05**	Taiwan granger cause Pakistan
Pakistan does not granger cause Taiwan	6.82	**0.0011**	Pakistan granger cause Taiwan
Indonesia does not granger cause Pak.	7.69	**0.0005**	Indonesia granger cause Pakistan
Pak. does not granger cause Indonesia	0.25	0.77	------
Taiwan does not granger cause Philipp.	24.6	**2.00E-11**	Taiwan granger cause Philippines
Philipp. does not granger cause Taiwan	12.16	**5.00E-06**	Philippines granger cause Taiwan
Indonesia does not granger cause Philipp.	75.9	**3.00E-33**	Indonesia granger cause Philippines
Philipp. does not granger cause Indonesia	6.7	**0.001**	Philippines granger cause Indonesia
Indonesia does not granger cause Taiwan	21.6	**4.00E-10**	Indonesia granger cause Taiwan
Taiwan does not granger cause Indonesia	6.5	**0.001**	Taiwan granger cause Indonesia

Source: Data Processed through E-Views Software Level of significance 5%.

There are some cases of bidirectional causal relationship of India with Japan, H.K., China, Philippines and Taiwan, of Taiwan with Pakistan, Indonesia, H.K. and Philippines, of S. K. with H.K. and Indonesia and of Indonesia with Japan and

Philippines. There exist unidirectional relationship between India and S.K., S. Lanka, Pakistan, and Indonesia. Also, there exist unidirectional relationship of Japan with H.K., S.K., Pakistan, Philippines and Taiwan, of H.K. with China, S.L., Pakistan, Philippines and Indonesia, of China with Pakistan, Philippines and Indonesia, of S.K. with S.L., Pakistan, Philippines and Taiwan, of S.L. with Taiwan and Indonesia and of Pakistan with Philippines and Indonesia. In the remaining cases, there are no cause and effect relationship between each other.

RESULTS AND DISCUSSIONS

The highest % mean return is 0.07% of Pakistan and the most risky stock exchange is of Japan (with the std. dev. of 1.44%). All the series are negatively skewed and leptokurtic in nature. We observe significant correlation of Japan with South Korea and Hong Kong. Hong Kong stock market is highly correlated with South Korea and Taiwan. There are also a significant positive correlation between South Korea and Taiwan.

All the series are stationary in nature at Ist difference. There are some cases of bidirectional causal relationship of India with Japan, H.K., China, Philippines and Taiwan, of Taiwan with Pakistan, Indonesia, H.K. and Philippines, of S. K. with H.K. and Indonesia and of Indonesia with Japan and Philippines. There exist unidirectional relationship between India and S.K., S. Lanka, Pakistan, and Indonesia. Also there exist unidirectional relationship of Japan with H.K., S.K., Pakistan, Philippines and Taiwan, of H.K. with China, S.L., Pakistan, Philippines and Indonesia, of China with Pakistan, Philippines and Indonesia, of S.K. with S.L., Pakistan, Philippines and Taiwan, of S.L. with Taiwan and Indonesia and of Pakistan with Philippines and Indonesia. The outcome of Johansen cointegration test indicates that there is also long run relationship among Asian stock markets. The study can be used in taking investment decisions keeping in mind the international portfolio diversification opportunities. But we see that there is cause and effect relationship in most of the cases and also exist long run as well as short term equilibrium relationship among the Asian stock markets so the investors cannot get abnormal gain by portfolio diversification. It concludes that there is no benefit of portfolio diversification in Asian stock markets.

The present work is not free from limitations. Firstly, because of lack of time only closing prices are included in the current study. Opening prices can also be taken in this study. All the limitations associated with various techniques used in the present study. The time period could also be extended in order to have a broader view of the scenario. Other macroeconomic variable factors can also be taken for future study. The future study can also be done to examine the short run relationship among Asian stock markets by using further econometric techniques namely Variance decomposition and Impulse response. It is also recommended to investigate integration among more markets of different regions.

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Received: Mar 20, 2023 Revised: Mar 25, 2023 Accepted: May 22, 2023

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Current Protein and Peptide Science, 2023, *24*, 285-287

PERSPECTIVE

Micelles-based Drug Delivery Systems: Implication, Challenges and Future Perspectives in Lung Cancer Therapy

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ARTICLE HISTORY

Received: August 01, 2022
Accepted: September 13, 2022

DOI:
10.2174/1389203724666221102164756

1. INTRODUCTION

The recent advancement in pharmaceutical and biotechnology research using nanotechnology has provided the significant improvements in early and real-time diagnosis, effective treatment of cancers by offering lower toxicity, specific targeting, and reduced treatment cost. Polymeric micelles (PM) in clinical trials have revealed better and effective clinical outcomes in small animals and human beings [1]. It is prepared by self-assembly of diblock of biodegradable copolymers into core-shell nanostructures and the diameter from 10-100 nm [2] which reduces their accumulation in reticuloendothelial system (RES) and facilitates overcoming physiological barriers and extravasation, deep penetration and high accumulation in solid tumors after systemic injection [3]. It provides a controlled and triggered release of therapeutic moiety in lung cancer tissue and have advantages such as nontoxic and nonimmunogenic composition, effective core size, surface functionalization and active targeting [4].

2. CLINICAL TRANSLATION OF POLYMERIC MICELLES

The small size (<100 nm) of PM not only avoids RES recognitions but also prevent rapid clearance of therapeutic or bioactive agents from systemic circulation. The metabolites of PM could be excreted in urine due to their lower molecular weight than the threshold of glomerular filtration which suggests the safety of micelles with a lower risk of chronic accumulation in the body [5]. PM can greatly accumulate in cancers cells and diseased tisuues followed by its EPR effect [6]. In a clinical trial at phase-I of doxorubicin-loaded micelles (NK911) in 23 patients with solid tumors has revealed a desired drug dose tolerance and its safety and proceeded for phase-II clinical trial. Similarly, genexol-PM synthesized from a PEG-poly (D,L-lactide) polymerization containing paclitaxel which have been approved for its clinical uses. Likewise, docetaxel-loaded PM were formulated by conjugation of docetaxel to PEG-b-poly(α,β-aspartic acid) through a hydrazone bond [8] after better preclinical results and further recommended for phase I study to investigate the safety, tolerability, and recommended dosage, in patients suffering from late stage of solid tumors [9]. Subsequently, phase I clinical trial of NC-4016 has been started in November 2013 at The University of Texas MD Anderson Cancer Center in US to find its therapeutic effect, associated toxicities, and dosage in patients with advanced solid tumors or lymphoma which have revealed the better clinical outcomes [10]. In conclusion, it has been observed that PM based nanoformulations may utilise for effective delivery of anticancer drugs, bioactive molecules, proteins, peptides, and diagnostic agents against a wide variety of cancers.

3. RECENT GENERATION OF POLYMERIC MICELLES

The block copolymers of PM can be adjusted for improving drug loading, release, pharmacokinetics and its tumor targeting ability. Accordingly, groups of PEG with different ligands can recognize specific surface receptors, also provides cell selectivity and intracellular delivery of drug through micelles. There are so many novel strategies by which PM can greatly improve clinical outcomes of therapeutic agents against cancers [10].

3.1. Stimuli Responsive Micelles

Lung cancer is characterized by lower pH in both intertumor and interstitial confines of tumor, overexpression of enzymes such as procathepsin D (pCD), fatty acid synthase (FASN), elevated cytoplasmic glutathione (GSH), and heightened temperatures employed as internal triggers in conjunction magnetic fields, ultrasound, and light to allow destabilization of stimuli-responsive PMs for the delivery and release of drugs in a controlled manner [11]. Liu et al, focused on sonodynamic nanotherapy in which reactive oxygen species (ROS)-responsive micelles of poly (ethylene glycol)-poly (propylene sulfide) (PEG-PPS) were delivered upon the irradiation with ultrasound waves. The oxidization

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of anticancer drug loaded sonosensitizer hypocretin due to ultrasound waves triggers the disassembly of amphiphilic PEG-PPS-HC to hydrophilic PEG-PPS. The separated drug-loaded HC escapes the polymer and was released in blood circulation near the tumor site [12]. Zhang *et al.* constructed dual enzyme/redox-responsive PMs with active targeting abilities for fast intracellular drug release in a recent study [13].

3.2. Dual Drug Combination Nanotherapy

Currently, etoposide (ETO) and platinum, and the combinational delivery system settles problems with ratio metric co-loading of hydrophilic and hydrophobic drugs to achieve their maximal anticancer efficacy in non-small cell lung cancer (NSCLC). Liang *et al,* reported that the successfully loading of gemcitabine and paclitaxel into targeted nanostructured PM in an accurate ratio of the two drugs achieved maximal anticancer efficacy in NSCLC [14].

3.3. Imaging Micelles

PM containing MRI contrast agents like gadolinium-diethylenetriaminepenta-acetic acid (Gd-DTPA) and superparamagnetic iron-oxide nanoparticles (SPIONs) have been created to serve the purpose of imaging of cancer. SPIONs micelles are self-assembled amphiphilic polymers which are modified and actuate by electrostatic ineraction prussian blue staining. However, there are limitations for SPIONS due to their surface hydrophobicity which attributes to macrophage engulfing and rapid decrease in concentration in blood circulation [15]. It has been observed that PM can used for the delivery of imgaing agents in cancer therapy.

4. FUTURE PROSPECTS

According to the research done till date into the development of various PM, it is clear that PM have enormous potential for drug delivery applications, particularly in cancer therapy. The polymer's toxicity, as well as their degradation kinetics, must be investigated to an extent. However, with these improved drug loading capabilities, easy scale-up, and a thorough understanding of the fate of the PM in the biological system would propel them from the bench to the bedside as a promising drug delivery candidate in cancer therapy.

CONCLUSION

PM have been extensively researched in recent decades due to their ability to load hydrophobic medicines efficiently and consistently in their core. Also it can be an ideal vehicle for enhancing the prolonged circulation of anticancer/therapeutic agents followed by avoidance of drug accumulation in RES. Furthermore, tumor-targeting ligands can be added to PM to improve tumor selectivity and intratumoral drug delivery. To date, clinical trials have looked into about nine drug-loaded PM, with genexol-PM being licensed in Korea in 2007 and commercialized in Europe for the treatment of breast cancer and lung cancer. The clinical translation and intended therapeutic outcomes of PM, however, remain unsatisfactory it need further extensive research. Based on published research it can surely be concluded that PM have proved its drug loading capabilities, enhanced biodistribution with lower risk of chronic accumulation, and easy scale-up process for the treatment of lung cancer.

LIST OF ABBREVIATIONS

PM	=	Polymeric Micelles
RES	=	Reticuloendothelial System
EPR	=	Enhanced Permeability and Retention
Pcd	=	Procathepsin D
FASN	=	Fatty Acid Synthase
GSH	=	Elevated Cytoplasmic Glutathione
ROS	=	Reactive Oxygen Species
PEG-PPS	=	Poly(ethylene glycol)-poly (Propylene Sulfide)
ETO	=	Etoposide
NSCLC	=	Non-small Cell Lung Cancer
Gd-DTPA	=	Gadoliniumdiethylenetriaminepenta-acetic Acid
SPIONs	=	Superparamagnetic Iron-oxide Nanoparticles

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

Author Rahul Pratap Singh acknowledges the Science and Engineering Research Board (SERB), Department of Science & Technology, Government of India for providing financial support (File No. EEQ/2019/000218) under the scheme of Empowerment and Equity Opportunities for Excellence in Science (EMEQ) that promoted his nanotheranostic research in cancer nanotechnology for effective and safe cancer therapy. He also acknowlwdges GD Goenka University, India for providing financial support (R&D:12/21/02) under the scheme of SEED Grant as per the University's Policy for Research Promotion.

CONFLICT OF INTEREST

Rahul Pratap Singh is the Associate Editorial Board Member of the Journal "Current Protein and Peptide Science".

ACKNOWLEDGEMENTS

Declared none.

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Current Physical Chemistry, xxxx, *xx,* x-x

REVIEW ARTICLE

Biopolymers and their Nanocomposites: Current Status and Future Prospects

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ARTICLE HISTORY

Received: April 18, 2023
Revised: May 22, 2023
Accepted: June 16, 2023

DOI:
10.2174/1877946813666230726125759

Abstract: For many years, petroleum-based polymers have been successfully enhanced by the addition of nanoparticles as additives. Carbon nanotubes, graphene, nanoclays, 2-D layered materials, and cellulose nano whiskers are a few of the several nano-reinforcements that are currently being researched. In comparison to unmodified polymer resin, the use of these nanofillers with bio-based polymers could improve a wide range of physical properties, including barrier, flame resistance, thermal stability, solvent uptake, and rate of biodegradability. This nano-reinforcement is a very appealing method to create new functional biomaterials for a variety of applications because these enhancements are typically achieved at minimal filler content.

Keywords: Biopolymers, nanocomposites, biocompatible, nano-science, non-toxic, biological polymers.

1. INTRODUCTION

As the name implies, biological polymers, such as plants, animals, and bacteria, or those created chemically from various saccharides, are known as biopolymers. They are functionally flexible, non-toxic, biocompatible, and degradable. Contrarily, nanocomposites are hybrids created by embedding components (referred to as the reinforcing phase) into another material (referred to as the matrix phase). Both or at least one of the materials must have nanoscale dimensions. While the filler materials give the matrix phase new qualities, the matrix material is in charge of sustaining the locations of the reinforcement components. In biopolymer-based nanocomposites, fillers that make up nanoparticles are mixed throughout the matrix of the biopolymer. Collaborating nanomaterials with biologically important molecules open new doors to the invention of novel physical therapies, as well as the drug delivery systems highly sensitive to the temperature and pH variation at pathological sites. Fillers produce strong interactions with the biopolymer matrix because they have distinct characteristics from the bulk and have a small number of atoms per particle [1, 2]. Furthermore, conducting polymers and a variety of proteins can be used to create nanocomposites. Additional benefits associated with nanocomposites include the options to involve them in distinct applications as pertinent functional materials can be crafted. Because of their exceptional mechanical, thermal, and electrical characteristics, nanocomposites are well suited to replace metals in a variety of industrial applications. A adjusted weight proportion of polymer network and nano fillers restrain the propensity of filler aggregation further maintaining the ductile and affect quality of composite materials. When it comes to barrier qualities, oxygen impermeability, solvent resistance, moisture permeability, thermal stability, and antibacterial traits, polymeric nanocomposites are noticeably superior to traditional packaging materials, 4 [3, 4]. (https://pubmed.ncbi.nlm.nih.gov/34883701)

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1877-9468/xx $65.00+.00

(https://ift.onlinelibrary.wiley.com/doi/10.1111/1541-4337.1283)

2. DISCUSSION

Only when nanoparticles are evenly disseminated within a polymer matrix can their advantages become apparent. The main obstacle to creating nanocomposites with desirable qualities is achieving a uniform and homogenous dispersion of nanofillers in biopolymer matrix. A significant amount of interfacial area in the composite matrix may result from the homogeneous dispersion of nanofillers. Its reinforcement depends on a number of variables, including particle size, distribution, orientation, and structure, as well as the characteristics and filler and polymer matrix concentrations [5]. Further processing method affects the thermomechanical properties of polymer based nanocomposites in expansion to have an impact on the aggregation properties and morphology of the end product. Combining two or more types of fillers with varying nature such as natural or synthetic along with the distinct packing arrangements of fibres broadens the arena of applications of concerned nanocomposites. Chemical nature of the fillers used which may be organic or inorganic further characterise thermomechanical, interfacial and optical properties proving the inorganic ones to be better choice. Nanofillers have been proven to be an ideal choice when compared with fillers in micro dimensions used in fibre reinforced composites owing to specific surface area of former resulting in improved intermolecular interactions of filler with the polymer [6].

3. CLASSIFICATION OF POLYMER NANOCOMPOSITES

Polymer nanocomposites can be categorized on the basis of:

- Dimensions of nanofillers,
- Type of nanofiller,
- Type of polymer matrix

3.1. Classification Based on the Dimension of Nanofillers

Nanofillers can be categorized into zero- (0D), one- (1D), two- (2D), or three dimensional (3D) nanoparticles according to their dimensions at the nanoscale (Fig. **1**). The 0D nanofillers have all the dimensions in the nanometer-scale. For example-quantum dots. Acquiring the spherical shapes, these nanoparticles having radius less than 50 nm offer excellent optical properties due to their wavelength dependent photoluminescence. Being biocompatible, these nanomaterials find diverse applications in the area of pharmaceuticals, cosmetic industry and biosensoring. Recently graphene based quantum dots have been utilized successful-

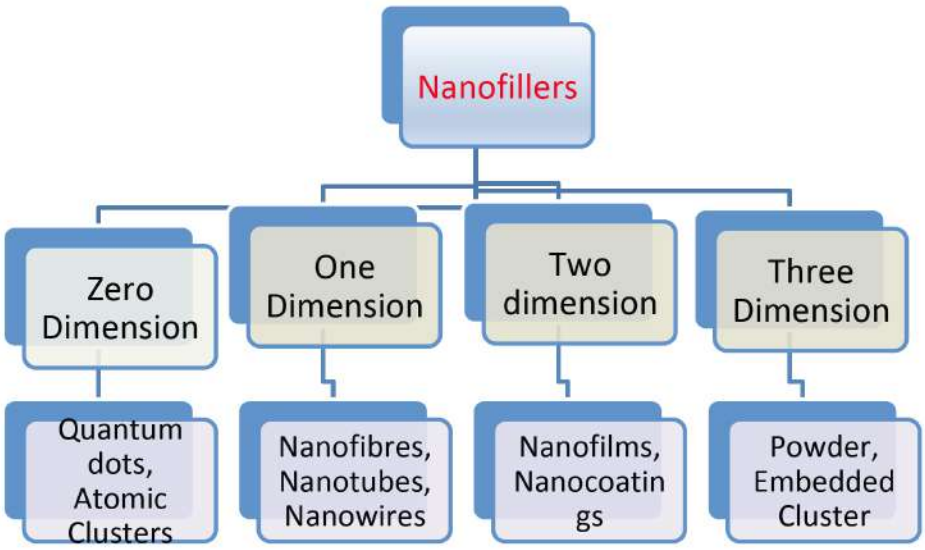

Fig. (1). Classification of nanofillers on the basis of dimensions. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

ly in the cancer diagnostic devices sensing particular excreted chemicals as well as pH changes in the affected tissues [7].

1D nanofillers have two dimensions in the nanometer range. For example- Nanofibers, nanowires, nanotubes, nanowhiskers and nanorods. Nanofillers of this particular type have been found to be extremely efficient in the area of energy storage devices. Further ZnO Nano rods, polyvinyl alcohol composite nanofibers, single walled carbon nanotubes are some other widely studied nanofillers with numerous applications.

2D nanofillers have only one dimension in the nanometer-scale. For example- nanofilms, nanocoatings, nanosheets, nanowalls, and nanoplates such as layered silicate clays and graphene. As mentioned earlier, conventional biopolymers cannot offer desirable thermal, mechanical and obstruction properties, however, nanocomposites in conjugation with these biopolymers lead to the formation of nanofilms having great degree of biocompatibility and biodegradability. In addition, potential applications in biomedical field as well as nourishment industry have made their area of research a much intrigued one.

The 3D nanofillers have no dimension in the nanometerscale, such as bulk powders [8].

3.2. Classification Based on the Types of Nanofillers

A uniform nanophase-separated structure is created on the biopolymer matrix by dispersing metal or metal oxide nanofillers (such as silicon, zinc, magnesium, titanium oxide, zirconium oxide, aluminium oxide, and iron oxide) to give the nanocomposites which enhance their flame retardancy and thermal stability.

Compared to pure polymer, metal-sulfide nanofillers have improved thermal, optical, electrical, and mechanical capabilities. Popular examples of this class are CdS, ZnS, and HgS [9, 10].

In order to create flame-retardant polymer nanocomposites, metal hydroxide nanofillers—such as aluminium and magnesium hydroxide or double hydroxides—such as zinc and alumina—have been deposited as fillers on the surfaces of polymer matrices. Increased number of active sites, enlarged surface area available for interaction, and porous structure are some additional merits of layered double hydroxides making them a material of top choice in textile industry. The mechanical and thermal properties of silicate polymer nanocomposites are respectable. Suitable treatment of nanoscale layered silicates augments the bonding of filler with the matrix resulting in the enhanced thermomechanical performance. Agglomeration of nanofillers in composites is not as important as in the case of microscale reinforcements, rather intercalation of dispersion matrix and filler resulting in uniform distribution matters more in this case. Balanced forces between particles and suspending medium results in the better reinforcement [11].

The easiest approach for creating these silicate polymer nanocomposites is the direct mixing of silica into polymers.

Classification Based on Type of Polymer Matrix

Biopolymer or biodegradable polymer matrices are softer and more malleable than other polymeric matrices. The primary sources of natural polymers are proteins, starch, cellulose, pectin, chitosan, collagen, and cellulose. Some examples of biopolymers that are utilised as matrices are polylactic acid (PLA], polyhydroxybutyrate (PHB), polybutylene adipate terephthalate (PBAT), and poly 3-hydroxybutyrate-co-3-hydroxyvalarate (PHBV) [12-14]. Embedded with lot of carboxylic functional groups, PLA has magnificent mechanical and optical characteristics, limiting applications owing to the economic issues as well as weak performance. Chitosan being biologically renewable, has become the centre of prime research interest due to widespread applications in drug delivery, bio sensing and in the field of super capacitors. Cellulose is another important biopolymer from commercial point of view and is hydrophilic in nature being a derivative of hydroxypropyl methyl cellulose. Polyhdroxy butyrate is a type of biopolymer that is renewable and biocompatible, have elastic properties, can be produced from solutions and can be used in biosensors and charge distribution applications.

4. PROCESSING TECHNIQUE OF BIO-NANOCOMPOSITES

The most commonly used methods in preparing bio-nanocomposites are briefly explained as follows.

4.1. Melt Extrusion

Because of its exceptional endurance and compatibility, extrusion is regarded as a cost-effective and ecologically benign method for producing bio-nanocomposites. The nanoparticles and polymer granules are combined while still in a molten state to create a uniform and homogeneous dispersion of nanoparticles for a predetermined amount of time. This technique takes advantage of the fact that the need of solvent is minimized, however high temperature conditions have to be maintained. A surface modification-related compatibility with the host polymer and the nanofiller's processing conditions is ensured by the uniform distribution [15, 16].

4.2. Solution Casting

In the lab, this method is mostly employed to create polymer nanocomposites. Typically, a suitable solvent is used to dissolve the polymer that contains uniformly dispersed nanoparticles. Mechanical stirring and ultrasonication are typically utilised to create a homogenous dispersion of nanostructure additions into the biopolymer [17, 18]. After the completion of homogenization, excess solvent can be removed by vaporization, making this method a convenient one as it requires low temperature conditions as compared with melt extrusion.

4.3. Spraying

It is possible to create bio-nanocomposites coatings with improved qualities by misting melted bio-nanocomposites—typically carbon-based, metallic, or ceramic—onto a substrate. This technique is appropriate for coating applications like thermal barrier, abatable seal, and corrosion and wear resistance. High deposition rates, cheap operating costs, simplicity, and portable equipment are some benefits of this approach [19-20]. Depending upon the temperature of deposited material technique can be further categorized as Physical Vapour Deposition (PVD) or Chemical Vapour Deposition (CVD). Moreover Coating comprised of particles in nanoscale has been proven to be possessing improved wear performance as compared to coatings with microcrystalline dimensions [21].

4.4. Electrospinning

The manufacture of scaffolds, synthetic organ materials, body implants, tissue engineering, and drug delivery vehicles using bio-nanocomposites has been documented. The spinneret is a syringe needle with the polymer solution loaded on it. A high voltage produces a direct current. Constant pressure is applied while a collection plate, which electrically functions as a ground plate, is positioned above the syringe tip. Electrospinning has been proved to be one of the best techniques to manufacture nanocomposites of different dimensionality which can certainly act as synthetic counterparts of natural matrix and find applications in medicinal as well as tissue engineering fields. Unfortunately, it is challenging to create huge amounts of fibres quickly, using the electrospinning method [22, 23].

5. PROPERTIES OF BIONANOCOMPOSITES

5.1. Mechanical Properties

The performance, mechanical characteristics, and dimensional stability of advanced composite materials are primarily determined by the polymer matrix interface. When materials like nanofiller and polymer matrix are mixed together to improve the dispersion of materials into matrices, interfacial adhesion takes place [24, 25]. To produce greater interfacial adhesion and a strong bond between the two materials, the combination of components must have the same qualities, such as hydrophobic fillers and hydrophobic matrices or hydrophilic and hydrophilic materials. Recent research has shown that a generalised analytical three-phase-series-parallel model can be used to predict the effective mechanical reinforcement of polymeric nanocomposites incorporating spherical nanofillers. The mechanical characteristics of bio-nanocomposites are also highly influenced by the particle size and loading. A study on the mechanical properties of carbon based reinforced compo-

site papers reveals that its challenging to maintain a balance between the strength and toughness which can be met by refining the dimensions of nano fibrils involved. Nature of intermolecular bonding involved in filler permits the stress transformation under pressure and results in enhanced strength and roughness [26]. A good adhesion between the fillers and the matrix is a prerequisite for high strength in the resulting composite.

5.2. Barrier Properties

The intrinsic propensity of polymers to allow the exchange of low-molecular-weight compounds is strongly connected with their barrier qualities. The degree of alteration of the barrier characteristics of nanocomposites can be influenced by the architectures of nanocomposites as well as the kind and size of nanofillers. Well-dispersed nanofillers in the polymer matrix have the potential to affect the solubility and diffusivity of penetrating molecules, particularly in interfacial domains, by lengthening the diffusion length and the tortuous path of penetrating molecules to form an impermeable structure in the polymer matrix due to their high aspect ratio. The barrier qualities are further controlled by the shape, polarity, and crystallinity of the diffusing molecule, the degree of cross linking, and polymer chains. Packaging of food materials require special types of properties as compared to wrapping of other materials as in case of former one has to take care of the safety aspects and comparatively short shelf life. Platelet-shaped fillers and latex membranes, when compared to clean membranes, offer greater permeability and improved gas barrier qualities, respectively. Similarly, in the category of biopolymers, wrapping sheets of proteins and carbohydrates act as excellent barrier against oxygen in normal humid conditions however the barrier against water vapours is not that good. Formation of bionanocomposites with filler as organoclays improve the above mentioned shortcomings to a great extent.

5.3. Thermal Properties

However, the majority of nanofillers or nanomaterials exhibit high thermal conductivities in the range of 100-400 W/m·K. Thermally stable plain polymers typically exhibit thermal conductivities in the range of 0.1-1.4 W/m·K. The thermal stability of polymeric nanocomposites is greatly influenced by the use of various types and concentrations of nanofillers, which have lower thermal expansion coefficients and greater E (Young's modulus) values than the polymer components. In order to prevent heat-induced polymer degradation, nanofillers operate as barriers to heat and mass transmission. They also lower the diffusion of gaseous products and the molecular mobility of polymers. The thermal stability of polymeric nanocomposites is also improved by the combined chemical and physical mechanisms [27-28]. The primary mechanisms underlying the thermal stability of polymeric nanocomposites are those described above.

5.4. Flame Retardency

The flammability and physical characteristics of polymeric nanocomposites can be simultaneously improved by nanofillers such CNTs and clay, making them appealing materials for flame retardants. Nevertheless, nanofillers are coupled with other fire retardants because they do not show notable fire retardancy on their own. By preventing the forceful bubble action during combustion-induced degradation, nanofillers like nanoclay particles or CNTs can reduce flammability. The physical properties of nanocomposites as compared to those of the polymer matrix are often improved by the inclusion of these nanofillers. As a result, nanofiller-incorporated nanocomposites can provide a solid, continuous layer of protection on the burning surface made of clay and CNTs.

5.5. Optical Properties

Dimensional quantization, local surface plasmon excitation, and dielectric limitation are all factors that contribute to nanomaterials' distinctive optical characteristics. Surface plasmon resonance has a very particular spectrum location and intensity for each type of nanomaterial and is very dependent on both the spatial organisation and characteristics of the nanomaterials or nanofillers. By adjusting the size, shape, concentration, and dielectric constant of the nanomaterials as well as the polymer matrix, it is possible to control the optical properties of nanocomposites. The activation of local SPR, particularly the collective oscillations of the conduction electrons, has an impact on the linear and non-linear optical characteristics of

nanocomposites including nanofillers. The sub-micrometer-scale ordering of nanocomposites is clearly related to their plasmon characteristics. Moreover, the addition of graphene and CNTs provides certain benefits to other useful optical properties.

CONCLUSION

Bio-nanocomposites is a rapidly expanding and highly intriguing field. The world is currently on the cusp of using bio-nanocomposites in large-scale technological applications. Biopolymers are crucial for a sustainable economic development since they are renewable, biodegradable, biocompatible, and environmentally benign. Their use lessened numerous difficulties created by synthetic polymers, including greenhouse gas emissions, reliance on fossil fuels, biodegradation to innocuous compounds, and many others. Excellent gas and water permeability, as well as a low thermal breakdown temperature, characterize biopolymers. The qualities and capabilities of biopolymers are enhanced by the addition of nanoparticles. Getting a homogeneous nanofiller additive dispersion in the biopolymer matrix is a serious problem. We will end up with a generic composite material that contains the filler if they are not properly disseminated during their preparation. It won't exhibit the appropriate nanocomposites-specific characteristics. Then, they ought to be referred to as biocomposites.

The family of nanomaterials known as bio-nanocomposites is yet not well defined. It is a rapidly expanding area at the moment. Biopolymers are the matrix that gives bio-nanocomposites their shape, structural organisation, and functioning. Dispersed nanoparticles alter the matrix by allowing the structure, characteristics, and functionality to be tuned. Composites for each desired application are made possible by a wide range of biopolymers and nanosized particles. Currently only few of them are used to create bio-nanocomposites.

Bio-nanocomposites are appropriate for a wide range of biomedical applications due to their exceptional biocompatibility. Nowadays, bio-nanocomposites are of utmost importance in this field [29,30]. They are employed in the production of biomaterials such as scaffolds and implants, drug delivery mechanisms, diagnostic tools, and biomedical equipment. They are also ideally suited for biotechnology and cosmetics due to their biocompatibility.

In addition to ensuring the quality and safety of food for consumers, the ultimate purpose of new food packaging technologies is to take environmental issues into account and lessen its effects. Using environmentally friendly biopolymers rather of standard petroleum-based polymers is one approach in this area [31].

Reinforcement of both man-made and natural fillers in order to make bio nanocomposites is a composition specific process as fillers embedded in the nanoscale dimensions grace the physical and chemical properties. However enhanced Vander Waals forces beyond a certain concentration, results in great cohesive forces of filler particles and have an adverse effect on the polished properties of bio nanocomposites. Fillers of organic and non-synthetic origin enhance the moisture resistance but reduce thermal resistance to some extent. On the other hand, interfacial as well as sound absorption properties get improved as optimum volume/weight ratio of filler and polymer with appropriate particle size of former is maintained. Depending on the required properties, a supposition to rule out the use of a certain type of filler (natural/ engineered) seems incomprehensible and a balance is to be kept to create the best of these. Further expanded applications are anticipated.

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

Not Applicable

ACKNOWLEDGEMENTS

None declared.

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Cover Page

DOI: http://ijmer.in.doi./2023/12.06.123
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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:6(6), June: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd June 2023
Publication Date:30th June 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

PEREGRINATION FOR FEMALE LIBERATION: STRUGGLE OF THREE GENERATIONS IN ARUNDHATI ROY'S *A GOD OF SMALL THINGS*

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Abstract:

The present paper critically analyses the meandering journey of three generations of women characters in Arundhati Roy's Booker Prize winner novel, The God of Small Things. It studies the effects of gender bias prevalent in Indian society on the psyche of women. The analysis focuses on the portrayal of various types of marginalisation and subjugation of women by their powerful and dominating counterparts. Further, it also throws light on the general condition of women throughout the country. The paper highlights the struggle of female characters for their liberation from discriminatory and exploitative patriarchal norms. It also underlines women's miserable condition in Indian society and their reaction to their social position. From the passive acceptance of their plight with a fatalistic attitude initially, the journey of women characters in the novel toward their amelioration and self-assertion has really been an arduous one. The present paper reflects this progression and evolution of the femme eternelle.

Keywords: Human Rights, Subjugation, Discrimination, Patriarchy, Liberation

Female life and experience have been seen mostly through the masculine lens. As a result, the female experience has been either ignored or completely wiped off the pages of history. It is a sad commentary on the existing affairs in the life of women that they have been denied their fundamental right to make decisions about their life. Article 1 of the United Nation's Universal Declaration of Human Rights states that all human beings are born free and are equal in dignity and rights ("Universal"). However, for ages, women's rights, freedom, equality, and dignity have been compromised in the male-dominated world. Though women represent one-half of the world population, they have been struggling to voice their opinions and assert themselves. As Simone de Beauvoir rightly comments: "They have gained only what men have been willing to grant; they have taken nothing, they have only received" (19). Women have been rendered voiceless, powerless, and even purposeless by the patriarchy. The struggle to bridge the gap between the powerful and powerless, voiced and voiceless, exploiter and exploited is one of the biggest challenges of modern times. Though there are no quick answers or easy solutions to such a practical problem, social activists and litterateurs from all corners of the world have tried to empower women by empathizing with them and by presenting their stories to the world. In India, women writers like Anita Desai, Shashi Deshpande, Anita Nair, Nayantara Sehgal, Manju Kapur, Amrita Pritam, Githa Hariharan, Arundhati Roy, etc. have beautifully showcased in their works the various nuances of female life, including their aspirations, sufferings, struggles, suppression, marginalisation, and also, their relentless efforts to lead a life of dignity. Arundhati Roy's name shines out in this list for presenting a realistic picture of the plight of women in her debut novel, *The God of Small Things* (1996). The book offers the readers a critique of male chauvinism through the portrayal of the suffering of females due to patriarchal ideologies and hegemony. Fittingly, the novel won the prestigious Booker Prize in 1997 for exploring how 'small' things which are seemingly insignificant and generally ignored can make a deep impact on the lives of 'big' people.

*The God of Small Thing*s deals with the lives of three generations of women who must pay the price of being women in a male-dominated world. The novel is set in Ayemenem in Kerala, a place that symbolizes a microcosm of the whole country in terms of the unfair treatment of women. The novelist gives a realistic portrayal of her women characters to highlight the fact that despite the tall claims of equality made in the constitution of India, women are still subordinated and marginalised on various fronts. Such suppression and gender inequality alienate them from the 'centre' and push them to the 'margins' of the social fabric.

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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:6(6), June: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd June 2023
Publication Date:30th June 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/12.06.123
www.ijmer.in

The book successfully portrays various types of marginalisation and subjugation of women by their powerful and dominating counterparts. The very title of the novel is an indication of the novelist's aim of underscoring "the intrinsic dignity, worth and beauty of all that is considered to be 'the small' (trivial or insignificant) by 'the big' (those who wield power over others…)" (Ghotra 227). Besides other things in her book, Arundhati Roy has represented the inferior status of female characters in the Keralite society in Postcolonial India. B.S. Jamuna remarks that the novel, *The God of Small Things is* a "neo-colonial text highlighting the struggle for liberation not from the colonial hegemony but from one's own oppressive neo-cultural set-up" (189).

The three generations of women characters in this novel become victims of double marginalisation at the hands of their male counterparts as well as their own native culture. In general, they are treated as mere objects of possession and are meant to be controlled by the 'man' of the house. Mammachi represents the first generation of women. She complacently accepts her subordinate existence and never thinks of questioning male sovereignty. She is truly a subdued Indian woman who has no control over her life and is not allowed to take decisions on her own. She cannot even think of questioning the authority of her father who gets her married to a man who is seventeen years older than her. After marriage, she silently bears all the tortures and beatings of her husband and never complains. Regarding the silent subordination of women, R.K. Mishra's comment seems apt in the Indian context. He says that in India, "there is a willing resignation of the women to the subordination of the male. They have accepted this subjugation as a traditional prescription of the Hindu society" (48). Mammachi's husband, Pappachi is a male chauvinist, jealous, possessive, and cruel man who beats his wife at the slightest pretext. He disallows her violin lessons just because her teacher tells him that his wife is "exceptionally talented and, in his opinion, potentially concert class." (Roy 50). The thought of subverting the arbitrary and atrocious male authority never crosses Mammachi's mind for she had been taught since her childhood that the husband is the God of a woman. Thus, she mutely resigns to her fate and, like Kamla Das' female persona, "Cowering / Beneath your monstrous ego I ate the magic loaf and / Became a dwarf". Frequent and severe beatings also affect the psyche of Mammachi's children, especially, her daughter, Ammu who observes, "Father Bear beat mother Bear" (180). It is only towards the fag-end of Mammachi's painful married life that her husband stops beating her because of the intervention of their son, Chacko. Symbolically, the novelist makes the reader hear the "long-smothered wail" of all the suffering females in the country (Sharma 178). Pappachi's male chauvinism becomes evident from the fact that he considers it below his dignity to help Mammachi in pickle-making though he has nothing else to do after his retirement. Even the fact that his wife has become nearly blind due to her conical corneas does not alter his decision. Though after Pappachi's death, she gains the legal right to be the mistress of the house, yet the de-facto master of the house in all matters is her son, Chacko.

The women workers' predicament at the pickle factory is even worse than that of Mammachi. Nobody sympathizes with them, nor hears their silent cries. They are doubly oppressed and marginalized. The whole narrative shows how the elite women become the centre and the factory women the margins. Vicious roots of patriarchy deeply ingrained in the female psyche affect these women's subconscious selves so adversely that even when, like Mammachi, they are free from male oppression, they turn into the female version of patriarchy. Mammachi, now the woman in power, accommodates and overlooks the sexual affairs of her son, Chacko with the factory women who have to become mere sexual objects for satisfying the "man's needs". Chacko's clandestine relationships receive silent approval from the mother as she constructs a separate entrance for his room, "so that the objects of his 'Needs' wouldn't have to go trespassing through the house" (169). Such commodified women suffer at the hands of both the men and women of the elite class. It is not only the patriarchal ideology, but also the hegemony which becomes instrumental in the oppression of such hapless women indiscriminately by both genders. Benita Parry is right to point out that such females are "positioned on the boundary between human and animal" (39).

The second-generation women are represented mainly by Ammu in the novel. Since her childhood, she suffers in a patriarchal household where there is a discriminatory attitude towards her. She is not allowed to get a college education while her brother Chacko is sent to Oxford to study as a Rhodes scholar. Ammu does not get equal treatment at home since she is a woman and such discrimination makes it unbearable for Ammu to spend her life at Ayemenem. Though Ammu

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ISSN:2277-7881; IMPACT FACTOR :8.017(2023); IC VALUE:5.16; ISI VALUE:2.286
Peer Reviewed and Refereed Journal: VOLUME:12, ISSUE:6(6), June: 2023
Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd June 2023
Publication Date:30th June 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

DOI: http://ijmer.in.doi./2023/12.06.123
www.ijmer.in

contributes to factory work, she is never paid anything, and her legal and natural right on the family property is also denied and usurped by her brother, Chacko who always refers to the property as "my Factory, my pineapples, my pickles" (57). He brazenly tells Ammu, "What's yours is mine and what's mine is also mine" (57). To such a statement, Ammu retorts: "Thanks to our wonderful male chauvinistic society" (57). Her ironic reaction indicates the root cause of gender bias prevalent in Indian society.

Ammu's life at her parental home has been bad, but her married life with a Bengali Hindu is worse than that. Her husband, besides being a habitual liar and a drunkard, is so mean that he stoops to the extent of forcing her to act as his boss' concubine for allowing him to remain absent from duty. When Ammu refuses to oblige him, he "lunged at her, grabbed her hair, punched her, and then passed out from the effort" (42). Under this exigency, Ammu decides to retaliate to reclaim her self-respect and distinct identity. She refuses to submit to the highly invidious patriarchal norms. She picks up "the heaviest book she could find in the book-shelf… hit[s] him with it as hard as she could. On his head. His legs. His back and shoulders" (42). Finally, she walks out on her husband for good and returns to Ayemenem. She expresses her fervent feministic stance by getting her wedding ring melted down and made into a bangle for Rahel, her daughter. Her family does not let any opportunity slip to make her realize that she and her children are unwelcome guests in the house. But by that time, Ammu has learned to assert herself and hit back at the chauvinistic world. She starts defying the social norms which use the silence of women as "a tool of oppression" because she has realized that women's retaliating voice is "a tool of resistance" (Faheem 63). Ammu is not ashamed of her divorce since she knows that it is better to live alone with dignity rather than keep dragging on a life of woes and innumerable insults at the cost of one's self-respect. She even establishes a sexual relationship with an untouchable Paravan, Velutha. Such a relationship is unacceptable in an orthodox caste-ridden society. When Ammu's aunt, Baby Kochamma comes to know about their affair, she cannot tolerate it and decides to punish them. She is, in a way, the doppelganger of elderly Mammachi who is unreasonably vicious and oppressive towards Ammu. Kochamma's unrequited love for Father Mulligan has turned her so bitter, self-centered, and vindictive that she makes a scheme of filing a false case of molestation, kidnapping of children, and murder of Chacko's wife, Sophie Mol against Velutha. She even forces Estha to testify against the poor Velutha, leading to his impending doom. Ammu fails to get her lover released from that charge since the attitude of the Police Inspector is also biased against him. She finds social and administrative forces to be against her. She is separated from her children and asked to leave the house at Ayemenem. She yearns to be united with her kids, but ultimately, she dies all alone in a cheap hotel room. Though she pays the price for her free-thinking attitude and assertive behaviour, she paves the way for the next generation of women who would put up a good fight against the stringent phallocentric norms.

Ammu's daughter, Rahel represents the third generation of women. She is also important in the story as she grows through her traumatic childhood experiences. Owing to the divorce of her parents, she does not enjoy a normal childhood. Deserted by her father and separated from her twin brother Estha, she becomes a victim of biased treatment in Ayemenem, by her maternal grandparents and maternal uncle who do not welcome Ammu and her daughter, Rahel after the former one's divorce. While Ammu raises her voice against the prejudicial social norms meekly, her daughter flouts the same openly since her childhood. Her expulsion from school and a virtual social boycott by her college mates do not make her a conformist. The neglect of her family gradually makes her an independent, bold, and free-spirited girl, ready to take the decisions of her life. She gets admitted to a college of Architecture in Delhi, not out of interest, but to stay away from Ayemenem. When she comes to understand that nobody at Ayemenem is taking an interest in finding a suitable match for her, she decides on her own and marries an American scholar, Larry McCaslin. Unfortunately, the marriage does not work and she gets divorced after some time, but she does not bother about this and asserts her right to follow her convictions. She becomes finally independent and starts earning by doing various jobs in New York without feeling any shame or moral scruples for getting a divorce. She leaves her job and returns to Ayemenem when she comes to know that her twin brother, Estha has 're-returned' to Kerala. The separation from Estha has taken a toll on the psychological health of both the brother and sister. While Estha turns speechless in the process of dealing with the painful memories of separation, Rahel becomes so mentally and emotionally wrecked that she fails to form a long-lasting healthy relationship with her husband or any other man and even ends up a pervert, committing incest with her brother towards the end of the novel.

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DOI: http://ijmer.in.doi./2023/12.06.123
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Online Copy of Article Publication Available (2023 Issues)
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: 2nd June 2023
Publication Date:30th June 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

Hence, Arundhati Roy has successfully depicted the plight of her female characters and how they subvert as well as assert their doubly marginalized identity. While older women become subdued under the phallocentric social set-up and conform to it, the younger generation's educated women claim their right over their lives by rebelling against the rigid patriarchal norms. Thus, the novelist seems to convey that women can reclaim their lost dignity and self-esteem with education and proper grooming. She skilfully portrays the revolutionary progress made by modern women in exploring new avenues, availing themselves of opportunities, making bold unorthodox decisions, taking life's challenges unwaveringly, and moving forward "…strong in will/ To strive, to seek, to find, and not to yield" (Tennyson).

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A RESEARCH JOURNAL OF HUMANITIES, COMMERCE AND SCIENCES OF DAV COLLEGE AMBALA

ISSN: 2348-0300 VOLUME : 10 Issue: 2022

DAV COLLEGE (LAHORE)
AMBALA CITY-134003

College with Potential for Excellence and
NAAC Re-accredited Grade 'A'
(Conferred Heritage Status by UGC)

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ISSN : 2348-0300 VOLUME: 10

ISSN : 2348-0300 Volume 10 Issue : 202

A MINI REVIEW ON NOVEL MATERIALS: SHAPE MEMORY POLYMERS

NEHA AGGARWAL, Assistant Professor, Department of Chemistry, Gandhi Memorial National College, Ambala Cantt.-133001, Haryana, **Email:** chem.nehaaggarwal@gmail.com

Abstract

Shape memory polymers are emerging class of smart materials which can change their shape when exposed to an external stimulus. These polymers belong to a group of 'actively moving' polymers which can change from shape A to shape B. Shape A is a temporary shape while shape B is the permanent one. The external stimuli may be heat, light, electric field, infra-red light, alternating magnetic field, water immersion or any other indirect actuation. These polymers are gaining popularity day by day because of its immense applications in everyday life. The shape-memory effect is not an intrinsic property but results from the combination of polymer morphology and specific processing. In this mini review, the author is mainly focusing on creating awareness among the readers regarding this class of novel materials. Since these materials are very good respondent of temperature, light, pH there are very interesting possibilities of innovations with them. For instance, innovating possibilities with clothing is in terms of aesthetics, sustainability, environmental protection, comfort etc.

Keywords: Polymers, Shape memory, stimulus, smart materials.

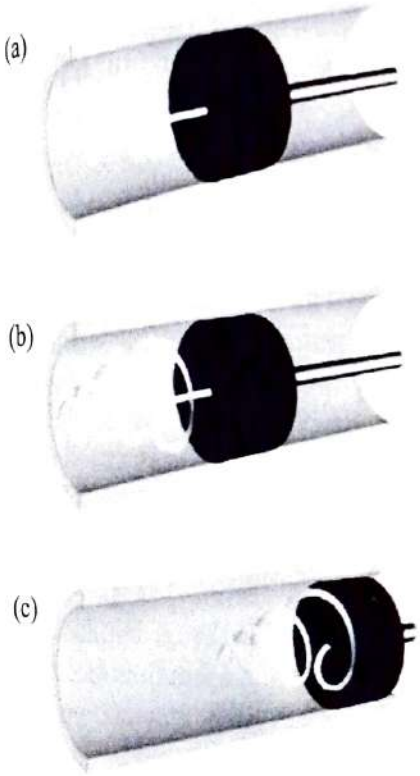
Introduction & Review

The shape memory polymers are materials that have a "memory" of their shape. Specifically, they are able to return to their original shape after they have been deformed in some way. They can return to their original shape when a particular stimulus is applied. These all belong to a wider set of "smart materials", also known as intelligent or responsive materials that have one or more properties that are affected in a controlled manner by some external stimuli (1-3).

The shape-memory effect is not an intrinsic property, meaning that polymers do not display this effect by themselves. Shape memory results from a combination of polymer morphology and specific processing and can be understood as a polymer functionalization. By conventional processing, e.g. extruding or injection molding, the polymer is formed into its initial, permanent shape B. Afterwards, in a process called programming, the polymer sample is deformed and fixed into the temporary shape A. Upon application of an external stimulus, the polymer recovers its initial permanent shape B. This cycle of programming and recovery can be repeated several times, with different temporary shapes in subsequent cycles. In comparison with metallic shape-memory alloys, this cycle of programming and recovery can take place in a much shorter time interval and polymers allow a much higher deformation rate between shapes A and B (4-7).

Some interesting applications:

1. *Use in active medical devices*: Shape memory polymers are widely used in laser devices for blood clot removal. Such device is inserted into the blood vessel with its temporary shape and then upon laser activation, gets converted to permanent shape which removes the blood clot from the vessel (8,9).



(Image source: W. Small, et al. Opt. Express, 13 (2005) 8204)

Depiction of removal of a clot in a blood vessel using the laser-activated shape-memory polymer microactuator coupled to an optical fiber.

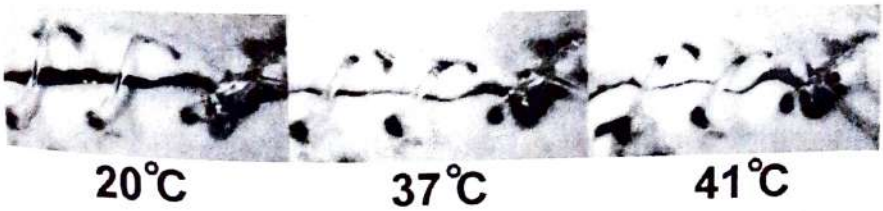
Shape memory polymers are also used in the stents for the prevention of strokes.

2. *Biodegradable shape memory polymers:* These biodegradable polymers are used in bulky human implants which retain their original shape after being inserted in the body through small incision. Another interesting example is as intelligent suture for wound closure. These sutures seal the wound through its shape memory effect and then degrade within a pre-defined interval of time (10-13).

Degradable shape memory suture for wound closure

3. *Industrial applications:* Shape memory polymers are used in building industry as shape memory foams which expand on heating sealing the window frames. These are used in sports wear like helmets, karate and judo suits. These are also useful in the production of functional and responsive photonic gratings (14-16).

4. *Automobile industry:* Shape memory polymers are used in self-repairing structural components such as e.g. automobile fenders in which dents are repaired by application of temperature. After an undesired deformation, such as a dent in the fender, these materials "remember" their original shape. Heating them activates their "memory." SMPs may also be useful in the production of aircraft which would morph during flight. Currently, the



(Image source: A. Landlein, R. Langer, Science, 296 (2002) 1673.)

Defense Advanced Research Projects Agency DARPA is testing wings which would change shape by 150°.

The field of actively moving polymers i.e. polymers that are able to perform movements by themselves is progressing rapidly. The application requirements can be complex in this area. Therefore, a trend toward the development of multifunctional materials can be seen.(17-18)

Conclusion and Prospects

Being regarded as smart materials, shape memory polymers are the best solution for a wide range of applications, such as artificial muscles, bio-inspired actuators, smart clothes, solar arrays, and deployable trusses due to their outstanding properties, such as structural versatility, lightweight, low cost, easy processing, mechanical, biocompatibility, and biodegradability.

Fundamental shape-memory research is focusing on the implementation of stimuli other than heat to actuate shape-memory polymers, or to actuate them remotely. First examples include the light-induced stimulation of shape-memory polymers or the use of alternating magnetic fields for remote actuation. It is assumed that these methods of stimulation will open up new fields of application. An important application area for shape-memory polymers is in active medical devices and implants, and initial demonstrations have been presented. The application requirements can be complex in this area. Therefore, a trend toward the development of multifunctional materials can be seen.

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A RESEARCH JOURNAL OF HUMANITIES, COMMERCE AND SCIENCES OF DAV COLLEGE AMBALA

ISSN: 2348-0300 VOLUME : 9 & 10 ISSUES: 2021 & 2022

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A RESEARCH JOURNAL OF HUMANITIES, COMMERCE AND SCIENCES OF DAV COLLEGE AMBALA

ISSN: 2348-0300 VOLUME : 10 Issue: 2022

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ISSN : 2348-0300 VOLUME: 10 ISSUE: 2

CLASSIFICATION, CHARACTERIZATION AND APPLICATION OF NANO PARTICLES: A REVIEW

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Abstract

Nanotechnology has been defined as research and development at the atomic, molecular or macromolecular scales. Nanoparticles are considered to be the building blocks for nanotechnology. Nanoparticles are a class of materials with properties distinctively different from their bulk and molecular counterparts and have sizes ranging from 1 to 100nm with one or more dimensions. The nanoparticles are generally classified into the organic, inorganic and carbon based particles in nanometric scale that has improved properties compared to larger sizes of respective materials. The nanoparticles show enhanced properties such as high reactivity, strength, surface area, sensitivity, stability, etc. because of their small size. Nanoscale materials find use in a variety of different areas, such as electronic, magnetic and optoelectronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic, and materials applications. This paper presents a review on nanoparticles, their types, properties and their applications.

Keywords: Nanotechnology, Research & Development, Nanoparticles.

Introduction & Review

The fundamental component of nanotechnology is the nanoparticles. Nanoparticles are particles between 1 and 100 nanometres in size and are made up of carbon, metal, metal oxides or organic matter [1]. The nanoparticles exhibit a unique physical, chemical and biological properties at nanoscale compared to their respective particles at higher scales. This phenomena is due to a relatively larger surface area to the volume, increased reactivity or stability in a chemical process, enhanced mechanical strength, etc. [2]. These properties of nanoparticles has led to its use various applications. The nanoparticles differs from various dimensions, to shapes and sizes apart from their material [3]. A nanoparticle can be either a zero dimensional where the length, breadth and height is fixed at a single point for example nano dots, one dimensional where it can possess only one parameter for example graphene, two dimensional where it has length and breadth for example carbon nanotubes or three dimensional where it has all the parameters such as length, breadth and height for example gold nanoparticles. The nanoparticles are of different shape, size and structure. It be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. or irregular and differ from 1 nm to 100 nm in size. The surface can be a uniform or irregular with surface variations. Some nanoparticles are crystalline or amorphous with single or multi crystal solids either loose or agglomerated [4].

...synthesis methods are either being ...eloped or improved to enhance the ...erties and reduce the production costs. ...methods are modified to achieve process ...ific nanoparticles to increase their optical, ...mical, physical and chemical properties ...A vast development in the instrumentation ...led to an improved nanoparticle ...acterization and subsequent application. ...nanoparticles are now used in every ...spect like from cooking vessel, electronics to ...ewable energy and aerospace industry. ...otechnology is the key for a clean and ...ainable future.

Classification of Nanoparticles

The nanoparticles are generally classified into the organic, inorganic and carbon based:

1. **Organic nanoparticles:** Dendrimers, micelles, liposomes and ferritin, etc. are commonly knows the organic nanoparticles or polymers. These nanoparticles are biodegradable, non-toxic, and some particles such as micelles and liposomes have a hollow ...ure, also known as nanocapsules and are sensitive to thermal and electromagnetic radiation such as heat and light [5]. These unique characteristics make them an ideal choice for drug delivery. The drug carrying capacity, its stability and delivery systems, either entrapped drug or adsorbed drug system determines their field of applications and their efficiency apart from their normal characteristics such as the size, composition, surface morphology, etc. The organic nanoparticles are most widely used in the biomedical field for example drug delivery system as they are efficient and also can be injected on specific parts of the body that is also known as targeted drug delivery.

2. **Inorganic nanoparticles:** Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide based nanoparticles are generally categorised as inorganic nanoparticles

(a) Metal based. Nanoparticles that are synthesised from metals to nanometric sizes either by destructive or constructive methods are metal based nanoparticles. Almost all the metals can be synthesised into their nanoparticles [6]. The commonly used metals for nanoparticle synthesis are aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn). The nanoparticles have distinctive properties such sizes as low as 10 to 100nm, surface characteristics like high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline and amorphous structures, shapes like spherical and cylindrical and colour, reactivity and sensitivity to environmental factors such as air, moisture, heat and sunlight etc.

(b) Metal oxides based. The metal oxide based nanoparticles are synthesised to modify the properties of their respective metal based nanoparticles, for example nanoparticles of iron (Fe) instantly oxidises to iron oxide (Fe_2O_3) in the presence of oxygen at room temperature that increases its reactivity compared to iron nanoparticles. Metal oxide nanoparticles are synthesised mainly due to their increased reactivity and efficiency [7]. The commonly synthesised are Aluminium oxide (Al2O3) Cerium oxide (CeO2), Iron oxide (Fe2O3), Magnetite (Fe3O4), Silicon dioxide (SiO2), Titanium oxide (TiO2), Zinc oxide (ZnO). These nanoparticles have possessed an exceptional property when compared to their metal counterparts.

3. **Carbon based:** The nanoparticles made completely of carbon are knows as carbon based [8]. They can be classified into fullerenes, graphene, carbon nano tubes (CNT), carbon nanofibers and carbon black and sometimes activated carbon in nano size.

(a) Fullerenes. Fullerenes (C60) is a carbon molecule that is spherical in shape and made up of carbon atoms held together by sp2 hybridization. About 28 to 1500 carbon atoms forms the spherical structure with diameters up to 8.2 nm for a single layer and 4 to 36 nm for multi-layered fullerenes.

(b) Graphene. Graphene is an allotrope of carbon. Graphene is a hexagonal network of honeycomb lattice made up of carbon atoms in a two dimensional planar surface. Generally the thickness of the graphene sheet is around 1 nm.

(c) Carbon Nano Tubes (CNT). Carbon Nano Tubes (CNT), a graphene nanofoil with a honeycomb lattice of carbon atoms is wound into hollow cylinders to form nanotubes of diameters as low as 0.7 nm for a single layered and 100 nm for multi-layered CNT and length varying from a few micrometres to several millimetres. The ends can either be hollow or closed by a half fullerene molecule.

(d) Carbon Nanofiber. The same graphene nanofoils are used to produce carbon nanofiber as CNT but wound into a cone or cup shape instead of a regular cylindrical tubes.

(e) Carbon black. An amorphous material made up of carbon, generally spherical in shape with diameters from 20 to 70 nm. The interaction between the particles is so high that they bound in aggregates and around 500 nm agglomerates are formed.

Characterization

The unique characteristic determines the potential and application of a nanoparticle.

(a) Size The particle is one of the most basic and important measurement for nanoparticle characterisation. It determines the size and distribution of the particle and whether it falls under nano or micro scale. The particle size and distribution is most commonly measured using electron microscopy. The images of Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) are used for the measurement of particles and clusters whereas laser diffraction methods are used for measuring bulk samples in solid phase [9]. The particles in liquid phase are measured using photon correlation spectroscopy and centrifugation. The particles in gaseous phase are difficult and irreverent to use the imaging techniques and hence a Scanning Mobility Particle Sizer (SMPS) is used which provides a fast and accurate measurements compared to other methods.

(b) Surface area The surface area is also a significant factor in nanoparticle characterisation. The surface area to volume ratio of a nanoparticle has a huge influence on its performance and properties. The surface area is most commonly measured using BET analysis. A simple titration is sufficient for the surface area analysis of particles in liquid phase, but it is a labour intensive process. Hence nuclear magnetic resonance spectroscopy (NMR) is used. A modified SMPS and differential mobility analyser (DMA) is used for the measurement of surface are of nanoparticles in gaseous phase.

Composition The chemical or elemental composition determines the purity and performance of the nanoparticle. Presence of higher secondary or undesired elements in the nanoparticle may reduce its efficiency and also lead to secondary reaction and contamination in the process. The composition measurement is usually carried out by X-ray photoelectron spectroscopy (XPS) [10]. Some techniques involve chemical digestion of the particles followed by wet chemical analysis such as mass spectrometry, atomic emission spectroscopy and ion chromatography. The particles in gaseous phase are collected either by filtration or electrostatically and spectrometric or wet chemical techniques are used for the analysis [11].

Surface morphology The nanoparticles possess various shapes and surface structures that plays a key role in exploiting its properties. Some of the shapes include spherical, flat, cylindrical, tubular, conical and irregular shapes with surface like crystalline or amorphous with uniform or irregularities on the surface. The surface is generally determined by electron microscopy imaging techniques like SEM and TEM [12]. The particles in liquid phase are deposited on a surface and analysed whereas the particles in gaseous phase are capture electrostatically or by filtration for imaging using electron microscopy.

Surface charge The surface charge or the charge of a nanoparticle determines its interactions with the target. Generally a zeta potentiometer is used for the measurement of surface charges and its dispersion stability in a solution [9]. A Differential Mobility Analyser (DMA) is used for the charge determination of nanoparticles in gaseous phase.

(f) Crystallography Crystallography is the study of atoms and molecules arrangement in crystal solids. The crystallography of nanoparticles are carried out by a powder X-ray, electron or neutron diffraction to determine the structural arrangement [13].

(g) Concentration The concentration of nanoparticles in gaseous phase is measured to determine the volume of air or gas required for the process. The concentration, size and distribution of nanoparticles in a unit volume of air or gas marks the performance or its efficiency. The concentration measurements are usually made through a Condensation Particle Counter (CPC).

Applications Below are some of the significant applications of nanoparticles.

(a) Cosmetics and Sunscreens The conventional ultraviolet (UV) protection sunscreen lacks long-term stability during usage. The sunscreen including nanoparticles such as titanium dioxide provides numerous advantages. The UV protection property of titanium oxide and zinc oxide nanoparticles as they are transparent to visible light as well as absorb and reflect UV rays found their way to be used in some sunscreens. Some lipsticks use iron oxide nanoparticles as a pigment [14].

(b) Electronics The higher necessity for large size and high brightness displays in recent days that are used in the computer monitors and television is encouraging the use of nanoparticles in the display technology. For example nanocrystalline lead telluride, cadmium sulphide, zinc selenide and sulphide, are used in the light emitting diodes (LED)

of modern displays [15]. The development in portable consumer electronics such as mobile phones and laptop computers led to the enormous demand for a compact, lightweight and high capacity batteries. Nanoparticles are the ideal choice for separator plates in batteries. A considerable more energy can be stored compared to traditional batteries due to their foam like (aerogel) structure. Batteries made from nanocrystalline nickel and metal hydrides, due to their large surface area require less recharging and last longer. The increase in electrical conductivity of nanoparticles is used to detect gases like NO2 and NH3 [16]. This is due to increase in the pores of nanoparticles due to charge transfer from nanoparticles to NO2 as the gas molecules bind them together making them a better gas sensors.

(c) Catalysis Nanoparticles contain high surface area that offers higher catalytic activity. Due to their extremely large surface to volume ratio the nanoparticles function as efficient catalyst in the production of chemicals [17]. One of the important application is the use of platinum nanoparticles in the automotive catalytic converters as they reduce the amount of platinum required due to very high surface area of the nanoparticles thus reducing the cost significantly and improving performance. Some chemical reactions for example, reduction of nickel oxide to metal nickel (Ni) is performed using nanoparticles.

(d) Medicine Nanotechnology has improved the medical field by use of nanoparticles in drug delivery. The drug can be delivered to specific cells using nanoparticles [18]. The total drug consumption and side effects are significantly lowered by placing the drug in the required area in required dosage. This method reduces the cost and side effects. The reproduction and repair of damaged tissue (Tissue engineering) can be carried out with the help of nanotechnology. The traditional treatments such as artificial implants and organ transplants can be replaced by tissue engineering. One such example is the growth of bones carbon nanotube scaffolds [19]. The use of gold in medicine is not new. In Ayurveda an Indian medical system, gold is used in several practices. One common prescription is the use of gold for memory enhancement. To enhance the mental fitness of a baby gold is included in certain medical preparations [20].

(e) Food The improvement in production, processing, protection and packaging of food is achieved by incorporating nanotechnology. For example a nanocomposite coating in a food packaging process can directly introduce the anti-microbial substances on the coated film surface [21]. One of the example is the canola oil production industry includes nanodrops, an additive designed to transfer the vitamins and minerals in the food.

(f) Renewable energy and environmental remediation The unique physical and chemical properties of nanoparticles has made them an ideal choice to be used nowadays in environmental remediation to enhancing the performance in renewable energy sector [22]. Nanoparticles occur in nature themselves and some of them are found to cure the environment. Environmental remediation using nanoparticles or Nano remediation is successfully being used to

treat or decontaminate the air, water and soil for over a decade [2]. Nano remediation is one of the effective solutions as it offers in situ treatment eliminating the necessity of pumping the ground water out for treatment and the need for excavation to reach the target destination. The nanoparticles are injected into the desired location and get carried along the groundwater flow and decontaminate the water by immobilising the contaminants. The general mechanism involving in decontamination is the redox reactions. The nanoparticles are used to treat the surface water by disinfection, purification and desalination. Some of the contaminants are most likely to be heavy metals, pathogens and organic contaminants. It has proven to be efficient and eliminating the need for chemicals that may sometime produce secondary reaction products. Oil spill is one of the major problems worldwide as it may spread over very long distances. Cleaning them by conventional methods is difficult and time consuming that makes the situation worse as it may spread more. The nanoparticles are also used to clean-up oil spills and has also established to be effective method. The major uses of nanoparticles are to treat municipal and industrial wastewater as well as the sludge produced. The replacement of nanoparticles for conventional chemicals is due to less cost, higher efficiency and lower quantity required for treatment. Nano filtration is a recent membrane filtration system for water purification widely used in food and dairy industries. Soil contamination is also an increasing concern. Contaminated soil is cleaned or treated using nanoparticles by injecting the nanoparticles into specific target locations for heavy metal contamination, toxic industrial waste, etc.

Conclusion

Nanotechnology is improving our everyday lives by enhancing the performance and efficiency of everyday objects. It provides a clean environment by providing safer air and water, and clean renewable energy for a sustainable future. Nanotechnology has gained a wide attention where more investment is made for the research and development by top institutions, industries and organizations. Nanotechnology has established to be an advanced field of science where extensive research is carried out to implement the technology. It is being tested for various new applications to increase the efficiency and performance of the object or process and subsequently reduce the cost so that it is accessible for everyone. The nanotechnology has a great future due to its efficiency and environmental friendly property.

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NANOPOROUS MATERIALS: A COMPREHENSIVE STUDY OF PROPERTIES AND APPLICATIONS

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Abstract
Nanoporous materials are a class of materials with a high surface area and pore volume. They are characterized by having pores that are on the order of nanometers in size. This gives them a number of unique properties, such as high adsorption capacity, tunable selectivity, and biocompatibility. Nanoporous materials have a wide range of applications, including catalysis, gas storage, water purification, and drug delivery. These materials have a very high surface area to volume ratio, which makes them ideal for a variety of applications, including catalysis, gas separation, energy storage, and sensing.

Keywords: Nanoporous Materials, Gas separation, sensing, energy storage

Introduction

Nanoporous materials are a type of porous material that has a pore size in the nanoscale range (<100 nm). They are characterized by a high surface area to volume ratio, which gives them a number of unique properties. These properties include:

- High adsorption capacity: Nanoporous materials can adsorb large amounts of gas, liquid, or solid molecules. This makes them useful for applications such as gas storage, water purification, and catalysis [1].
- Tunable selectivity: The pore size and surface chemistry of nanoporous materials can be tuned to achieve a desired selectivity for a particular molecule or type of molecule. This makes them useful for applications such as separations and purifications[2,3].
- Biocompatibility: Some nanoporous materials are biocompatible, meaning that they can be used in contact with living tissue. This makes them promising candidates for applications such as drug delivery and tissue engineering [2,3].

Objectives of the study

- To analyze types of Nanoporous Materials
- To study properties Nanoporous Materials
- To study applications of Nanoporous Materials

1. Types of Nanoporous Materials

There are a number of different types of nanoporous materials, each with its own unique properties. Some of the most common types of nanoporous materials include [4,5]:

- Zeolites: Zeolites are crystalline aluminosilicates with a regular pore structure. They are known for their high adsorption capacity and selectivity.
- Metal-organic frameworks (MOFs): MOFs are organic-inorganic hybrid materials that are formed by the coordination of metal ions or clusters with organic linker molecules. They have a wide range of pore sizes and surface chemistries, which makes them versatile for a variety of applications.
- Activated carbons: Activated carbons are carbon materials that have been treated to increase their surface area. They are known for their high adsorption capacity for a variety of molecules.
- Carbon nanotubes: Carbon nanotubes are hollow cylindrical structures made of carbon atoms. They have a high surface area and a high electrical conductivity, which makes them useful for a variety of applications.

2. Properties of Nanoporous Materials

The properties of nanoporous materials are determined by their pore size, surface chemistry, and the nature of the material itself. Some of the key properties of nanoporous materials include [6]:

- Surface area: The surface area of a nanoporous material is the total area of all the pores in the material. It is a measure of the amount of surface available for adsorption or reaction.
- Pore size: The pore size of a nanoporous material is the diameter of the pores in the material. It determines the size of molecules that can be adsorbed or transported through the material.
- Surface chemistry: The surface chemistry of a nanoporous material is the chemical composition of the surface of the material. It determines the types of molecules that can be adsorbed or reacted with the material.
- Mechanical properties: The mechanical properties of a nanoporous material are determined by the nature of the material itself and the pore structure. They are important for applications where the material needs to be strong or durable.

3. Applications of Nanoporous Materials

- **Catalysis :** Catalysis is the process of accelerating a chemical reaction without being consumed in the reaction. Nanoporous materials are excellent catalysts because they have a large surface area that can provide active sites for the reaction to occur. In addition, the confined environment of the pores can help to control the reaction and improve the selectivity of the catalyst. One example of a nanoporous catalyst is zeolites. Zeolites are a type of crystalline aluminosilicate with a honeycomb-like structure. The pores in zeolites are typically in the range of 2-20 nanometers, which makes them ideal for catalyzing reactions involving small molecules. Zeolites have been used to catalyze a wide variety of reactions, including the production of gasoline, plastics, and pharmaceuticals[7-9].
- **Gas Separation:** Gas separation is the process of separating a mixture of gases into its individual components. Nanoporous materials are well-suited for gas separation because they have a high selectivity for different gases. This is due to the fact that the pores in nanoporous materials can be tailored to the size and shape of the molecules that need to be separated.One example of a nanoporous material used for gas separation is carbon molecular sieves (CMS). CMS are a type of carbon with a highly ordered pore structure. The pores in CMS are typically in the range of 0.3-1.0 nanometers, which makes them ideal for separating small molecules such as hydrogen, oxygen, and nitrogen. CMS have been used to separate gases in a variety of applications, including the production of hydrogen for fuel cells and the purification of air [10].
- **Energy Storage:** Nanoporous materials are also being investigated for use in energy storage applications. One potential application is in the development of hydrogen storage materials. Hydrogen is a promising fuel for the future, but it is difficult to store because it is a gas at ambient temperature and pressure. Nanoporous materials with a high pore volume and surface area could be used to store hydrogen in a more compact and efficient way.Another potential application of nanoporous materials in energy storage is in the development of supercapacitors. Supercapacitors are devices that can store and release electrical energy very quickly. Nanoporous materials with a high surface area could be used to make the electrodes in supercapacitors, which would improve their performance[11-12].
- **Sensing:** Nanoporous materials are also being used in sensing applications. Sensors are devices that can detect the presence of a specific molecule or substance. Nanoporous materials with a high surface area can be used to create sensors that are highly sensitive to specific molecules.One example of a nanoporous sensor is a gas sensor. Gas sensors can be used to detect the presence of harmful gases such as carbon monoxide and methane. Nanoporous materials with a high surface area can be used to create gas sensors that are very sensitive to these gases [13].

Conclusion

By boosting the functionality and efficiency of ordinary items, nanotechnology is enhancing our daily life. It offers a clean environment by supplying cleaner renewable energy for a sustainable future, as well as safer air and water. Widespread interest in nanotechnology has led to increased funding for research and development from prestigious institutions, businesses, and organizations. In order to utilize the technology, substantial study is being done in the advanced scientific field of nanotechnology. To improve the effectiveness and performance of the thing or process and consequently lower the cost so that everyone can use it, it is being evaluated for a variety of new

applications. Considering its effectiveness and ability to be environmentally benign, nanotechnology has a bright future.

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Journal of Drug Delivery Science and Technology 87 (2023) 104808

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Development and characterization of micelles for nucleolin-targeted co-delivery of docetaxel and upconversion nanoparticles for theranostic applications in brain cancer therapy

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ARTICLE INFO

Keywords:
AS1411 aptamer
Brain distribution
Brain-targeted drug delivery
Histopathology of the brain
Nanomedicines
Pharmacokinetics

ABSTRACT

Despite the existence of several treatment modalities and advancements in cancer research, brain cancer is still incurable. Over-expression of nucleolin receptors on cancer cells has been explored in several studies. The study aimed to develop and characterize nucleolar -targeted theranostic pluronic F127-TPGS micelles for brain cancer therapy. The theranostic agents i.e., Docetaxel; DTX as a therapeutic agent, and the upconversion nanoparticles; UCNP as a diagnostic agent, were loaded into micelles by a slightly-modified solvent casting method. Micelles were further decorated with synthesized TPGS-AS1411 aptamer conjugate for targeting brain cancer cells. The prepared micelles were found between 90 and 165 nm, with a uniform homogeneous and narrow distribution in formulations. DTX and UCNP encapsulation efficiencies of micelles were found 74–88% and 38–40%, respectively. Micelles have depicted sustained release of DTX for as long as 72 h. Hemolytic assay confirmed that DUTP-AS1411 aptamer micelles were found more biocompatible than Taxotere®. The cytotoxicity results revealed that DTP, DUTP, and DUTP-AS1411 aptamer micelles achieved 4.20, 11.70, and 17.54-fold more effectiveness than Taxotere®, after 24 h of therapy, respectively. In addition, DUTP-AS1411 aptamer micelles achieved higher t_{max} and C_{max} of DTX up to 8- and 1.5-fold, respectively, compared to Taxotere® treated group. A similar trend was observed for the brain-distribution study as DUTP-AS1411 aptamer micelles were found more efficacious than Taxotere®. The histopathology studies showed no toxicity and cellular damage even after the 14th and 28th day post *i.v.* administration of normal saline, DTP, DUTP, and DUTP-AS1411 aptamer micelles formulations whereas Taxotere® has reported to cause toxicity in brain tissues. The study revealed that DUTP-AS1411 aptamer micelles inherit promising and improved therapeutic efficacy, reduced toxicity, dosing frequency, and sustained drug release behavior which can be further exploited as a potential therapeutic approach for brain cancer.

1. Introduction

Brain cancer is a progressive form of cancer and has high fatality globally. Although there are several treatment modalities available, still brain cancer is uncurable due to its certain limitations. The survival of brain tumor patients is only 5 years or less as a consequence of the

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https://doi.org/10.1016/j.jddst.2023.104808
Received 29 May 2023; Received in revised form 25 July 2023; Accepted 1 August 2023
Available online 5 August 2023

combination of surgery, radiotherapy, and systemic chemotherapy [1,2]. Although, chemotherapy is most preferred cancer treatment modality, it also targets healthy cells due to the haphazard distribution of anticancer drug in the body [3]. It highlights the need for targeted delivery of chemotherapeutic agents in brain cancer therapy. Radiotherapy-mediated redox homeostasis-modulating nanomedicine was developed to enhance ferroptosis susceptibility in tumor therapy. Nanomedicine devised in combination with radiation therapy has demonstrated potent tumor growth suppression through an enhanced ferroptosis approach [4,5]. Last decade has seen a meteoric rise in attention towards nanomedicine as targeted drug delivery candidate for favourable outcomes in anticancer studies. The term "nanomedicines" can be explained as nano-size ranged colloidal drug carriers for effective diagnosis and treatment of cancer [6]. There are several nanomedicines such as nanoparticles, niosomes, liposomes, dendrimers and micelles etc. are utilized for the delivery of therapeutic agents for treatment of brain cancer. Among all nanomedicines, micelles are one of the best suitable nanocarriers which facilitate higher blood-brain barrier (BBB) penetration of therapeutic drugs, reduce multidrug-resistance and inhibit tumor recurrence post surgery [7]. It can be prepared from amphiphilic di/tri-block or from graft copolymers. It also offers high pay-load and greater biocompatibility with *in-vitro* and *in-vivo* biological systems. Micelles are highly capable to load and administer both hydrophilic and hydrophobic drugs to the body and can help increase their pharmacokinetic and bioavailability profiles [8]. Micelles absorb through passive diffusion and receptor-based endocytosis mechanism citing its enhanced permeability and retention effect (EPR) into tumor tissues [9].

D-α-tocopheryl polyethylene glycol 1000 Succinate (TPGS) is an excellent emulsifier, solubilizer, and nonionic surfactant composed of a desirable combination of natural vitamin E (tocopherol) and polyethylene glycol 1000 chains. The chemical structure of TPGS consists of a hydrophobic alkyl tail and a polar hydrophilic head. TPGS has a hydrophilic-hydrophobic balance (HLB) value of 13.2 and a relatively low critical micelle concentration (CMC) of 0.02% w/w. These are exceptional properties that suggest that TPGS is an ideal molecule for use in developing drug delivery vehicles. It inhibits the multidrug resistance (MDR) and P-glycoprotein (P-gp; an ATP-binding cassette (ABC) transporter) efflux mechanism in cancer cells and prolongs the bioavailability of anticancer drugs and increases the clinical outcomes of anticancer agents in cancer therapy [10,11]. Similarly, Pluronic F127 (PF127) is also one of the FDA approved eco-friendly polymers which construct through a block copolymer of three poly(ethylene oxide)-poly (propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) segments at 3:7 ratio of PPO:PEO. It possesses several excellent properties including biocompatibility, degradability, and flexible mechanical properties, enabling the creation of versatile chemical conjugates with proteins and ligands. It also helps in enhancing the extended bioavailability of nanodrugs [12,13]. Meng et al. demonstrated the clinical effects of effect of PF-127 TPGS micelles. The cellular uptake studies revealed the greater cellular internalization of mixed micelles loaded with rhodamine 123 and DiR in rat brain. In the pharmacokinetic study, PF-127-TPGS micelles increased potency and increased β-galactosidase accumulation in rat brain [2].

Docetaxel (DTX) is an anticancer drug that is having more effectiveness in treating cancers of the brain, breast, prostate, lung, stomach, head and neck etc. It is a broad-spectrum anticancer drug that stops cancer growth by inhibiting tubulin depolymerization and causes microtubule aggregation and cell death. It is a poorly water-soluble drug (6–7 μg/mL) and is considered as a BCS class VI drug, thus limiting its clinical uses and efficacy [14]. Recently, nanomedicine-based theranostic approaches for simultaneous treatment and diagnosis of cancer have been investigated. Recently, upconversion nanoparticles have attracted attention due to its uniqueness such as minimum level of background noise, greater cellular internalization, minimal photodamage, high resistance to photobleaching, and easy surface modification with ligands that propose suitable bioimaging/biosensing agents [15]. Rare earth-doped upconversion nanoparticles (UCNP) offer several advantages such as excellent photostability, deep tissue penetration, and excellent biocompatibility etc. The exceptional protection of the 5s and 5p orbits in the structure results in stable and high fluorescence spectra with narrow emission peaks [16].

Aptamers are used in cancer research to recognize cancer cells and to effectively and targetally deliver cancer and diagnostic agents [17]. These are single-stranded RNA or DNA oligonucleotide sequences based on targeting ligands that have greater affinity and specificity for binding to specific nucleolin receptors on cancer cells [18]. Among all the aptamers, AS1411 aptamer has been reported to inhibit glioma xenograft growth and prolonged the survival in glioma-bearing mice. It is a synthetic 26-base DNA aptamer that has been investigated in cancer research because of its convenient synthesis process, non-immunogenicity, and malleability. It tightly binds to external domain of the membrane protein nucleolin (~76 kDa protein) and facilitates the intracellular entry of drug-containing nanocarriers [19,20]. It also has anticancer activity and prevents tumor growth by upregulating p53 (tumor suppressor gene) and downregulating Bcl-2 (B-cell lymphoma 2) protein in cancer cells [21]. Alibolandi et al. investigated the effects of AS1411-modified polyethylene (PEG) polymersomes for targeted delivery of gemcitabine in lung therapy. This study demonstrated potential applications of AS1411-modified polyethylene (PEG) polymersomes in lung cancer therapy [22]. Despite all these exceptional features, no one has demonstrated the targeting effectiveness for brain cancer therapy using AS1411 aptamer-engineered TPGS-PF127 theranostic micelles containing DTX and UCNP.

In this investigation, the PF127-TPGS diblock copolymer was used to develop micelles through the solvent casting method. DTX and UCNP were loaded into inner core of micelles. To target brain cancer cells, the surface of micelles was decorated with TPGS-AS1411 aptamer conjugate. TPGS-AS1411 aptamer conjugate was synthesized using activated TPGS (TPGS-COOH) which was synthesized by a ring-opening polymerization reaction. The objectives of the study were to i) develop a TPGS-AS1411 aptamer conjugates to enhance nucleolin-targeted delivery of DTX and UCNP in brain tumor cells, ii) investigate the physicochemical characteristics of PF127-TPGS micelles and iii) evaluate the effectiveness and biocompatibility effects of the developed AS1411 aptamer engineered PF127-TPGS micelles through pharmacokinetic, brain distribution, hemolysis and histopathology studies and compared with Taxotere® (a marked DTX injection).

2. Materials and methods

2.1. Materials

Docetaxel (DTX) was gifted from Alembic Research Center, Vadodara, India. Acetonitrile, chloroform, pluronic-F127 (PF127), succinic anhydride, 4-(Dimethylamino) pyridine (DMAP), DAPI (4′,6-diamidino-2-phenylindole; DAPI) and rare earth-doped upconversion nanoparticles (UCNP) were purchased from Sigma Aldrich Chemicals, St. Louis, MO, USA. D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was gifted from Antares Health Products., St. Charles, U.S.A. AS1411 (5′-GGT-GGT-GGT-GGT-TGT-GGT-GGT-GGT-GG-3′) aptamer was procured from Eurogentec, Kaneka Eurogentec S.A., Rue Bois Saint-Jean, Seraing, Belgium, with High-Performance Liquid Chromatography (HPLC) grade purification and used without further treatment. The PVDF syringe filters (filtration rating 0.45 and 0.22 μm) were procured from Axiva Sichem Pvt. Ltd., Sonipat, India. HPLC grade water and methanol, and tris-hydrochloride buffer (pH 8.0) were purchased from Sisco Research Laboratories (SRL) Pvt. Ltd. Mumbai, India. WhatmanTM glass microfiber filters with a diameter of 47 mm was procured from GE Healthcare UK Limited, Buckinghamshire, HP7 9NA, UK. Dialysis membrane (Spectra/Por 6®) pre-wetted RC tubing of 1 kDa and 10 kDa molecular weight cut-off (MWCO) was procured from Spectrum Laboratories Inc.,

U.S.A. All other solvents if not specified and solvents were of analytical grade and supplied by reputed manufacturing organizations. U-87MG cancer cells (human malignant GBM multiforme cells) were procured from ATCC (Manassas, VA, USA).

2.2. Methods

2.2.1. Synthesis of TPGS intermediate (TPGS-COOH) and its conjugate

TPGS was activated as TPGS-COOH through ring-opening polymerization reaction in the presence of DMAP and succinic anhydride exhibited in Fig. 1. The prepared TPGS-COOH intermediate was used to synthesize TPGS-AS1411 aptamer conjugate.

2.2.2. Activation of TPGS into TPGS-COOH

A 0.77 g of TPGS (equivalent to 0.5 mM), 0.10 g of succinic anhydride (equivalent to 1 mM) and 0.12 g of DMAP (equivalent to 1 mM) were added in a round bottom flask and then heated at near about 100 ± 5 °C under inert nitrogen atmosphere for 24 h. Then the whole resultant mixture was cooled at 25 °C and further, it was dissolved in 15 ml of dichloromethane (DCM). Then the solution was filtered through the Whatmann filter to remove unreacted succinic anhydride and DMAP. Furthermore, the product was precipitated in 50 ml of diethyl ether approximately at −10 °C overnight. Finally, the obtained white precipitate of product was filtered by filter paper (150 mm) and dried under vacuum [23,24]. The resulting product as TPGS-COOH intermediate was stored in the refrigerated condition in an Eppendorf tube till further analysis and synthesis of micelles formations.

2.2.3. Synthesis of TPGS-AS1411 aptamer conjugate

In the next set of experiments, TPGS-AS1411 aptamer conjugate was synthesized through a physical adsorption technique displayed in Fig. 1B. A 200 mg of TPGS-COOH was dissolved in 1 M tris-hydrochloride buffer (pH 8) and stirred at room temperature, 200 rpm till forming a homogeneous solution. After that, 1.82 ml of AS1411 aptamer (equivalent to 100 μM/mL) was added into 6 ml of 1 M tris-hydrochloride buffer (pH 8.0) under stirring condition. The solution was added in a dropwise manner into TPGS-COOH solution under stirring conditions for 6–8 h at room temperature for surface adsorption on TPGS. Product was concentrated by using a rotavapor (Rotapaor® R100, Buchi, Switzerland) at 474 mbar pressure at 40 °C temp. At last, the TPGS-AS1411 aptamer conjugate was stored at 4 °C till further used for characterization and synthesis of DUTP-AS1411 aptamer micelles [25,26]. Further, activated TPGS and its conjugate were characterized for their functional groups using Fourier-Transform Infrared (FTIR) Spectroscopy, and ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy techniques.

2.2.4. Characterization of TPGS-COOH and its conjugate

2.2.4.1. Fourier-Transform Infrared (FTIR) spectroscopy. To confirm the

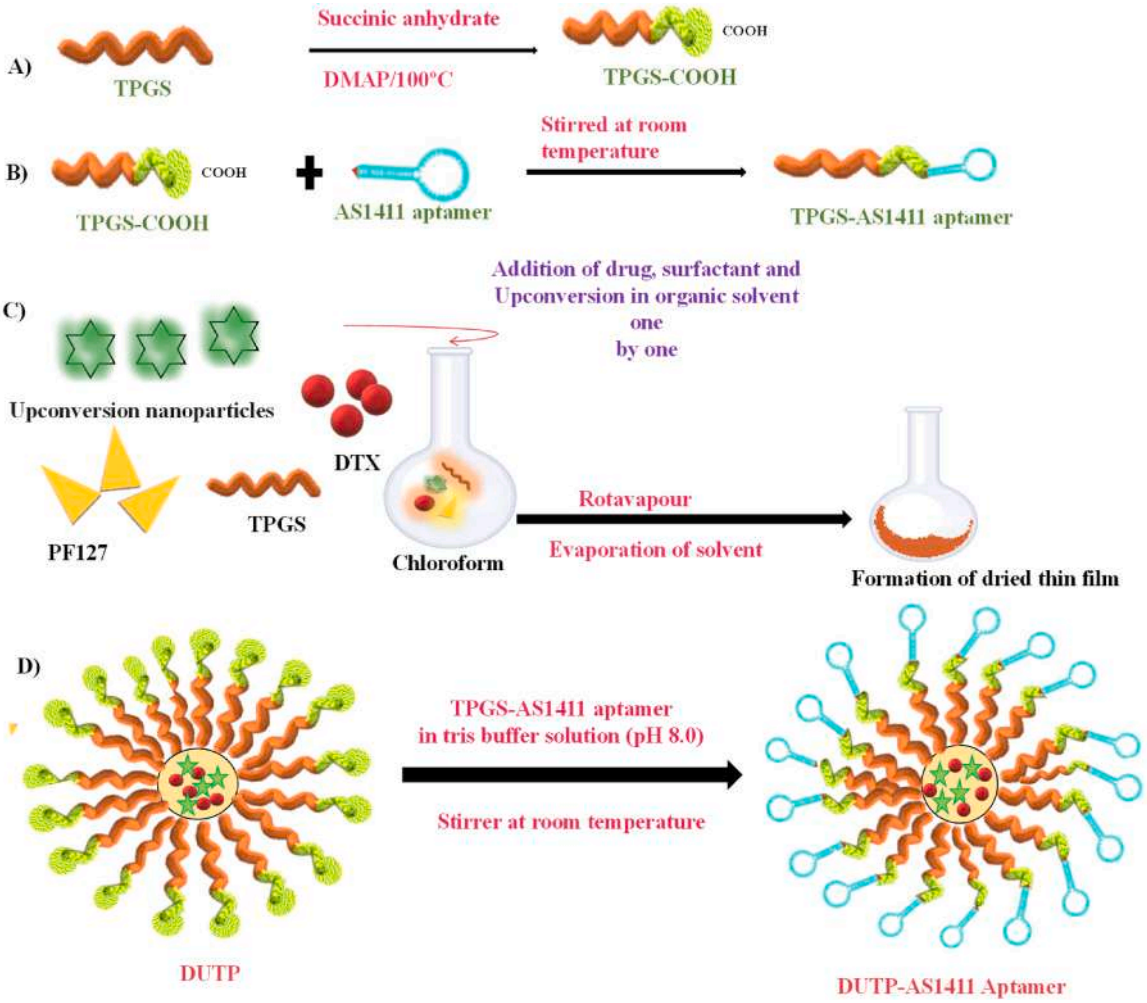

Fig. 1. Schematic illustration for A) activation of TPGS using succinic anhydrate (TPGS-COOH), (B) synthesis of TPGS-AS1411 aptamer conjugate, (C) synthesis of TPGS-PF127 micelles and DUTP: Theranostic TPGS-PF127 micelles containing DTX and UCNP, and (D), preparation of DUTP-AS1411 aptamer: AS1411 aptamer-engineered TPGS-PF127 theranostic micelles containing DTX and UCNP.

successful activation of TPGS to TPGS-COOH and synthesis of TPGS-AS1411 aptamer conjugate, an FTIR spectrophotometer (PerkinElmer Spectrum Two, Waltham, Massachusetts, U.S.A) was used. In brief, very few quantities of TPGS, TPGS-COOH, AS1411, and TPGS-AS1411 aptamer were blended with KBr individually and then detected for their transmittance at scanning range 450–4000 cm^{-1} under resolution at 2 cm^{-1}. The peaks of the major functional groups of each sample were obtained by running the spectra. Then spectrum of the samples was compared with their reference FTIR spectrum [27].

2.2.4.2. ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) spectrophotometery. Furthermore, the chemical structure of TPGS, TPGS-COOH, AS1411, and TPGS-AS1411 aptamer conjugate, was recorded at 25 °C through a ^{1}H NMR spectrometer (Bruker Avance III HD 400, Billerica, MA. United States). Each sample of TPGS, TPGS-COOH, AS1411, and TPGS-AS1411 aptamer conjugate were solubilized in an appropriately suitable solvent (i.e., water/$CDCl_3$/D_2O), separately before the analysis [28].

2.2.5. Fabrication of micelles formulations

The micelles were synthesized through a slightly modified solvent casting technique and demonstrated in Fig. 1 (C-D). In brief, the TPGS and PF127 (at 1:1 ratio) were dissolved in 5 ml of chloroform in a round-bottom flask (RBF) under stirring condition, and then 3 mg of DTX was added into it under sonication condition for 20 min at room temp. Then chloroform was removed from the solution using a rotavapor (Rotapaor® R100, Buchi, Switzerland) at 474 mbar pressure at 40 °C temp. to form a thin film of drug-dispersed surfactant. Finally, 10 ml phosphate buffer saline (PBS) at pH 7.4 was added to disperse thin film and then the resultant colloidal dispersion was transferred into a 15 ml of falcon tube. The colloidal dispersion was incubated into an orbital water bath shaker (Remi Elektrotechnik Ltd. India) at 37 ± 2 °C for 24 h. Later, the developed micellar dispersion (DTP: DTX loaded TPGS-PF127 micelles) was filtered by a 0.22 μm syringe filter to remove unloaded drug and aggregates before characterization [3,29]. For the preparation of DUTP (theranostic TPGS-PF127 micelles containing DTX and UCNP), 100 μg/ml of UCNP was added along with DTX in the preparation, and the rest of the procedure was same as mentioned above. A similar procedure was used for the preparation of DUTP-AS1411 (AS1411 aptamer-engineered TPGS-PF127 theranostic micelles containing DTX and UCNP) using additional 32 mg of TPGS-AS1411 aptamer conjugate (equivalent to 15 μM/mL conc. of AS1411 aptamer) to enhance drug loading and targeting of micelles for effective and brain cancer therapy (**Table 1**).

2.2.6. Characterization of micelles formulations

2.2.6.1. Micelles size, polydispersity, and zeta (ζ) potential measurements of micelles. The average particle size, polydispersity index, and ζ potential of plain PF127, DTP, DUTP, and DUTP-AS1411 aptamer micelles formulations were analyzed using Litesizer™ 500 (Anton Paar GmbH, Austria, Europe). Before performing sample analysis, the micelles samples were diluted 50 times with deionized water and then evaluated at 658 nm with an angle of 165°. The value of each report is the sum of its three measurements [27].

2.2.6.2. Transmission Electron Microscopy (TEM). The surface morphology was studied by a Transmission Electron Microscope (TEM-2100Plus LaB6 TEM, JEOL USA, Inc. Peabody, MA, USA). A drop of each micelle i.e., plain micelles, DTP, DUTP, and DUTP-AS1411 aptamer was placed onto a copper TEM grid (mesh size 200 and 3 mm) with a carbon coating with a mesh size of 200 and a diameter of 3 mm. Later, the solvent was evaporated by lamp heat for 30 min. The dried layer of micelles was stained with 1% phosphotungstic acid for 30 s then images were visualized at 80 kV under TEM [30].

2.2.6.3. Atomic force microscopy (AFM). Furthermore, the surface properties of plain micelles, DTP, DUTP, and DUTP-AS1411 aptamer micelles were visualized by an Atomic Force Microscope (Solver P-47-PRO, MDT; Moscow, Russia) under normal atmospheric conditions. The samples were diluted 50 times with distilled water. Then a drop of each micelles formulation was dropped onto glass slides and the solvent was dried through a vacuum drying technique at 25 °C for 24 h. The measurements were obtained using AFM image analysis software [31,32].

2.2.6.4. Determination of encapsulation of micelles. The DTX content in the developed micelles formulation was measured by a UV–visible spectrophotometer (Shimadzu 1800, Tokyo, Japan) at 230 nm. Briefly, 0.5 ml of prepared DTP, DUTP, and DUTP-AS1411 aptamer micelles formulations were dried using a rotary evaporator at 40 °C under reduced pressure. Then 10 ml of methanol was used to dissolve the residue and further drug absorbance was taken by UV–visible spectrophotometer at 230 nm [32]. The UCNP encapsulation efficiency of theranostic micelles, DUTP, and DUTP-AS1411 aptamer micelles is analyzed with toluene as solvent and the absorbance was measured at 245 nm [33]. The spectrophotometric absorbance measurements were carried out to determine the DTX and UCNP contents in micelles formulation by using the following formula:

$$DTX\ encapsulation\ efficiency\ (\%) = \frac{amount\ of\ drug\ loaded\ in\ micelles}{amount\ of\ drug\ added\ during\ fabrication} X100$$

2.2.7. In-vitro drug release studies

The *in-vitro* drug release from Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles was assessed by dialysis method in PBS at pH 7.4 using a water bath shaker. The micelles/Taxotere® formulations volume was kept equivalent to 300 μg of DTX and were placed in the dialysis membrane (MWCO: 1 kDa) and hermetically sealed to avoid leakage from the membrane. Then dialysis membrane was immersed in 100 ml of PBS at pH 7.4 and kept inside the water bath shaker at 37 ± 2 °C for 72 h under constant shaking. At predetermined time intervals

Table 1
Formulae of TPGS- PF127 preparation of micelles.

Formulation	DTX (in mg)	TPGS (in mg)	PF127 (in mg)	UCNP (μg/ml)	TPGS-AS1411 aptamer Conjugate (in mg)
DTX	3	–	–	–	–
DTP	3	50	50	–	–
DUTP	3	50	50	100	–
DUTP-AS1411aptamer	3	50	50	100	32

DTX: Docetaxel.
PF127: Pluronic-F127.
TPGS: Tocopheryl polyethylene glycol 1000 succinate.
UCNP: Upconversion nanoparticles.
DTP: TPGS-PF127 micelles containing DTX.
DUTP: Theranostic TPGS-PF127 micelles containing DTX and UCNP.
DUTP-AS1411 aptamer: AS1411 aptamer-engineered TPGS-PF127 theranostic micelles containing DTX and UCNP.

such as 2, 4, 6, 8, 24, 48, and 72 h, the 5 ml of samples were withdrawn from the receptor compartment and replaced with preheated at 37 °C as the same medium to maintain the sink conditions [34]. Then samples were filtered using a 0.45 μm PVDF syringe filter and analyzed through a UV–visible spectrophotometer at 230 nm. Data at each time point was presented as a mean of triplicate samples.

2.2.8. Haemolysis study

For the evaluation of hemolytic blood compatibility study of prepared DTP, DUTP, DUTP-AS1411 aptamer micelles and Taxotere®, the freshly collected blood was purchased from a registered Rotary Club, Gurugram, India. In brief, 20 ml of blood was centrifuged at 1344×*g* in a 50 ml centrifuge tube for 15 min at 25 °C. The supernatant was carefully removed using a micropipette and an equal volume of normal saline was added to the RBCs pellet before the suspension was centrifuged and washed with normal saline. This step was performed at least three times. Finally, RBCs were diluted with 50 ml of 0.9% NaCl (normal saline) and further preceded by gentle shaking. In this study, a few microlitres of 100% lysed with Triton X-100 (TX100) exposed erythrocytes (positive control) and normal saline (spontaneous negative control) were used as standards. An appropriate volume of DTP, DUTP, DUTP-AS1411 aptamer micelles, and Taxotere® equivalent to 100 μg of DTX 100 μg/ml was added separately into 2 ml of RBC suspension and then the samples were incubated for 1 h at 37 °C. At intervals of 10–15 min, then samples were gently mixed for homogeneous dispersion of micelles in RBC suspension. Then 1 ml supernatant of aliquots was taken and collected in a tube at predetermined time intervals (i.e., 0.5, 2, and 4 h) and centrifuged for 10 min at room temp. Then it is incubated for 30 min at room temperature for oxidation of hemoglobin into oxyhemoglobin [33,35]. The samples were then evaluated for absorbance at 540 nm with a UV–visible spectrophotometer. The percentage hemolysis was determined by using the formula:

$$(\%)\ Haemolysis = A\ sample - Aspontaneous\ control / Apositive\ control\ * 100$$

Where the sample absorbance is the supernatant of RBC suspension incubated with micelles formulations and Taxotere®, Aspontaneous control is the absorbance of the supernatant of RBC suspension incubated with normal saline equivalent to the volume of samples. A positive control is the absorbance of the supernatant of RBCs incubated in Triton X-100. This study was repeated three times and results were represented in mean with standard deviations.

2.2.9. Cell culture and cytotoxicity study

U-87MG cells were cultured in DMEM supplemented with 10% Fetal Bovine Serum (FBS) (Gibco, CA, USA), and grown in a 5% CO_2 humidified atmosphere at 37 °C temp. To demonstrate the therapeutic efficacy of prepared DTP, DUTP DUTP-AS1411 aptamer micelles and Taxotere®, U-87MG cells were used. The 96-well plate was plated with 5×10^4 cells/wall with DMEM using a multichannel microtiter plate and then incubated for 24 h to allow cell adhesion at 37 °C under 5% CO_2 (humidified atmosphere). After 24 h, the spent culture medium was discarded and cells were incubated with all DTX micelles formulations with a series of its concentrations i.e., 0.025, 0.25, 2.5, and 25 μg/ml for 24 h, and cytotoxicity was assessed. The obtained resultes were compared with Taxotere®. Later, culture media was replaced and 10 μL of 5 mg/ml 3-(4, 5-dimethylthiazolyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) in PBS, pH 7.4 was incubated for additional 2 h. The MTT with medium was removed and 100 μL of DMSO was added to dissolve the formed MTT formazan crystals into the cell well and the absorbance of samples was measured at 570 nm using an ELISA microplate reader. The following formula was used to determine the percentage of cell viability [36,37].

$$\%\ \text{Cell viability} = \text{Absorbance of treated cells} / \text{Absorbance of control cells}) \text{ x } 100$$

2.2.10. In-vivo pharmacokinetic studies

Male *Albino Wistar* rats were procured from the School of Medical and Allied Sciences, G.D. Goenka University, Gurugram, India. All animals were maintained in environmentally controlled rooms at 22 ± 3 °C and 55 ± 5% humidity with 12 h light and 12 h dark cycle allowed fed, and water *ad libitum*. The experimental procedures were agreed upon, reviewed, and approved by the Institute Animal Ethics Committee (IAEC) of the GD Goenka University, Sohna, Gurugram, India. All animal experiments were strictly performed according to Committee for Control and Supervision of Experiments on Animals (CCSEA) guidelines with approval no. GDGU/IAEC/2022/11.

To perform the pharmacokinetic study, male *Albino Wistar* rats with an average weight of 190 ± 10 g were categorized into five different groups (n = 5) and exposed to DTP, DUTP DUTP-AS1411 aptamer micelles, and Taxotere® at the constant DTX conc. (5 mg/kg of body weight). The control group of animals was treated with normal saline. All formulations were administered through *i.v.* route by tail vein. At predetermined time points i.e., 0.5, 1, 2, 4, 8, and 24 h of post-administration, the 200 μl of blood samples were collected by *i.v.* route into 2 ml of heparinized tubes and the collected blood samples were centrifuged at 7,000 rpm for 15 min at 4 °C to separate plasma as supernatant. Then, 1 ml of High-Performance Liquid Chromatography (HPLC) grade mobile phase containing acetonitrile and 0.002 M of ammonium acetate at the ratio of 50:50 v/v was added into each tube and vortexed, separately. Furthermore, the samples were centrifuged and supernatant was collected followed by filtration using 0.22 μm PVDF syringe filters. Finally, the DTX-containing plasma samples were kept at −20 °C till further HPLC analysis.

Prior to performing the HPLC analysis of blood plasma samples, the HPLC system (LC2010HT, Shimadzu Corporation, Tokyo, Japan) was equipped with a UV detector with an auto-sampler, RP-C18 (4.6 × 250 mm i.d., and 5 μm particle size) and LC-2010 Utility Software. For the preparation of internal standard, 1 mg of standard (free DTX) was dissolved in 0.1 ml of plasma (collected from the control group) and the volume was maintained up to 1 ml using the same HPLC mobile phase as mentioned above which resulted in a stock solution. Further serial dilutions were prepared from a stock solution in the range of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 μg/ml conc. of DTX. All samples were then filtered through 0.22 μm PVDF syringe filters and injected into the HPLC column under a flow rate of 1.0 ml/min. The area under the curve of the standard was measured at 230 nm and the calibration curve of the standard (free DTX) was established. To estimate DTX concentration in blood plasma, 0.22 μm PVDF syringe filters were used to filter plasma samples [38]. From each sample, 100 μl of plasma was reconstituted in 900 μl of HPLC mobile phase, separately as mentioned earlier. Then, 200 μl was injected into the column under standard conditions. The total run time was kept at 20 min. The resulting DTX content in the blood plasm was then calculated based on the pre-established DTX calibration curve in blank blood plasma. The column effluent was detected with a UV-VIS detector at 230 nm. Pharmacokinetic software (PK Solver v2.0) was used to calculate all pharmacokinetic parameters [39].

2.2.11. In-vivo brain distribution study

To assess the brain distribution of DTX from Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles (at the constant DTX conc. 5 mg/kg of body weight) were injected into rats through *i.v.* tail vein. All animals were regularly monitored for their general health conditions. The animals were grouped into five groups and treated with the above-mentioned formulations and results were compared with normal saline-treated control and Taxotere® treated groups. At predetermined time intervals i.e., 0.5, 1, 2, and 4 h, the rats were anesthetized using urethane (1.5 g/kg of rat) and brains were harvested after decapitation from different treatment groups. The brain samples were stored at −80 °C until further experiment. Further, brain samples were homogenized in 5 ml of ethyl acetate and vortex for 20 min to leach out the DTX

from brain samples. This process was performed separately for each brain sample under standard conditions. Then the resulting solution was centrifuged at 7,000 rpm for 15 min at 4 °C for separation of DTX containing organic layer. The formed organic layer was transferred to a 2 ml of sterile eppendorf tube for drying at room temp. 1 ml of HPLC mobile phase (acetonitrile and 0.002 M of ammonium acetate at the ratio of 50:50 v/v) was added and vortexed for 20 min, then the resulting solution was filtered using a 0.22 μm PVDF syringe filter. Then 100 μl samples were transferred to HPLC insert for analysis and 20 μl sample was injected into the HPLC column under standard conditions. The total run time was kept at 20 min and the area under the curve was obtained at 230 nm using a UV–visible detector [40].

2.2.12. *In-vivo brain theranostic evaluation of micelles in rats*

The imaging application of developed DUTP, DUTP-AS1411 aptamer micelles and free UCNP was evaluated in rats. The formulations and free UCNP were injected to the rats through *i.v.* route. After 0.5 h of post-administration of formulations into rats, rats were anesthetized using urathane (1.5 g/kg of rat) and brains were harvested after decapitation from different treatment groups. The brain samples were collected in 10% formaline solution and stored at −80 °C till further processing of brain tissues. Then the frozen brain tissues were sliced to a 10 μm thickness using a Cryotome (Leica CM 1520, Leica Biosystem Nussloch GmbH, Heidelberger Strasse, Nussloch, Germany) and mounted on gelatine-coated glass plates. The nucleus of brain cells was stained with 100 μl of 1 μg/ml DAPI (diamidino-2-phenylindole) and incubated for 30 min at room temperature. Later, sectioned tissues were washed with PBS and images were observed by a Confocal Microscope (LSM 900, Carl Zeiss Microscopy, Germany) [29,38].

2.2.13. *Histopathology of brain tissues*

To demonstrate the biocompatibility of the DTX formulations (DTP, DUTP and DUTP AS1411 aptamer micelles and Taxotere®), histopathological studies were performed using standard methods. Male albino Wistar rats (mean body weight 190 ± 10 g) were divided into 5 different groups (n = 4) and treated with DTP, DUTP and DUTP-AS1411 aptamer micelles and the results were compared with Taxotere®, It was compared with a control group treated with normal Saline. All animal groups were exposed to DTX-containing formulation at a fixed dose of 5 mg/kg body weight. Groups of animals were administered individually via the tail vein at up to 3 intervals every 7 days. At the end of the study, exposed animals were sacrificed under anesthesia (urethane 1.5 g/kg rat, intraperitoneal) and brains were harvested from each group on days 14^{th} and 28^{th} after administration of the first dose. The collected tissue was thoroughly washed, preserved in a 10% formalin solution, and embedded in paraffin. The 5-μm-thick paraffin sections were then cut, mounted on glass slides, and separately stained with hematoxylin and eosin (H&E). The all the prepared slide sections were examined under a light microscope for pathological changes in the brain of DTX treated animals [38].

2.2.14. *Statistical analysis*

Results are presented as mean ± standard deviation (S.D.). The average particle size, polydispersity index, zeta potential and encapsulation efficiency were analyzed through two-way ANOVA (post hoc by Bonferroni). For the in-vitro cytotoxicity assay results, the statistical analyses were performed using an unpaired two-tailed Student's t-test. The analyses with p values <0.05 at a 95% confidence interval were considered significantly different.

3. Results and discussion

3.1. *Activation of TPGS and synthesis of its conjugates*

The hydroxylic (-OH) end of TPGS was converted into a carboxylic (-COOH) group in the presence of succinic anhydride through the ring-opening polymerization method as summarized in Fig. 1A. The resulting TPGS-COOH was used as to synthesize TPGS-AS1411 aptamer conjugate through physical adsorption method as shown Fig. 1B. For the preparation of AS1411 aptamer based product, tris-hydrochloride buffer (pH 7.5–8) is normally used, which promotes the 3D conformational change of aptamers upon binding to the substance [25]. However, TPGS-COOH have shown high solubility and stability with tris-hydrochloride buffer (pH 7.5–8) at room temp., therefore, a common solvent was choosen to dissolve both the TPGS-COOH and AS1411 aptamer and stirred for 6–8 h at room temp. to form TPGS-AS1411 aptamer conjugate [26]. Further, the synthesis of TPGS-COOH and TPGS-AS1411-aptamer conjugation was confirmed through FTIR and ^{1}H NMR spectroscopy techniques.

3.2. *Characterization of TPGS and its conjugate*

3.2.1. *FTIR spectroscopy*

FTIR spectra of TPGS, TPGS-COOH, AS1411 aptamer, and TPGS-AS1411 aptamer conjugate are shown in Fig. 2. In the FTIR spectrum of TPGS, the characteristic bands were observed at 1736 and 2867 cm^{-1} indicating the presence of carbonyl groups and –CH stretches in the TPGS molecule [3]. Furthermore, two major characteristic peaks were found at 3520.04 and 1099.13 cm^{-1} which confirming the presence of terminal hydroxyl and a strong C–O stretching band in the TPGS [41]. In FTIR spectrum of TPGS-COOH confirms the activation of TPGS by showing a characteristic peak of the –C═O stretching of the ester group at 1732.44 cm^{-1} and a characteristic band appears at 3433.28 cm^{-1} that confirms the successful formation of –COOH group in TPGS molecule [23,42]. These observations are similar to a reported work [31]. The FTIR spectra of AS1411 aptamer showed a district band at 764.13 cm^{-1} due to the presence of an amine group of aptamer sequence. Another absorption band appeared at 1032.22 cm^{-1}, confirming intramolecular C–O stretching. The AS1411 aptamer has a phosphate backbone, confirmed by a characteristics band at 1137.45 cm^{-1}, also indicating the presence of a thiolated N–O stretching in the compound. The FTIR spectrum of TPGS-AS1411 aptamer conjugate have showed successful conjugation of TPGS-COOH to the AS1411 aptamer due to the presence of a strong –C═O stretching at 1734.54 cm^{-1} which reveals the formation of TPGS-AS1411 aptamer conjugate.

3.2.2. *^{1}H NMR spectrophotometry*

Fig. 3 shows the ^{1}H NMR spectra of TPGS, TPGS-COOH, AS1411 aptamer and TPGS-AS1411 aptamer conjugate. A signal was observed at δ 3.698, confirming the $–CH_2$ proton of poly(ethylene oxide) in the TPGS structure. A lower peak was observed in the spectrum, confirming the presence of an aliphatic region corresponding to TPGS structure due to the various moieties of vitamin E. A single peak was observed at δ 1.89 due to the presence of $–CH_3$ group tocopherol aromatic ring in the TPGS molecule [43]. Similar proton signals were observed in TPGS-COOH spectrum, except for new signals at δ 2.5–2.6, and 3.4, confirming the presence of succinylmethylene ($-CH_2$) within the structure. These observations have confirmed the formation of TPGS-COOH. Sonali et al. also confirmed similar observations for TPGS-COOH with triplet signals at δ 3.156, 2.932, and 2.910, corresponding to the $–CH_2$ protons (polyethylene oxide) moiety of TPGS-COOH [3]. In the spectrum of the AS1411 aptamer shows peaks appeared at δ 3.2331, 3.2131, 3.2031, and 3.1881 due to the presence of sugar moieties within AS1411 aptamer molecule. In the spectrum of the TPGS-AS111 aptamer shows extended peaks at 3.6153, 3.5943, and 3.1139, confirming the successful conjugation of TPGS-COOH to the AS1411 aptamer and the formation of TPGS-AS1411 conjugate. The observed results have confirmed the successful activation of TPGS to TPGS-COOH and synthesis of the TPGS-AS1411 aptamer conjugate.

3.3. *Preparation of micelles formulations*

The solvent casting method is one of the simplest methods for

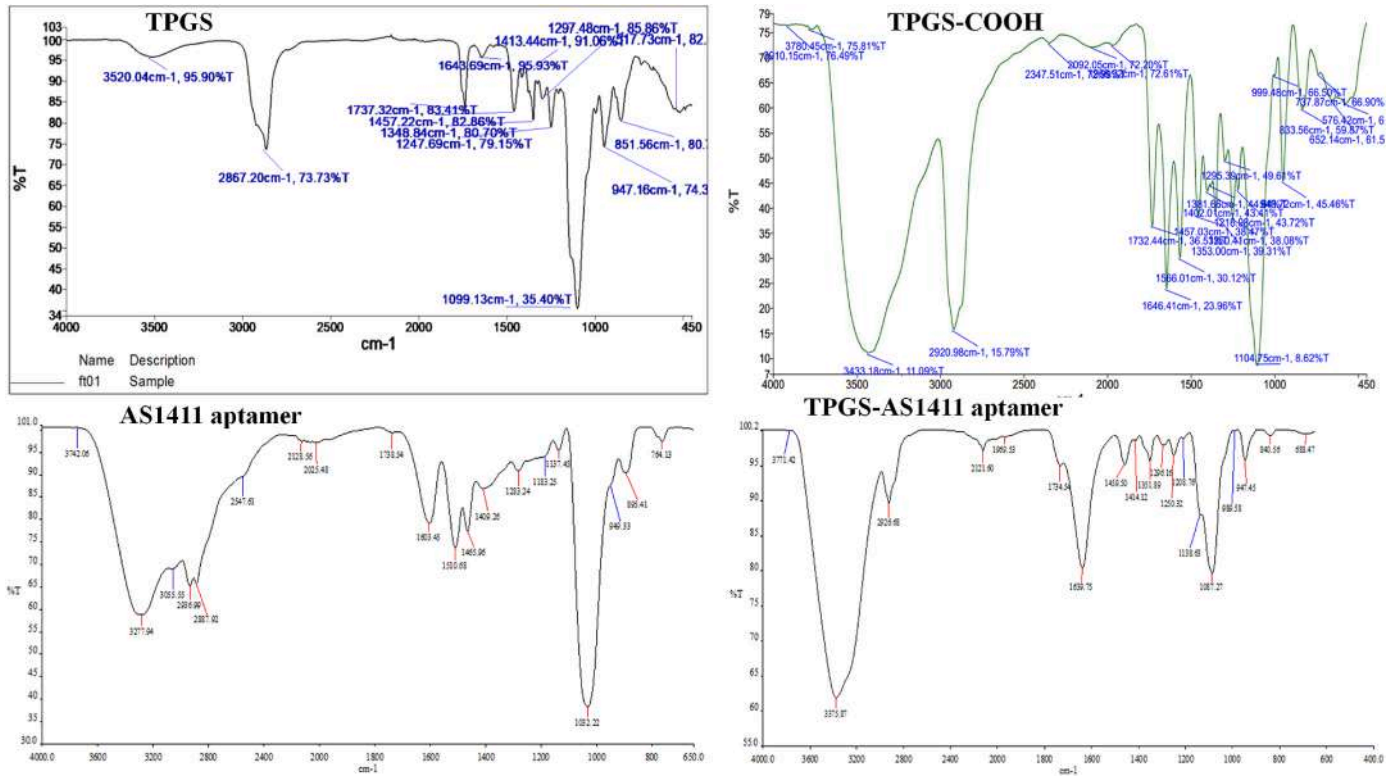

Fig. 2. FTIR spectra of TPGS, TPGS-COOH, plain AS1411 aptamer and TPGS-AS1411 aptamer conjugate.

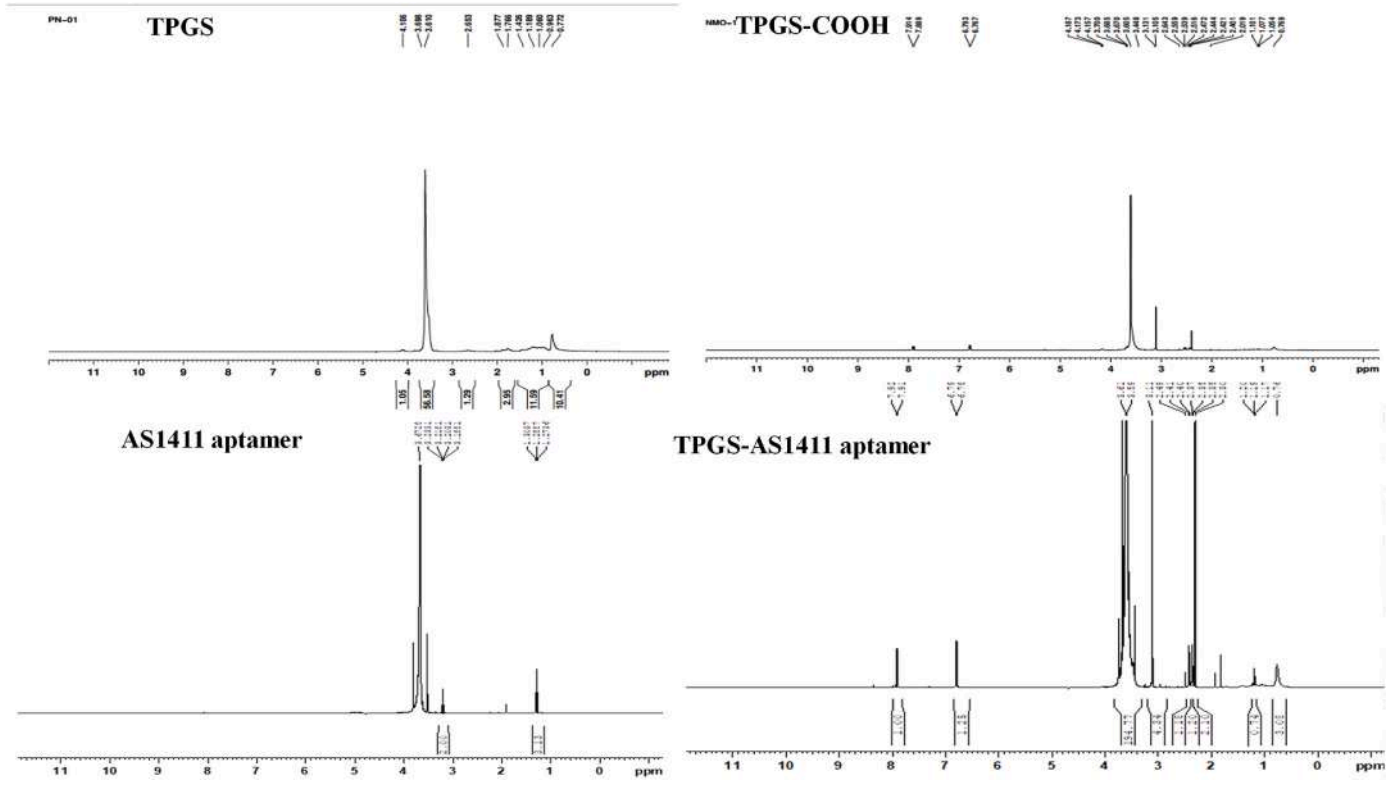

Fig. 3. [1]HNMR spectra of TPGS, TPGS-COOH, plain AS1411 aptamer and TPGS-AS1411 aptamer conjugate.

preparation of micelles formulations displayed in Fig. 1C-D. The micelles were prepared by a slightly modified solvent-casting method using optimized concentrations of TPGS and PF127, as described in Table 1. Subsequently, the particle size and drug encapsulation efficacy of the prepared DTP, DUTP and DUTP-AS1411 aptamer micelles were determined and proved their suitability for use as drug delivery vehicles in

brain tumor therapy. TPGS and PF127 was used at 1:1 ratio to achieve the desired micelle size with a narrow polydispersity index. TPGS has been shown to have a low relative critical micelle concentration (CMC) of 0.02% w/w, while PF127 has very low relatively CMC (0.0031%, w/w). The resulting cmobination of TPGS and PF127 could potentially influence P-gp inhibition through reducing efflux of DTX and UCNP from brain tumor cells.

3.4. Characterization of micelles formulations

3.4.1. Particles size, polydispersity index, ζ potential, and morphology of micelles

The mean particle size and polydispersity index and ζ potential values of plain micelles, DTP, DUTP, and DUTP-AS1411 aptamer micelles are listed in Table 2 and demonstrated in Fig. 4. The average hydrodynamic particle size of plain micelles was found to be 96.63 ± 2.86. The particle sizes of DTP and DUTP micelles were found 156.13 ± 2.26, and 158.23 ± 4.26 nm, respectively. DTP micelles have shown significant improvement in particle size which might be due to the loading of hydrophobic DTX into the core structure of formed micelles shows in Fig. 4A. Furthermore, the addition of UCNP (a hydrophobic molecule), into the DUTP and DUTP-AS1411 aptamer micelles has significantly increased particle size. The particle size of DUTP-AS1411 aptamer micelles was found to be 163.68 ± 2.28 nm (Fig. 4 B [i-iv]). The particle size result of the DUTP-AS1411 aptamer micelles indicates the incorporation of targeting ligand (TPGS-AS1411 aptamer) on the micelle surface which leads to a slight increase in the particle size. It is a canonical fact that small particle size improves the retention and accumulation of micelles in tumor tissues by avoiding clearance through the reticuloendothelial system. It might be significant that particle-sized developed micelles can affect drug uptake/delivery processes, diffusion rates, and immune responses [14]. The polydispersity index of all DTP, DUTP, and DUTP-AS1411 aptamer micelles was found to be between 0.2 and 0.4 which showed a moderately narrow size distribution of formed micelles into homogenous colloidal dispersion system. The ζ potential value is an important characteristic of nanoformulations that affects their physical stability in both *in-vitro* and *in-vivo* environments. All micelles (plain micelles, DTP, DUTP, and DUTP-AS1411 aptamer) formulations exhibited a negative ζ potential (−3.9 ± 0.03, −3.6 ± 0.04, -5.0 ± 0.02, and −24.6 ± 0.05 mV, respectively) shown in Fig. 4C which was influenced by the presence of non-ionic polyethylene glycol chains and phenolic groups of TPGS and PF-127 on surface of the micelles. In case of DUTP-AS1411 aptamer micelles ζ potential, it can be noted that the AS1411 aptamer is 26-base paired single-stranded nucleotides DNA carrying a strongly negative ζ potential due to the presence of a thiol group it shows strong negative ζ potential. Therefore, the surface conjugation of micelle formulation with TPGS-AS1411 aptamer may be attributed to the significantly lower ζ potential (−24.6 ± 0.05 mV) observed for DUTP-AS1411 aptamer micelles. It is well documented that cationic-charged nanoparticles are more susceptible to rapid elimination from the biological system, whereas neutral and negatively charged nanoparticles can greatly improve half-lives of bioactive molecules in circulation [44,45]. Furthermore, electrostatic repulsion and steric hindrance could prevent the aggregation of the micelles and provide an important role in the stability of the nanoformulation [46].

The surface morphology of plain micelles, DTP, DUTP, and DUTP-AS1411 aptamer micelles was confirmed through TEM and revealed that all the formed micelles were spherical in shapes with 200 nm in size in Fig. 5 (A-D). This study exhibited the spherical structure of the micelle into the corona and shell structure. The micelle-core was formed due to the hydrophobic PPO from PF127 and vitamin E from TPGS, respectively, and the outer micelle-shell responded due to hydrophilic PEO from PF127 and PEG in TPGS. Furthermore, the observations were strongly matched with the particles size measurement report.

3.4.2. Atomic force microscopy(AFM)

The 3D AFM images of developed plain micelles, DTP, DUTP, and DUTP-AS1411 aptamer micelles have shown in Fig. 5 (E-H) which exhibited that micelles had smooth surfaces without any noticeable pinholes or cracks. Furthermore, it confirmed that all the micelles were spherical in shape of size in the range of less than 200 nm in size. Smaller-sized micelles would be an excellent applicant for passive targeting of solid tumor tissue by the EPR effect that could facilitate higher extravasation from circulation and distribution of DTX and UCNP into the brain tumor mass [47].

3.4.3. Determination of encapsulation of micelles

The encapsulation efficiency of prepared micelles formulations was calculated as the percentage ratio between the amount of DTX/UCNP loaded in prepared micelles and the drug added during the preparation of micelles which is given in Table 2 and Fig. 4 (D). It is one of the most crucial factors in the development of nano-drug carriers. DTP and DUTP have exhibited 88.62 ± 4.02%, and 79.73 ± 5.09% encapsulation of DTX, while DUTP-AS1411 aptamer micelles exhibited 74.90 ± 5.21%. Drug encapsulation efficiency of DTP was found higher as compared to DUTP and DUTP-AS1411 aptamer micelles. DUTP and DUTP-AS1411 aptamer micelles have shown a reduction in DTX encapsulation due to the additional inhabitancy of UCNP in micelles-core. DUTP micelles compassed 40.4 ± 5.04% of encapsulation for UCNP. Moreover, DUTP-AS1411 aptamer micelles compassed 38.18 ± 2.07% encapsulation of UCNP in micelles. Furthermore, DUTP-AS1411 aptamer micelles exhibited a reduction in DTX and UCNP encapsulation in the core of micelles. It might be because of the co-encapsulation of both the hydrophobic molecules (DTX and UCNP) and additional coating of TPGS-AS1411 aptamer on the surface of micelles under stirring conditions [48,49].

Table 2
Particle size, polydispersity, zeta potential, encapsulation efficiency and IC_{50} value of drug and imaging agent loaded TPGS-PF127 micelles.

Batches	Particle size (nm ± S.D.*)	Polydispersity (mean ± S.D.[a])	Zeta Potential (mV)	DTX Encapsulation Efficiency (%) (mean ± S.D.[a,*])	UCNP Encapsulation Efficiency (%) (mean ± S.D.[a,*])	IC 50 μg/ml on U-87MG cells
Plain micelles	96.63 ± 2.86	0.26 ± 0.01	−3.9 ± 0.03	–	–	
DTP	156.13 ± 2.26	0.25 ± 0.01	−3.6 ± 0.04	88.62 ± 4.02	–	2.086 ± 0.021
DUTP	158.23 ± 4.26	0.13 ± 0.03	−5.0 ± 0.02	79.73 ± 5.09	40.4 ± 5.04	0.854 ± 0.006
DUTP-AS1411 aptamer	163.68 ± 2.28	0.37 ± 0.02	−24.6 ± 0.05	74.90 ± 5.21	38.18 ± 2.07	0.585 ± 0.001
Taxotere®	–	–	–	–	–	10.848 ± 0.86

Plain micelles: Without drug/UCNP loaded micelles
DTP: TPGS-PF127 micelles containing DTX.
DUTP: Theranostic TPGS-PF127 micelles containing DTX and UCNP.
DUTP-AS1411 aptamer: AS1411 aptamer-engineered TPGS-PF127 theranostic micelles containing DTX and UCNP.
Taxotere®: Marketed docetaxel injection.
* n = 3.
[a] *S.D: Standard deviation.*

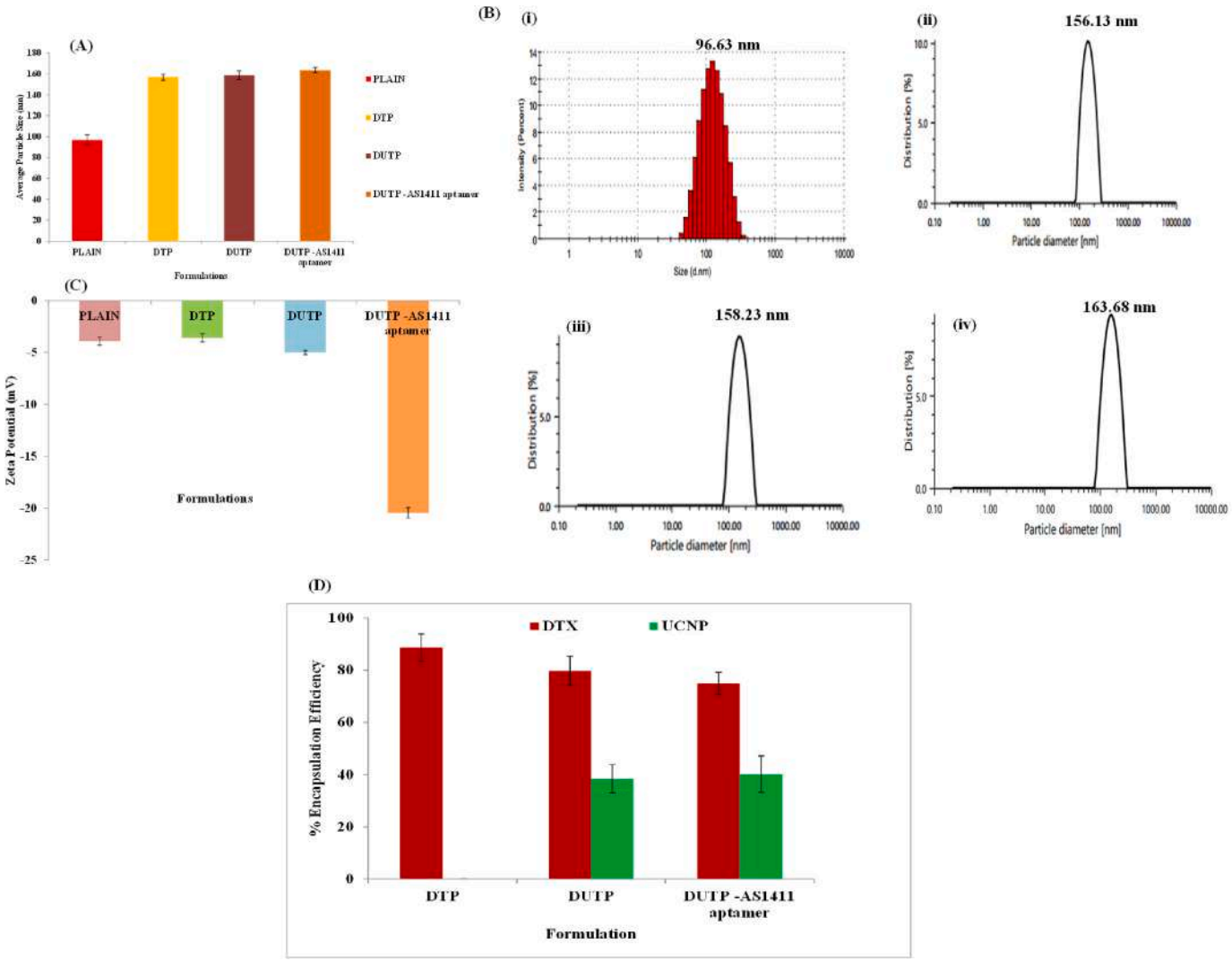

Fig. 4. (A) The average hydrodynamic particle size of plain micelles, DTP, DUTP and DUTP-AS1411 aptamer micelles, (B) histogram of particle size distribution of (i) plain micelles, (ii) DTP, (iii) DUTP and (iv) DUTP-AS1411 aptamer micelles, (C) bar graph representing the average ζ potential of plain micelles, DTP, DUTP and DUTP- AS1411-aptamer micelles, and (D) the average % encapsulation of DTX and UCNP in DTP, DUTP and DUTP-AS1411aptamer micelles formulations (±SD, n = 3); the significance levels were set at probabilities of * $p < 0.05$.

3.4.4. In-vitro drug release studies

Fig. 6 (A) shows the cumulative percentage release of DTX from Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles by dialysis membrane in the PBS medium at pH 7.4. Approximately 99.76 ± 2.32% of DTX amount was released from Taxotere® within 4 h of span. All micelles formulations have shown sustained release for ≥24 h followed by biphasic burst release with up to 30–45% in the first 4 h. After 24 h of drug release from micelles formulations, 62.15 ± 3.22%, 58.47 ± 3.22% and 46.39 ± 2.45% of DTX was released from DTP, DUTP, and DUTP-AS1411 aptamer micelles, respectively, which indicate the sustained release of DTX. A similar trend was continued till 48 h under the same conditions. Furthermore, the observed data have demonstrated that 98.27 ± 4.38 and 92.24 ± 8.24% of DTX was released from DTP and DUTP micelles after 72 h of the drug release study. While DUTP-AS1411 aptamer micelles have significantly restricted the drug diffusion and only 75.17 ± 3.31% of DTX was released in 72 h. T_{50}% at which 50% of the drug has been released in PBS (pH 7.4) was about 5.84, 7.95, and 19.02 h for DTP, DUTP, and DUTP-AS1411 aptamer micelles, respectively. It is noteworthy that coating of TPGS-AS1411 conjugate on micelles surface sustained drug release pattern as compared to DTP and DUTP micelles. In contrast, DTX-loaded micelle formulations have sustained the DTX release pattern as compared to Taxotere® up to 72 h. In DUTP-AS1411 aptamer micelles TPGS-AS1411 aptamer was coated on micelles surface which lead to much more slower kinetics. Moreover, In this study, the observed difference in the drug release kinetics between the DUTP-AS1411 aptamer and other counterparts may be attributed to the different molecular weights of the TPGS-AS1411 aptamer conjugate that was coated on the surface of the micelles [50]. As a result, the diffusion of DTX was prevented subsequently, which could be concluded from the sustained release behavior of DTX-loaded DUTP-AS1411 aptamer micelles.

3.4.5. Hemolysis study

As the prepared micelles were purporting for i.v. administrations, therefore it is highly necessary that micelles should not show hemolytic behavior with blood during and after administrations. Hemolysis was assessed with 100 μg/mL conc. of DTX in formulations at different time intervals (0.5, 2, and 4 h). Fig. 6B illustrates the hemolysis profile of prepared DTP, DUTP, and DUTP-AS1411 aptamer micelles and compared them with Taxotere®. The negative control group did not show any hemolytic effect compared to the positive control and prepared micelles formulations. After 0.5 h of exposure with Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles showed 75.10 ± 6.54, 3.84 ± 0.15, 3.21 ± 0.11 and 1.97 ± 0.09%, respectively RBCs were

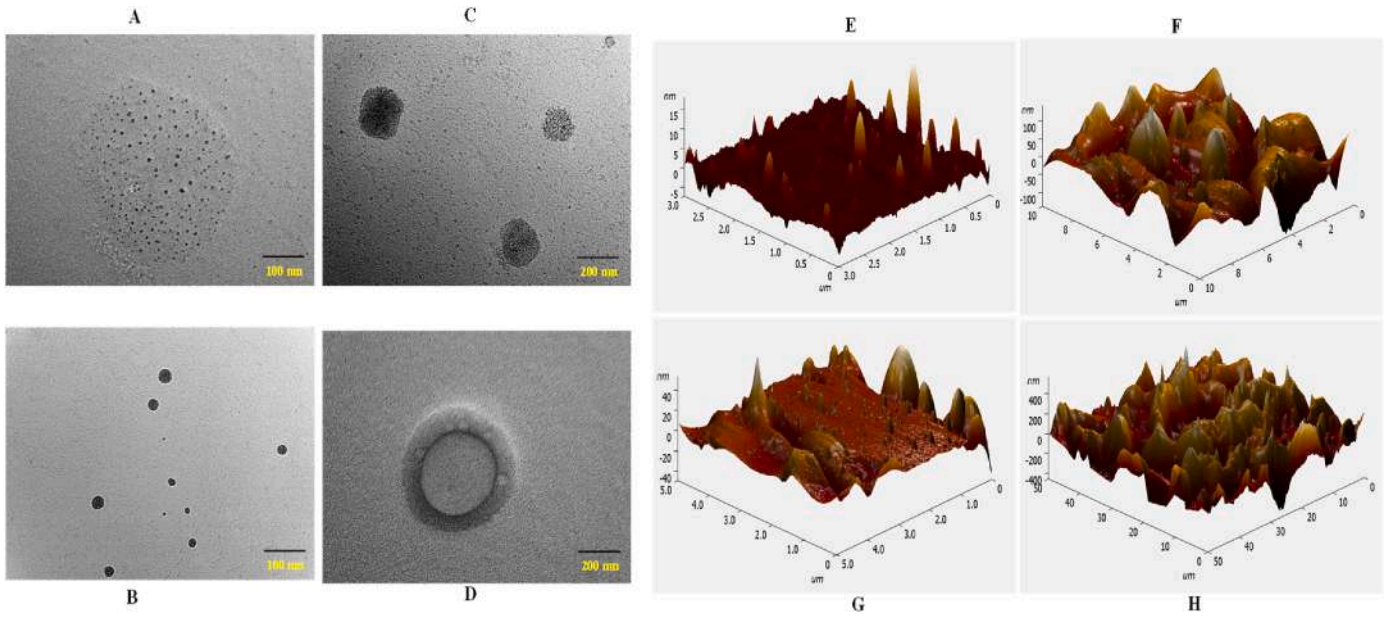

Fig. 5. TEM image of plain micelles at 100 nm scale (A) DTP at100 nm scale (B) DUTP at 200 nm scale and (C) DUTP-AS1411 aptamer at 200 nm scale (D), and 3D AFM images of plain micelles (E) DTP (F) DUTP (G), and DUTP-AS1411 aptamer micelles (H).

hemolysed. The observations have revealed that Taxotere® caused more damage to RBCs as compared to all TPGS-PF127 micelles. A similar trend was obtained at 2 and 4 h, respectively. Furthermore, After 2 h of exposure with Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles showed approx. 79.88 ± 5.33, 4.09 ± 0.21, 3.87 ± 0.27, and 2.01 ± 0.12% hemolysis was found in RBCs respectively. At the termination of the study, 84.62 ± 5.98, 4.69 ± 0.42, 4.89 ± 0.39, and 2.14 ± 0.07% hemolysis was caused due to the Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles exposure in RBCs. In context, samples with hemolytic rates less than 5% are considered non-hemolytic and can be useful for clinical benefits [51]. Thus, DTP, DUTP, and DTP-AS1411 aptamer micelles exhibited ≥5% less hemolysis compared to Taxotere®. The observed results of micelles have shown high biocompatibility and safety for i.v. administration. Researchers cited that cationic-charged particles or drugs may interact with anionic-charged RBCs, leading to hemolysis. However, the electroelution between the negative/neutral charges of the TPGS surface and the negatively charged surface of blood cells reduces the interaction between micelles and RBCs, which prevents hemolysis. Moreover, in micelles formulation, the non-ionic natures of both TPGS and PF127 have reduced hemolytic effects in RBCs. Therefore, it can be concluded that these developed micelles formulations are more biocompatible than Taxotere®. Notably. in this investigation, DUTP-AS1411 aptamer micelles exhibited negligible hemolytic activity due to the mechanism of AS1411 aptamer, which facilitated the internalization of the therapeutic agent into target cells via nucleolin-mediated delivery. Hence, the internalization of AS1411 aptamer into target cells does not destruct the cell membrane [52].

3.4.6. Cytotoxicity study

Fig. 7A demonstrates the cytotoxicity of DTX formulations i.e., DTP, DUTP, and DUTP-AS1411 aptamer micelles, and results were compared with Taxotere® in U-87MG cells, after 24 h post-exposure. The study exhibited DTX concentration-dependant cytotoxicity from Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles in U-87MG cells. An increase in the DTX concentration range from 0.025 to 25 μg/ml resulted in the reduction of cell viability and improved cytotoxicity, followed by passive diffusion, phagocytosis, and nucleolin-mediated endocytosis mechanisms [53]. Toxetere® has exhibited higher IC_{50} values compared to DTP, DUTP, and DUTP-AS1411 aptamer micelles in U-87MG cells. The IC_{50} values for Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles were found to be 10.848 ± 0.86, 2.086 ± 0.021, 0.854 ± 0.006 and 0.585 ± 0.001 μg/ml, respectively, as mentioned in Table 2 on U-87MG cells. DTX formulated in the DTP, DUTP, and DUTP-AS1411 aptamer micelles could be 4.20-, 11.70-, and 17.54-fold more therapeutic efficient than Taxotere® after 24 h treatment, respectively. It was possible that micelles of PF127-TPGS have elevated delivery of DTX and UCNP while suppressed p-glycoprotein mediated efflux mechanism in U-87MG cells. Furthermore, DUTP has shown a significant reduction in IC_{50} value compared to DTP and Taxotere®. DUTP-AS1411 aptamer micelles have significantly reduced cell viability because of the targeted delivery of loaded bioactive molecules through nucleolin receptors and stabilized microtubules in brain cancer cells. AS1411 aptamer has anticancer activity in almost every cancer cell line including U-87MG cells, as it binds with overexpressed nucleolins and facilitates improve drug penetration into U-87MG cells [54].

3.4.7. In-vivo pharmacokinetic studies

Pharmacokinetic study results of DTP, DUTP, and DUTP-AS1411 aptamer micelles were compared with Taxotere® and displayed in Table 3. The obtained pharmacokinetic data were evaluated using the non-compartmental model (PkSolver 2.00). Herein, it was noted that Taxotere® achieved short $t_{1/2}$ that was found at 5.14 ± 1.02 h. While, DTP, DUTP, and DUTP-AS1411 aptamer micelles have shown extended drug $t_{1/2}$ such as 21.11 ± 1.51, 33.49 ± 2.01, and 40.13 ± 2.87 h, respectively due to the sustained drug release action of micelles. The micelles formulations have significantly improved t_{max} up to 3-, 6-, and 8-fold as compared to Taxotere® injection. It exhibited the remarkable sustainability of DTP, DUTP, and DUTP-AS1411 aptamer micelles in systemic blood circulation. Furthermore, $AUC_{0\text{-}t}$ of DTX was significantly improved from 163.77 ± 11.37 μg/ml × h for Taxotere® to 318.91 ± 23.83, 389.12 ± 19.68 and 434.81 ± 29.09 μg/ml × h for DTP, DUTP and DUTP-AS1411 aptamer micelles which corresponds to the significant reduction of the body clearance from 0.029 ± 0.001, 0.008 ± 0.005, 0.004 ± 0.002, 0.003 ± 0.002 μg/ml/h, respectively. The C_{max} (μg/ml) of all prepared micelles DTP, DUTP, and DUTP-AS1411 aptamer micelles were observed at 22.54 ± 2.08, 24.18 ± 1.61, and 25.28 ± 1.50 μg/ml, respectively as compared to 9.47 ± 0.76, μg/ml for Taxotere® treated group. It revealed that the micelles were highly capable of delivering the desired amount of DTX as compared to

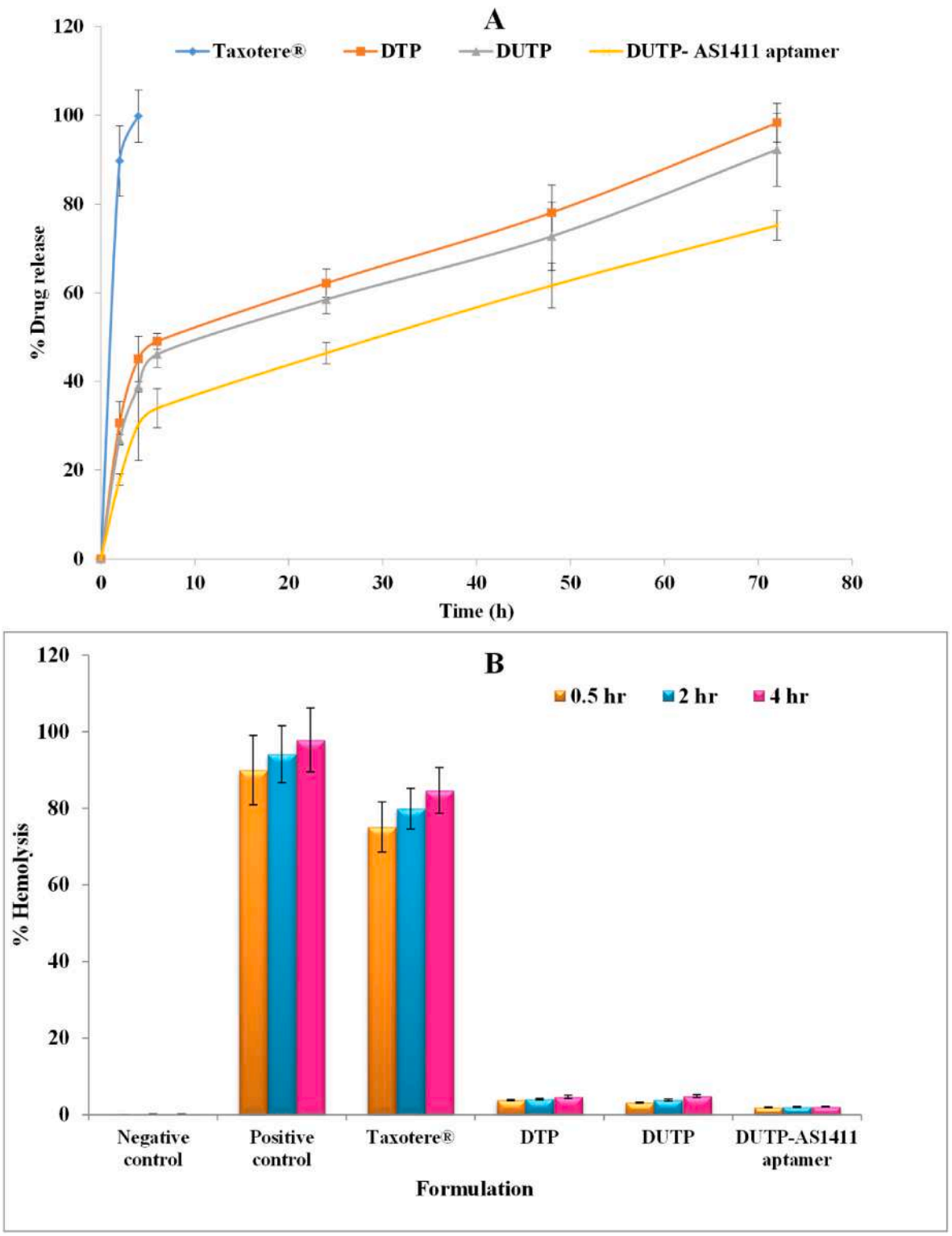

Fig. 6. (A) *In-vitro* drug release from the DTX from DTP, DUTP and DUTP-AS1411 aptamer micelles and Taxotere® in phosphate buffer saline at pH 7.4. Bar represent mean ± S.D ($P < 0.05$, $n = 3$), and (B) *in-vitro* hemolysis of negative control (normal saline), positive control (Triton X100), Taxotere®, DTP, DUTP, DUTP-AS1411 aptamer TPGS-PF127 micelle at 0.5, 2 and 4 h time point interval, data is represented as mean ± SD ($n = 3$); the significance levels were set at probabilities of * $p < 0.05$.

Taxotere® treated group. The obtained results of DTP and DUTP micelles may vacillate given the presence of TPGS and PF127 on the surface of micelles which enhanced the permeability of micelles and extended sustained DTX release. TPGS inhibits the binding of plasma protein which subsequently prevents recognition by the reticuloendothelial system which ultimately facilitates elevated systemic circulation time [53]. The results of V_{ss} (steady-state volume of distribution) of DTX containing DTP, DUTP, DUTP-AS1411 aptamer micelles, and Taxotere® were found to be 0.25 ± 0.154, 0.23 ± 0.092 and 0.21.012, 0.39 ± 0.019 μg/ml, respectively. The overall observation

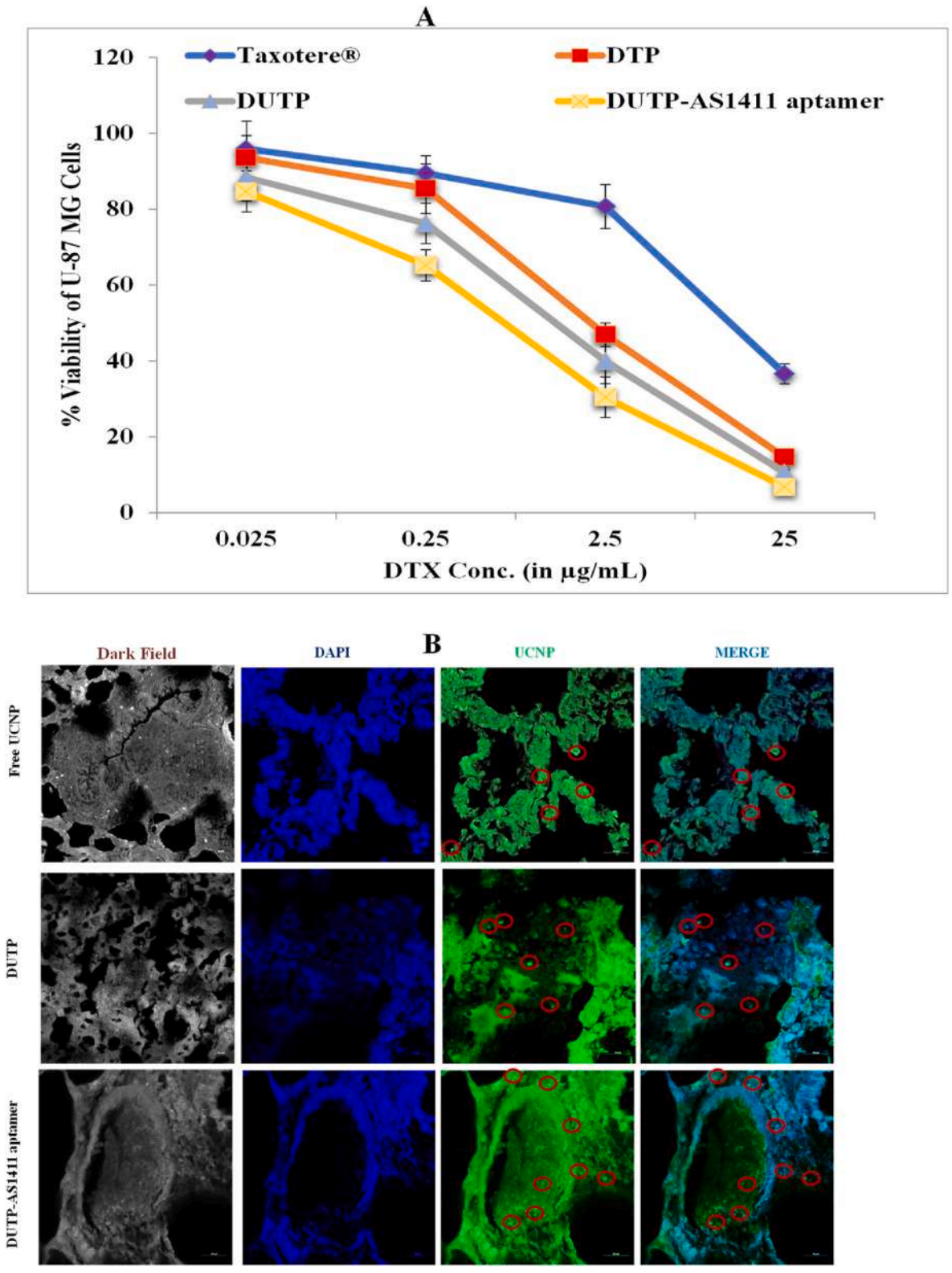

Fig. 7. (A) Cytotoxicity profile of DTX loaded TPGS-PF127 micelles DTP, DUTP, DUTP-AS1411 aptamer and Taxotere® for U-87MG cells (n = 4). Bars represent ± S. D. ($P < 0.05$) at 37 °C after 24 h and (B) Confocal UCNP fluorescence imaging of Free UCNP, DUTP and DUTP-AS1411 aptamer micelles in rat brain cells. Green and blue colors represent upconversion fluorescence signals and blue fluorescence from UCNPs and DAPI, respectively. Scale bar is 20 µm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Pharmacokinetic parameters of Taxotere®, DTP, DUTP and DUTP-AS1411 aptamer micelles after *i.v.* administration equivalent concentration of DTX (at 5 mg/kg).

	Pharmacokinetic parameters	Taxotere®	DTP	DUTP	DUTP-AS1411 aptamer
$t_{1/2}$	**h**	5.14 ± 1.02	21.11 ± 1.51	33.49 ± 2.01	40.13 ± 2.87
C_{max}	**μg/ml**	9.47 ± 0.76	22.54 ± 2.08	24.18 ± 1.61	25.28 ± 1.50
AUC_{0-t}	**μg/ml × h**	163.77 ± 11.37	318.91 ± 23.83	389.12 ± 19.68	434.81 ± 29.09
$Cl_{\_obs}$	**(μg/ml)/h**	0.029 ± 0.001	0.008 ± 0.005	0.004 ± 0.002	0.003 ± 0.002
V_{ss_obs}	**(μg/ml)**	0.39 ± 0.019	0.25 ± 0.154	0.23 ± 0.092	0.21 ± 0.012

n = 6.
S.D.: Standard deviation.
DTP: TPGS-PF127 micelles containing DTX.
DUTP: Theranostic TPGS-PF127 micelles containing DTX and UCNP.
DUTP-AS1411 aptamer: AS1411 aptamer-engineered TPGS-PF127 theranostic micelles containing DTX and UCNP.
Taxotere®: Marketed docetaxel injection.

of higher $t_{1/2}$, AUC, C_{max}, and lower V_{ss} and Cl of DUTP-AS1411 than Taxotere® showed potential therapeutic effects in *in-vivo* experimentations.

3.4.8. *In-vivo brain distribution study*

BBB is touted as a major limitation for conventional drug delivery systems in the brain citing its inherent ability to restrict various therapeutic agents. Ever changing and progressing nature of cancer forces the research community to find a novel passage to minimize BBB-associated restrictions. Current studies recognize that nanoformulations including micelles potential as BBB-evading carriers to deliver anticancer drugs to brain tissues. Micelles are spherical and amphiphilic structured nanodrug carriers that have a hydrophobic core and a hydrophilic shell. Several studies exhibited micelles have an advantage over other anticancer formulations citing their elevated drug localization ability in brain tissue. The findings of the current study are well corroborated with previously reported studies on brain accumulation of micelles. The brain distribution studies of DTX containing DTP, DUTP, and DUTP-AS1411 aptamer micelles were performed and compared with the Taxotere® group after *i.v.* administration. Fig. 8A represents the DTX distribution through prepared micelles and Taxotere® in the brain. After 0.5 h of dose administration into the different animal groups, 0.26 ± 0.014, 0.68 ± 0.034, 0.58 ± 0.038, and 0.23 ± 0.014 μg/gm DTX reached the brain tissue through Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles formulations, respectively. Furthermore, after 1 h of dose administrations approximately 2.07 ± 0.021, 1.51 ± 0.099, 1.92 ± 0.124, and 1.29 ± 0.054 μg/gm of brain tissues were accumulated in rat brain through Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles formulations. The observed results indicate that Taxotere® is highly efficient to deliver a higher concentration of DTX within 1 h as compared to prepared micelles formulations. It might be possible due to the quick release of DTX from polysorbate 80-based Taxotere® injection in brain tissue. In context, PF127-TPGS-based micelles have delayed the DTX release and followed sustained drug release patterns in the systemic circulation. Moreover, TPGS-AS1411 aptamer has reduced drug release citing TPGS is additionally present on the micelle's surface. After 2 h of dose administrations, it had been observed that DUTP-AS1411 aptamer micelles were highly efficient to deliver an effective concentration of DTX up to 1.84 ± 0.074 μg/gm as compared to Taxotere® (1.60 ± 0.084), DTP (0.84 ± 0.006), and DUTP micelles (1.11 ± 0.054) μg/gm of brain tissue. After 4 h of dose administration, Taxotere® was impuissant to deliver an effective DTX concentration to the brain, which advocates the quick release with rapid elimination of DTX from systemic circulation. While DTP and DUTP micelles were delivered 0.60 ± 0.001 and 0.46 ± 0.021 μg/gm DTX into the brain which was significantly higher than Taxotere®. It is noteworthy that DUTP-AS1411 aptamer micelles have achieved higher DTX concentrations of 1.42 ± 0.097 μg/gm in the brain. It might be possible that nucleolins receptors have facilitated the delivery of micelles which resulted in a higher accumulation of drugs [29,48]. Interestingly, it was noted that the mean concentration of DTX in brain tissues at 0.5, 1, 2, and 4 h after *i.v.* administration were found to be ascending order of DUTP-AS1441≥DUTP ≥ DTP ≥ Taxotere®, respectively.

3.4.9. *In-vivo brain theranostic evaluation of micelles in rats*

The brain theranostic observation of developed micelles formulations demonstrated a greater internalization of UCNP into brain tissues compared to the free UCNP exposed group. This was most probably due to the significantly enhanced transportation of the UCNP across the BBB by TPGS and PF127 surfactant. Brain tissue localization was highlighted in red color which confirms the delivery of UCNP it may have increased the concentration of DTX after the brain observed for DUTP- AS1411 aptamer micelles in comparison to DUTP micelles and free UCNP at even 0.5 h of *i.v.* administration shown in Fig. 7B. While DUTP micelles have shown more fluorescence than free UCNP, it might be possible because micelles were reached into brain cells through the phagocytosis mechanism. DUTP-AS1411 aptamer micelles have facilitated greater internalization with more accumulation of UCNP into brain tissues it may be due to the nucleolin-targeted delivery of UCNP. This study has demonstrated a potentially useful multifunctional nanoconstruct that can carry combinations of therapeutic and diagnostic modalities using developed DUTP-AS1411 aptamer micelles.

3.4.10. *Histopathology of brain tissues*

The *in-vivo* biocompatibility and toxicity of DTX-loaded micelles were analyzed by histopathological study and compared with Taxotere®. Fig. 8B demonstrates the histological micrographs of brain tissues after the 14th day and 28th day *i.v.* administered doses with Taxotere®, DTP, DUTP, DUTP-AS1411 aptamer micelles formulations. Interestingly, it has been observed that all the prepared micelles formulations such as DTP, DUTP, and DUTP-AS1411 micelles showed minimal or no signs of inflammation, shrinkage along with nuclear smudging and degeneration of dentate gyrus and cornu ammonis. While Taxotere® treated group exhibited lymphocytic inflammation and nuclear shrinkage, nuclear smudging and degeneration, and neuronal damage along with apoptotic cell death in some parts of the brain. All groups showed no changes in cerebellar structure [55,56]. The saline-treated group did not show any sign of inflammation or toxicity in brain tissues.

4. Conclusion

The current study focuses on the effective and targeted deliverance of DTX and UCNP through AS1411 aptamer -targeted theranostic micelles. Further characterization of TPGS, and its conjugate by FTIR and ^{1}H NMR spectroscopy reiterates the successful conjugation of TPGS-AS1411 aptamer, authenticity of micelle size in a range of 90–170 nm, spherical and uniform having narrow distribution. The ζ potential of DUTP-AS1411 aptamer micelles was found to be −24.6 ± 0.05 with comparatively higher stability. *In-vitro* drug release studies exhibited biphasic sustained release of DTX from all the micelles formulations, while Toxotere® released DTX rapidly. The $t_{50\%}$ of DTP, DUTP, and DUTP-AS1411 aptamer micelles were found 5.84, 7.95, and 19.02 h, respectively, indicating the sustained drug release effect of micelles. The hemolysis study exhibited 79.88 ± 5.33, 4.09 ± 0.21, 3.87 ± 0.27, and 2.01 ± 0.12% RBCs damage due to exposure with Taxotere®, DTP, DUTP, and DUTP-AS1411 aptamer micelles, respectively, after 4 h of treatment. The cytotoxicity study has revealed that the DUTP-AS1411

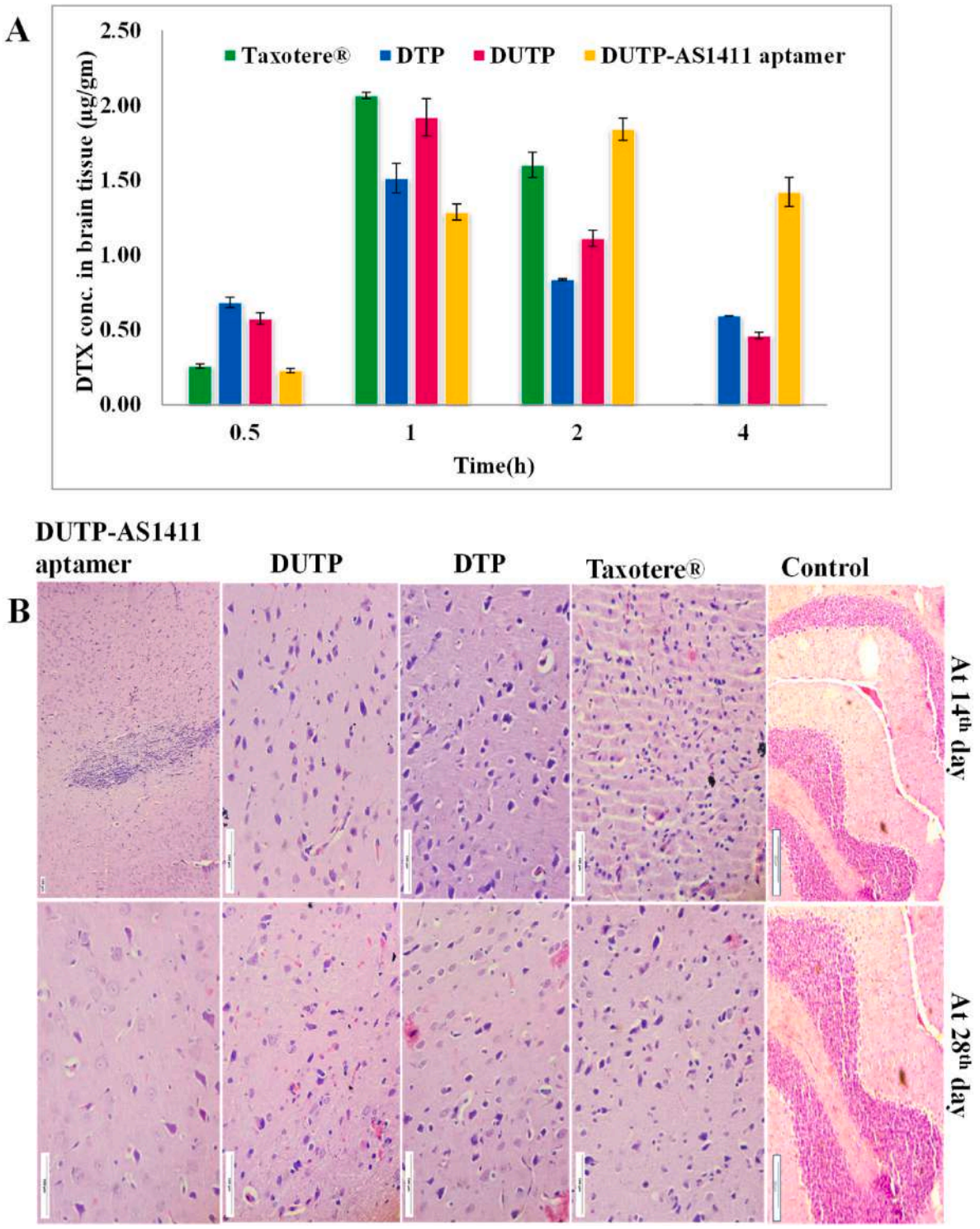

Fig. 8. (A) Graphical representation of brain distribution studies, concentration of DTX in brain after i.v administration of TPGS-PF127 micelles DTP, DUTP, DUTP-AS1411 aptamer and Taxotere® injection and free drug to rats at a dose of 5 mg/kg of DTX. Data show mean ± standard deviation (n = 3), and (B) Histopathology examination of rat brain tissues for the effect of TPGS-PF127 micelles in the brain cancer after *i.v.* administration of normal saline solution (control), Taxotere®, DTP, DUTP and DUTP-AS1411 dose of 5 mg/kg. The section was stained with Hematoxylin and Eosin and examined by light microscopy. Scale bar is 100 µm.

aptamer micelles achieved higher cytotoxicity compared to Taxotere® in U-87MG cells. DTX formulated in the DTP, DUTP, and DUTP-AS1411 aptamer micelles could be 4.20-, 11.70-, and 17.54-fold more therapeutic efficient than Taxotere® after 24 h treatment, respectively. Furthermore, DTP, DUTP, and DUTP-AS1411 aptamer micelles were found to be 4.20, 11.70, and 17.54-fold more therapeutic efficient than Taxotere®, respectively after 24 h of treatment. Interestingly, it was noted that the average concentration of DTX in brain tissues was found at 0.5, 1, 2, and 4 h after *i.v.* administration in the order of DUTP-AS1441≥DUTP ≥ DTP ≥ Taxotere®, respectively. The histological micrographs of brain tissues were observed at 14day and 28th day for first *i.v.* doses of DUTP-AS1411 aptamer micelles did not produce inflammation or irritation, nuclear shrinkage, nuclear smudging, and degeneration of dentate gyrus and cornu ammonis. This corroborates that DUTP-

AS1411 aptamer micelles have the potential for effective and safer theranostics for brain cancer therapy.

CRediT authorship contribution statement

Mahima Chauhan: Methodology, Validation, Investigation, Writing-original draft. Rahul Pratap Singh: Methodology, Supervision, Conceptualization, Investigation, Writing – review & editing, Funding acquisition. Sonali: Methodology, Supervision, Conceptualization, Investigation, Writing – review & editing. Bhavna Yadav: Methodology and Validation. Saurabh Shekhar: Methodology, Validation, Writing-original draft. Abhitinder Kumar: Methodology and Validation. Abhishesh Kumar Mehata: Methodology and Validation. Amit Kumar Nayak: Methodology, Validation and animal studies. Rohit Dutt: Methodology, Writing – review & editing. Vandana Garg: Methodology, Writing – review & editing. Vikas Kailashiya: Methodology, Writing – review & editing. Madaswamy S. Muthu: Methodology, Validation, Investigation, Writing – review & editing. Biplob Koch: Methodology, Validation, Investigation, Writing – review & editing. Dharmendra Kumar Pandey: Methodology and Validation.

Author statement

- We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.
  •We confirm that the manuscript has been read, revised and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.
- We further confirm that the order of authors listed in the manuscript has been approved by all of us.
- We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property.
- In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.
- We further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval (approval no. GDGU/IAEC/2022/11) of all relevant bodies and that such approvals are acknowledged within the manuscript.
- We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

Author Rahul Pratap Singh acknowledges to the Science and Engineering Research Board (SERB), New Delhi for their generous funding support through the project EEQ/2019/000218 under the scheme of Empowerment and Equity Opportunities for Excellence in Science. The authors extend their hertfelt appreciation to the CSIR-Central Drug Research Institute (CDRI), Lucknow, India for their invaluable expertise in FTIR and ^{1}H NMR Spectroscopy techniques to analyze our prepared polymeric intermediate and conjugates. We acknowledge Indian Institute of Technology, New Delhi and Indian Institute of Technology, Varanasi for performing TEM and AFM analyses, respectively. The authors would also like to thank the Oniosome Healthcare Pvt. Ltd., Mohali, Sas Nagar, India for HPLC method development and performing HPLC analysis of blood plasma samples.

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Current Drug Targets, XXXX, *XX*, 1-11

REVIEW ARTICLE

Nano Architect-Based Targeted Delivery Systems for Diabetic Nephropathy: A Review

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ARTICLE HISTORY

Received: November 17, 2022
Revised: March 31, 2023
Accepted: May 04, 2023

DOI:
10.2174/1389450124666230601163338

Abstract: Diabetes mellitus is a long-lasting disease that is very common in the age group above 20 years and is characterized by hyperglycemia with other complications like Diabetic Nephropathy (DN). The management of DN focuses on mainly four regions: reduction of cardiovascular risks, control of blood glycemic levels, control of the blood pressure (BP) profile, and the use of therenin-angiotensin system (RAS). Although BP management and RAS-acting agents can postpone the onset of DN, they cannot prevent it. In the modern era, nanotechnological interventions have spread rapidly in the field of medicine. Patient defiance is considered important in diabetes management when long-term or continuous management is required. Nano pharmaceuticals have been shown to increase compliance of diabetic patients by providing multiple ways of drug delivery, controlling release profile, increasing biological steadiness, targeting efficacy, and decreasing toxic profile. Nanoscale formulations of botanical antidiabetic molecules improve clinical efficacy and treatment compliance by overcoming associated biopharmaceutical and pharmacokinetic barriers. Therefore, the development of nanopharmaceuticals can be considered to be a possible answer to attain the finest scientific effect of the plant-based anti-diabetic molecule. Nevertheless, further studies are needed to create clinical research-based and therapeutically effective nanoforms of antidiabetic plant-based molecules to combat the most dreaded disease of diabetes and its known present complications.

Keywords: Diabetic nephropathy, nano architect, solubility enhancement, pathophysiology.

1. INTRODUCTION

Diabetes mellitus is a long-lasting disease that is very common in the age group above 20 years and is characterized by hyperglycemia [1]. It is two types; Type 1 Diabetes mainly takes place in the young generation of people (≤30 years). It is an auto-immune disorder that is related to the devastation of pancreatic beta cells that produce insulin ultimately. Insulin deficiency occurs and external insulin is required by the patient's body. Type 2 Diabetes is a progressive metabolic disorder in which a deficiency of insulin takes place [2]. Data given in the Global Burden of Disease (GBD) reveals that the increase in cases of chronic kidney disease associated with type 2 diabetes mellitus has gone up by 74% from 1990 to 2017 [3].

Albuminuria in diabetic patients may lead to DN also known as End-Stage Kidney Disease [4]. In 20-50% of patients, Diabetes mellitus induces some other complications in the body as several pathways independently or collectively get activated during diabetes that induces DN [5, 6], and 40% of diabetic nephropathy patients develop chronic kidney disease (CKD) [3]. Arterial hypertension is also generally associated with DN [7]. Figs. (**1** and **2**) shows the inflammatory consequences of diabetes-induced vascular damage and the progression of various diabetic complications like DN [8, 9]. There are no such clinical indicators for accurately distinguishing diabetic nephropathy from non-diabetic renal diseases but some indicators can be used for the examination of Diabetic Nephropathy that is mentioned as follows [10]:

- Microalbuminuria: Urine Albumin range of 30-299 mg/g creatinine is regarded as incipient nephropathy.
- Macro albuminuria: Urine Albumin ≥ 300 mg/g of creatinine is regarded as overt nephropathy
- GFR < 30 ml/min./1.73m^2 is regarded as kidney failure [11-13].

According to the Kidney Disease Outcomes Quality Index guidelines, CKD is distinguished into five stages based on the estimated glomerular filtration rate (eGFR) thresholds [2] within the CKD range or renal structural changes such as proteinuria: Stage-1, kidney damage with normal GFR (greater than 90 mL/min/1.73 m^2); Stage-2, mild reduction in GFR (60-89 mL/min/1.73 m^2); Stage-3, a moderate reduction in GFR (30-59 mL/min/1.73 m^2); Stage-4, a severe reduction in GFR (15-29 mL/min/1.73 m^2); and Stage-5, renal failure, also considered an end-stage renal disease (ESRD)

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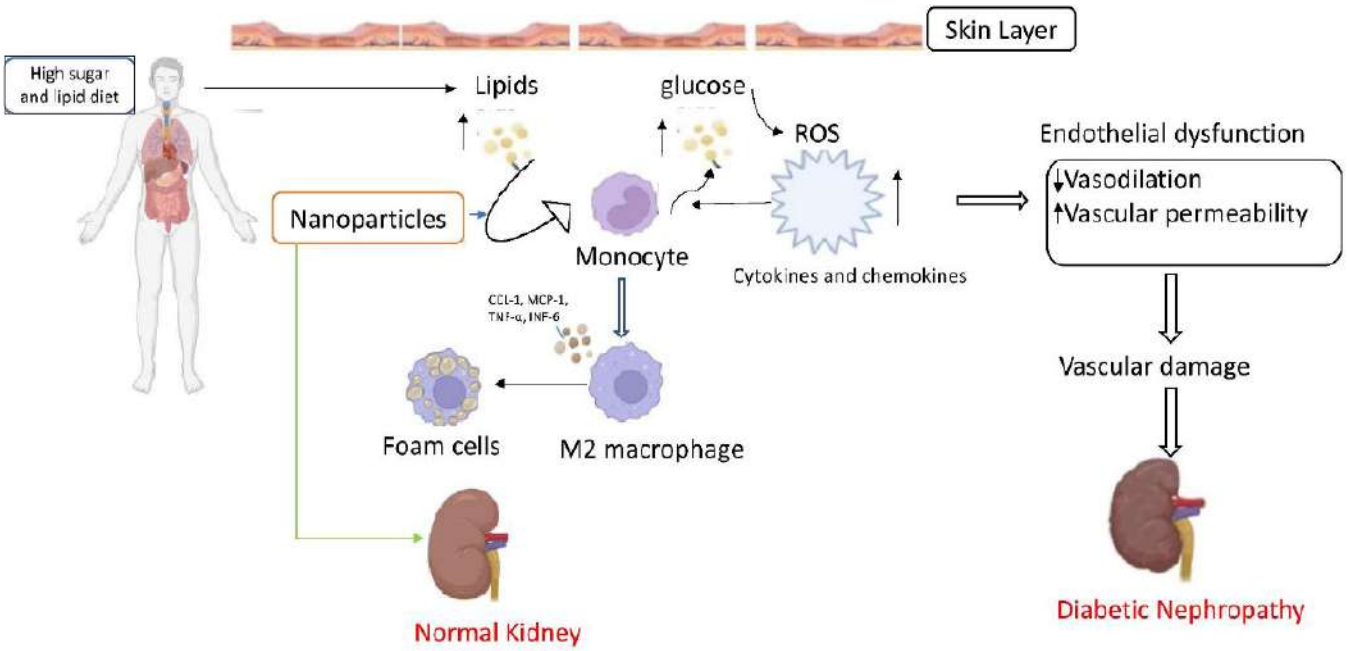

Fig. (1). Effect of nanoformulations on inflammatory consequences of diabetes-induced vascular damage and the progression of diabetic nephropathy. (**Abbreviations:** CCL1: C-C motif chemokine ligand 1; MCP-1, monocyte chemoattractant protein-1; ROS, reactive oxygen species; TNF-α, tumor necrosis factor; IL: interleukin-6). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

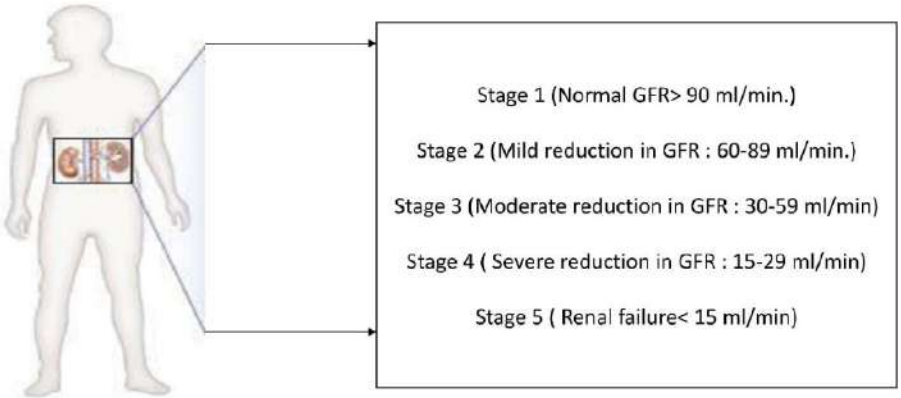

Fig. (2). Five stages based on the estimated glomerular filtration rate (eGFR) thresholds. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

(CKD5d, GFR less than 15 mL/min/1.73 m^2) [14]. CKD Stage-5 characterizes patients with chronic renal failure (CRF), who suffer from progressive loss of renal function and are dependent on dialysis [15].

Several drugs are frequently needed to establish sufficient glycemic control in the majority of patients; glycemic control frequently depends on a variety of techniques, including lifestyle modifications, low-sugar diets, chemical hypoglycemic agents, and herbal treatments [16]. However, frequently used medications like metformin, long-acting and short-acting insulin, renin-angiotensin system blockers, and others frequently have high blood clearance, low effective circulating drug concentrations, and non-targeted drug delivery, which causes end-stage renal disease in patients with DN [12]. Many treatments are available to treat DN, according to the most recent survey, but they are all quite expensive and have unpleasant side effects. Plant-derived chemicals play a vital part in the development of effective medicinal treatments [17]. Recent studies have focused on the nephroprotective properties of herbal-based nutraceutical products since they offer several pharmacological benefits

and low toxicity [18]. Nanotechnology and biochemistry advancements have enabled the development of a nano platform drug delivery system that allows anti-diabetic medications to be dissolved, implanted, encapsulated, or bound to nanoparticles [19, 20]. Furthermore, active targeting molecules modified nanoparticles may also help with effective medication delivery. Transported medications are cleared by the immune system, removing any unintended side effects and enhancing treatment outcomes [21].

Nanotechnology is the combined study of science, medicine, engineering, and technology at a nano-range [1-100 nm] [22]. The smallest-sized atoms in the aforementioned range are bound together in nanoscale particles, which increases the surface area and improves bioavailability [23, 24]. According to the Ostwald-Freundlich and Noyes-Whitney equations, with the decrease in particle size, there is an increase in the saturation solubility and dissolution rate [25, 26]. Therefore, for the improvement of dissolution of poorly water-soluble drugs, it can be seen that small size and large surface area are beneficial [27].

This review offers a fresh perspective on the development of nanoplatforms in the future, as well as the most recent developments in a number of nanoparticle technologies, phytochemicals, and their uses in the management of DN.

1.1. Pathophysiology of Diabetic Nephropathy

Diabetic nephropathy is characterized by the symptoms like oxidative stress, systemic blood pressure, proteinuria, glycemic control, hyperlipidemia, inflammation, growth factors, increase in growth factors [28-31]. Such metabolic and hemodynamic aberrations may sum up several pathways that lead to the production of reactive oxygen species (ROS) and that ultimately influence Gene regulation and transcription factors activation as shown in Fig. (**3**) [32].

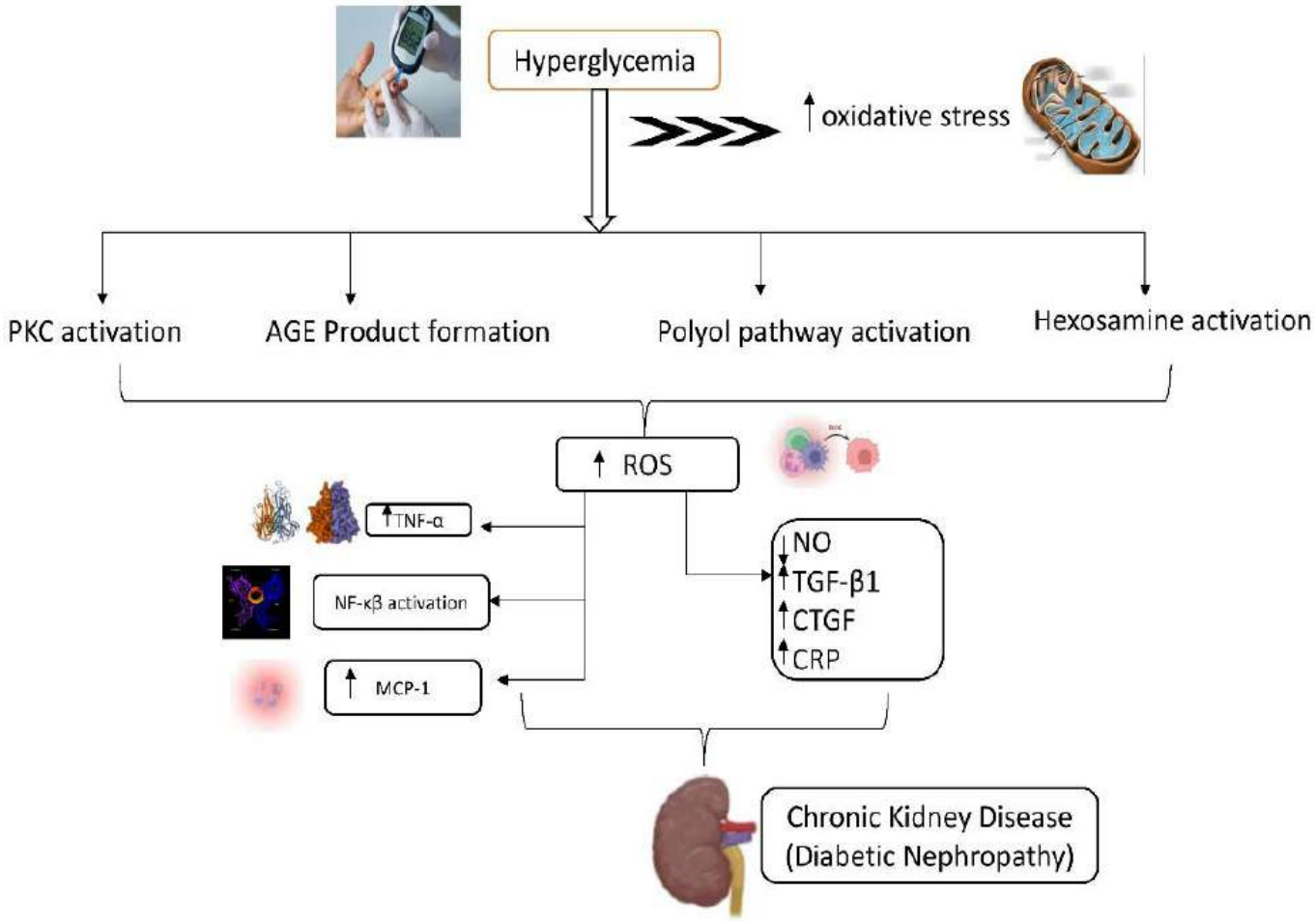

Fig. (3). Schematic representation of the pathogenesis of diabetic nephropathy. **Abbreviations:** PKC (protein kinase C); AGE (Advanced glycation end products); ROS (Reactive Oxygen species); TNF-alpha (Tumor Necrosis Factor alpha); NF-κB (Nuclear factor kappa B); MCP-1 (Monocyte chemoattractant protein-1); NO (Nitric oxide); TGF-β1 (Transforming growth factor-β1); CTGF (Connective tissue growth factor); CRP (C-reactive protein). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

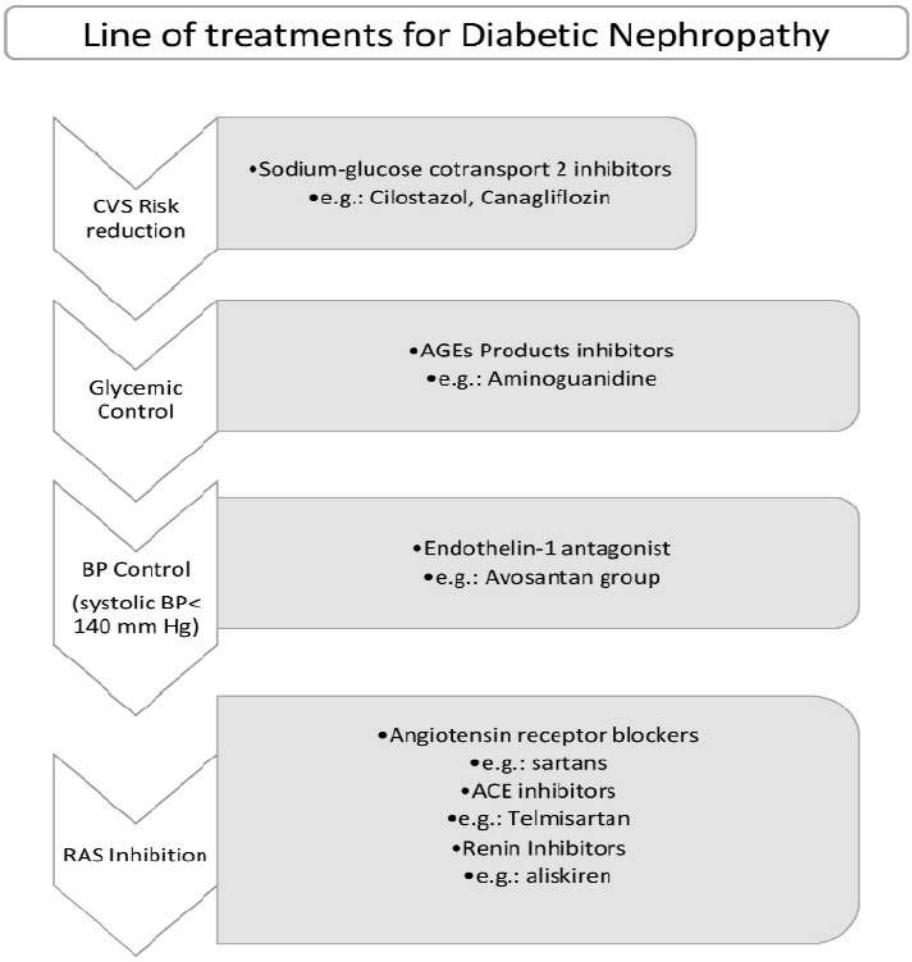

Fig. (4). Treatment of diabetic nephropathy.

- TNF-α, IL-1, and IL-6 are examples of pro-inflammatory cytokines that can be produced by renal cells (endothelial, epithelial, mesangial, and tubular cells), and these cytokines, acting in a paracrine or autocrine manner, may have a variety of effects on various renal structures contributing significantly to the onset and progression of a number of renal disorders like DN [33, 34].
- The afferent and efferent arterioles are both dilated by NO, which may also increase the glomerular filtration rate (GFR) and affect how sodium is handled by the kidneys along different tubule segments, from the thick ascending limb through the distal tubule and the collecting duct. Chronic kidney disease (CKD) causes a decrease in total NO production, which accelerates kidney damage and cardiovascular events [35].

1.2. Treatment

DN can be treated by using the following approaches as shown in Fig. (**4**) [36-38]. Some incipient therapies like RAS inhibition, Sodium-glucose cotransporter-2 inhibitors, and angiotensin receptor blockers (ARB] therapy are the favored therapies for treating DN [39, 40]. Some phytochemicals have also been shown to be emphatic like *Curcuma Longa, Ocimum Sanctum*, etc [41-45]. Though, the main apprehension is the physical unpredictability which is due to their high energy state and the intrinsic property of getting recrystallized while storage, dissolution, and manufacturing [46].

Some studies like EDIC (Epidemiology of Diabetes Interventions and Complications study) and DCCT (Diabetes Control and Complications Trial) have been done on type I diabetes mellitus patients competently controlling their sugar can conquest proteinuria and microalbuminuria [47]. The United Kingdom Prospective Diabetes Study (UKPDS) boasted that by lowering the risk of microvascular dysfunction and nephropathy, HbA1C may be targeted in people with type II diabetes [48].

Some studies, such as IDNT (the irbesartan trial for diabetic nephropathy] and RENAAL (lowering the NIDDM endpoint with the angiotensin II antagonist losartan trial), have also demonstrated the beneficial effects of ARBs in postponing the improvement in DN. It was done to prove it.

This study also highlighted the need for BP regulation [49, 50]. The JNC 8 (8th National Joint Committee) guidelines require that BP be maintained below 140/90 mmHg. With individualization, it applies to most patients with type 2 diabetes and DN [51].

A study, namely Randomized Olmesartan and Diabetic Microalbuminuria Prevention (ROADMAP), exhibited that the onset of microalbuminuria in T2DM patients can be prevented using RAS blockers [52].

High concentrations of drugs must be absorbed by the kidney in order to treat nephropathic diseases; nevertheless, these medications are frequently toxic and have other side effects. Higher drug doses are not always sufficient to ensure that the desired tissue receives the ideal dose of the medicine due to the reduced tubular secretion and glomerular filtration processes in DN [53]. Drug delivery that targets the kidneys assists in the resolution of this problem. To treat kidney illnesses, a variety of drug delivery strategies have been devised. They comprise antibodies, nanoparticles (NPs) and peptide carriers, small molecule prodrugs, and macromolecular carriers such as liposomes, lipid particles, and micelle polymers. A drug's impact on the damaged tissue is minimized through targeted delivery. Targeted distribution and controlled drug release are made possible by the use of nanotechnology [54, 55].

New drugs such as Finerenone, a 3rd-race mineralocorticoid receptor antagonist, have been revealed to reduce albuminuria in DN at 90 days in diabetic individuals previously treated with ARBs [56]. CANVAS and EMPAREG research work has shown that sodium-glucose cotransporter 2 (SGLT) inhibitor, which averts glucose re-absorption in the kidney renal tubule, reduces mortality which is related to cardiovascular diseases [57]. In the cardiovascular risk-related consequence studies, SGLT2 inhibitor had a beneficial effect on the renal outcome. That is, a decrease in albuminuria is observed, and the frequency of complex renal blood flow decreases. However, since this is secondary to studies of cardiovascular benefits, numerous studies are currently being conducted to experiment with the true perspective of this class of drug in preventing the development of DN.

Nano-curcumin is the greatest alternative for boosting the therapeutic efficiency and reducing unwanted side effects of curcumin since it has greater water solubility and possesses antioxidant capabilities [58]. Its antioxidant property aids in controlling the amount of Reactive Oxygen Species (ROS) in stressed cells [59]. A key element of employing plant-based remedies, such as those derived from Ocimum sanctum/Ocimum tenuiflorum (Holy basil/Tulsi) and Ocimum basilicum (Sweet basil), that seem to have the same effect as the antihyperglycemic medication commercially available [60]. Our comprehension of their complete therapeutic efficacy is constrained by their diverse bioactive chemical components, which include polyphenols, flavonoids, alkaloids, terpenoids, steroids, and glycosides that cooperatively coexist. In order to combat this, functionalizing green AgNps generated from these pharmacologically significant plants is a practical option to utilize in the creation of colloidal pharmaceuticals, enhancing the properties of well-known medicinal plants in combination with the many specificities of nanotherapy [61].

Recent guidelines for diabetes patients are listed below:

1. Blood glucose control must be ameliorated

2. ACE inhibitors or ARBs for controlling blood pressure

3. Optimum protein intake (0.8 g/kg body weight)

4. BUN and serum creatinine levels must be observed

5. The urine albumin-creatinine ratio must be monitored

6. Diabetic patients with normal GFR, urinary albumin to creatinine ratio, and BP are not to be prescribed ACE inhibitors and ARBs.

7. Chronic kidney disease is to be said when GFR falls below 60 ml/min and if below 30 ml/min., a nephrologist may recommend kidney replacement.

8. For the treatment of chronic kidney diseases, a nephrologist must be consulted [21, 23].

1.3. Nanosystem-Driven Therapeutic Pathways: A Modern Aptitude

Objects having the dimensions of 10^{-9}-10^{-7} m are compromised by nanotechnology. Contemporarily, nanoscience has emerged for diagnosis and therapies in many medical areas. Materials of nanoscale procure few designated physical, chemical, and biological properties, which spawn biomedical appositeness. Nanotechnology works under two conditions, the first is concerned with controlling the structure and size at the nanoscale and the second is the novelty that is because it uses the properties of its cargoes in their nano sizes [62]). Remedying agents also in nanoscale dimensions have been a boon for therapeutic efficacy and also gashed the confines between the therapeutic effects and pharmaceutical ineptitude (Fig. **5**). Chrysalis of certain kinds of nanocarriers, like niosomes, nanoparticles, micelles, dendrimers, and liposomes, have remodeled an original methodology as compared to the conventional drug delivery system as these are more effective, target specificity, bioavailability, stability, and release of drug [63]. Such nanocarriers are comprehensively useful in delivering a variety of drug molecules having flexible physic-chemical properties, and also, show distinctive active targeting by functionalizing their surfaces with the used polymer or with the suitable ligand (Fig. **4**). Nanomedicines are also finding their place in treating cancer and the confinement of tumors. Their applications are yet to be explored in many other therapeutic areas [64, 65]. Nanomedicines are widely used for the treatment of DN also nowadays drug-loaded nano-liposomes are used for the treatment [66].

Contrarily, some challenges are encountered while using a nano carrier-based drug delivery system loading capacity is poor, cellular uptake endowment becoming missing, sometimes becoming toxic, apocryphal biodegradation, and ligand tagging capacity. Biocompatibility is the major

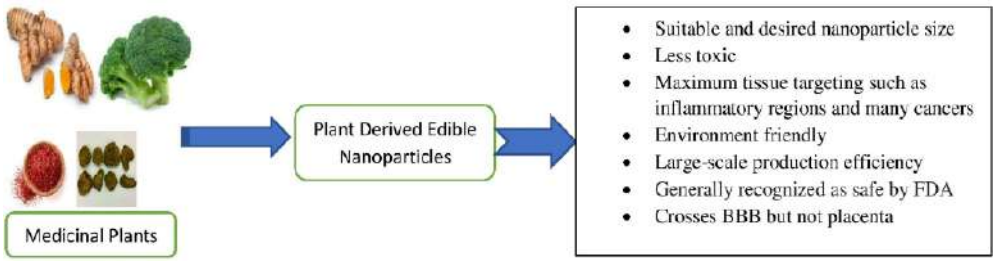

Fig. (5). Advantages of plant-derived nanoparticles. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

problem for such nanocarriers as the body's immune system recognizes them and may extract them from the body [67] Though, formulation scientists have intimated some disparate approaches to extenuate such impediments and can acquire real-world applications of these Nano formulations. A contemporary report imparted that the global market of such nano-carrier-based drug delivery is rising sequentially with an annual growth rate of 22%.

1.4. Nano Carrier-based Formulations for Diabetes Treatment

Diabetes mellitus has certain acute and chronic aggravations that can be better treated with the help of nanoformulations. Many nano carrier-based formulations with different structures are used for treating diabetes mellitus. As such types of formulations have the target specificity with congruous release patterns. In annexation to this, these formulations are also helpful in delivering the drugs *via* various routes [68, 69]. To enhance the targeting efficiency of nanocarriers they can be artifacts with certain ligands and ultimately systemic availability and stability of the drug get increased and it also abates the drug dosing and administration frequency. Conclusively, these artifacts comprising nano carrier-based formulations can abate the peril of toxic revelations [70]. Hence, appropriately prepared nanoformulations of antidiabetic agents may give efficient therapeutic efficacy for DN.

Consequentially, the review also enunciates the amelioration and the cogency of nanoformulations of hypoglycemic agents from plant resources for the treatment of DN and diabetes.

- DN is End Stage Kidney Disease that is characterized by many symptoms and many types of metabolic pathways involved. Therefore, if we use curcumin-tagged solid nanodispersion of drugs that act on the RAS, Sodium-glucose cotransporter-2 inhibitors, treatment would be better and more targeted. It can reduce the chances of concomitant Diabetes and Nephropathy. These are several nanoformulations that have been demonstrated to be effective against DN (Table **1**).

Table 1. Examples of preclinical research on various nanoplatforms for DN treatmen.

S. No.	Description	References
1.	Liponanoparticles loaded with Rhein having polycaprolactone-polyethyleneimine-based cores showed a better capability of directing and the accession of Rhein in the renal tissues along with the efficient therapeutic effects and a pittance of urinary excretion in diabetic nephropathic mice. Rhein lipo-nanoparticle showed decent steadiness in the tissue environmental conditions and a continuous prolonged drug-releasing contour up to the 48-h period with no occurrence of any toxicological effects on the renal tissues	[71]
2.	Pingping Li *et al.* also prepared Apigenin-loaded solid lipid nanoparticles that were shown to be attenuated diabetic nephropathy in rats by altering the signaling pathways.	[72]
3.	Administration of taurine in nanoparticle form greatly decreased positive effects on diabetic nephropathy were indicated by raised serum glucose levels, creatinine levels, and BUN levels caused by STZ. Administration of taurine causes a considerable increase in urine creatinine and a large decrease in urine albumin. Moreover, lipid indicators like cholesterol, triglycerides, and HDL levels are elevated.	[73]
4.	Chunrong Huang *et al.* presented in their study that novel treatments based on calycosin nano-formulated can ameliorate DN by restoring mitochondrial activity. They analyzed their results for TEM, DLS, FTIR methods, and animal activity.	[74]
5.	Xiaodong Yang showed that in STZ-induced diabetic rats, crocetin-loaded poly (lactic-co-glycolic acid) PLGA NPs had a kidney antifibrotic and anti-inflammatory impact.	[75]
6.	Akram Ahangarpour *et al.* showed that by lowering oxidative stress and raising antioxidant enzyme levels, myricitrin and its Solid lipid nanoparticles (SLN) treatment improved DN changes. These benefits were more pronounced in the animals who received the SLN.	[76]
7.	Yanfang Yu *et al.* showed that AuNPs exhibited a nephroprotective role *via* the SIRT3-SOD2 signaling pathway.	[77]
8.	Jing-Bo Hu *et al.* showed that increased accumulation of HA-CUR in kidneys with 13.9-fold was higher than that of free CUR. Pharmacodynamic studies indicated HA-CUR effectively ameliorated AKI, and the exact mechanism was that HA-CUR protected renal tubule epithelial cells from oxidative stress damage *via* inhibiting the PtdIns3K-AKT-mTOR signaling pathway.	[78]

Here are some other uses of nanotechnology for treating diabetes and its other complications:

- Nanoformulations of hypoglycemic agents containing curcumin have emerged as an effective approach for bioavailability, stability, solubility, and therapeutic efficiency enhancement. Allam *et al.* prepared curcumin encapsulated self-nano-phospholipid suspension using miglyol 812 and Phosal®53 using inconsistent surfactants ratios and formulated a Phosal®53 MCT is soybean lecithin derived phosphatidyl-choline which acts as the estimable solvent for poorly water-soluble compounds. They showed that Curcumin-self-nano phospholipid dispersions enhanced the bioavailability of poorly water-soluble curcumin as compared to the conventional formulations in rats [79].
- Emulsion- A diffusion-evaporation method used for the formulation of curcumin nanoparticles was found to be effective as the formulation reduced the fasting blood glucose and glycosylated hemoglobin levels factually *via* enhancing the rendition of insulin and insulin receptor (IR) mRNAs in diabetic rats [80].
- In type 2 diabetic model rats, diabetes therapy was given by curcumin- ZnO nanoparticles (10 mg/kg, for 21 days). It showed there is an abatement in blood glucose levels, serum insulin increased, and glucokinase and GLUT2 genes in the liver and pancreas get activated
- Curcumin fabricated with poly-(ethylene glycol), and poly-(-benzyl 1-glutamate) poly-(-benzyl l-glutamate) also proved to have increased bioavailability and water solubility. Curcumin-loaded multi-polymer nano-carrier attained exceeding pharmacologically driven activity in crossover regulation of, calcium-sensing receptor gene, Ca2+/calmodulin, and endogenous cystathionine -lyase/H2S as compared to the orthodox curcumin-based several formulations for the treatment of diabetic induced cardiac myopathy in rats [81].
- Curcumin-loaded PLGA nanoparticles also showed enhanced bioavailability (approx. 5.6 times than crude curcumin) and also increased biological half-life. Nanoparticles also had enhanced water solubility, eliciting the drug release profile prominently in the intestinal juice, improved penetration, arresting P-GP efflux, and elevated residence time in the intestinal cavity [82].
- For perpetuating plasma association and enhanced oral bioavailability of curcumin in diabetic neuropathic rats, self-nano emulsification of curcumin was used [83].
- Sameer Sharma *et al.* also demonstrated that curcumin has motley therapeutic targets as it shows antioxidant activity, and also prevented some metabolic chains in the rat's body for the prevention of the onset of diabetic nephropathy [84].
- The antisolvent precipitation method was used for preparing Betulin nanoparticles for improving oral bioavailability (2.2 times) and also showed the antidiabetic effect effectively as compared to the conventional form [85].
    - Qiong Ma *et al.* also prepared copolymerized antihyperglycemic nanoparticles with the help of a plant extract named HPA (p-hydroxyphenyl ethyl anisate). Scientists injected insulin-loaded injections of prepared nanoparticles and that showed the alleviation of the blood glucose level of mice within 48 hours [86].
    - PEG-block nanocarriers loaded with quercetin-[poly-(ethylenediamine-glutamate)--graft-poly-["-benzyloxy-carbonyl-1-lysine)] for the treatment of the quercetin-loaded system *versus* free molecule of quercetin in diabetes and related treatments Potential for nephropathy by increasing serum levels of quercetin in rats [87].
    - Mohd. Adnan Kausar *et al.* also showed two phytochemicals (*i.e.*, gentisic acid and michelalbine) that are supposed to target human amylin peptide and dipeptidyl peptidase-4, respectively, have an efficient binding capacity [88].
    - Several plant-based nanopharmaceuticals were established in the latest years and are known to be efficient in relieving diabetic conditions and their related problems. Though, significant investigation efforts are needed to invent new plant-based antidiabetic nanopharmaceuticals that can be applied clinically in diabetes.

CONCLUSION

DN is the end stage of the kidney-related disease that is characterized by many symptoms and many types of metabolic pathways involved. So, if we use curcumin--tagged solid nanodispersion of drugs that act on the RAS, Sodium-glucose cotransporter-2 inhibitors. So, treatment would be better and more targeted. It can reduce the chances of concomitant Diabetes and Nephropathy.

CURRENT SCENARIO AND FUTURE POINT OF VIEW

In the modern era, nanotechnological interventions have spread rapidly in the field of medicine. Patient defiance is considered important in diabetes management when long-term or continuous management is required. Nanopharmaceuticals have been shown to increase compliance of diabetic patients by providing multiple ways of drug delivery, controlling release profile, increasing biological steadiness, targeting efficacy, and decreasing toxic profile. As a result, attention to emerging nanomedicines to treat diabetes is growing rapidly. In addition, various naturally derived and product-based nanoforms and naturally product-based anti-diabetic nanoforms have attracted researchers' attention for

many years. With this in mind, it can be argued that there is a potential for the development of nanomedicines suitable for alleviating complications such as diabetes and diabetic nephropathy using natural antidiabetics or herbal medicines.

Nanoforms of several natural compounds with a known anti-diabetic perspective have been developed to advance pharmacokinetics and therapy effectiveness in the treatment of diabetic conditions. In addition, nano pharmaceuticals derived from plant molecules have been developed with many antidiabetic activities thought to be etiologically similar to diabetes or effective in other diseases [89-93]. Therefore, dose adjustment of these nano pharmaceuticals has the potential to treat diabetes. In this context, nano pharmaceuticals derived from natural hypoglycemic agents are expected to have great potential for the treatment of diabetes in the future, increasing patient-related factors such as compliance, providing effective costing, and decreasing toxicity.

In conclusion, nanoscale formulations of botanical antidiabetic molecules improve clinical efficacy and treatment compliance by overcoming associated biopharmaceutical and pharmacokinetic barriers. Therefore, the development of nanopharmaceuticals can be considered to be a possible answer to attain the finest scientific effect of the plant-based anti-diabetic molecule. Nevertheless, further studies are needed to create clinical research-based and therapeutically effective nanoforms of antidiabetic plant-based molecules to combat the most dreaded disease of diabetes and its known present complications.

LIST OF ABBREVIATIONS

AGE	=	Advanced Glycation End Products
AgNPs	=	Silver Nanoparticles
AKI	=	Acute Kidney Injury
ARBs	=	Angiotensin Receptor Blocker
ASD	=	Amorphous Solid Dispersion
AuNPs	=	Gold NPs
BCS	=	Biochemical Classification
BP	=	Blood Pressure
CKD	=	Chronic Kidney Disease
CRP	=	C-Reactive Protein
CTGF	=	Connective tissue growth factor
Cur	=	Curcumin
DN	=	Diabetic Nephropathy
ESRD	=	End-stage Renal Disease
GFR	=	Glomerular Filtration Rate
GFR	=	Glomerular Filtration Rate
HA	=	Hyaluronic Acid
IL	=	Interleukins
MCP-1	=	Monocyte Chemoattractant protein-1
NCE	=	New Chemical Entity
NF-κB	=	Nuclear Factor Kappa B
NO	=	Nitric Oxide
NPs	=	Nanoparticles
PKC	=	Protein Kinase C
PLGA	=	Poly(Lactic-co-glycolic Acid)
RAS	=	Renin-Angiotensin System
ROS	=	Reactive Oxygen Species
SGLT-2 Inhibitor	=	Sodium-glucose Cotransporter 2 Inhibitors
STZ	=	Streptozotocin
TGF-β1	=	Transforming growth factor-β1
TNF-α	=	Tumor Necrosis Factor- α

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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Effect of Extraction Methods on the Antioxidant Potential and Cytotoxicity of the Combined Ethanolic Extracts of *Daucus carota* L., *Beta vulgaris* L., *Phyllanthus emblica* L. and *Lycopersicon esculentum* against Gastric Adenocarcinoma Cells

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Abstract: Frequent consumption of fruits and vegetables in the daily diet may alleviate the risk of developing chronic diseases. *Daucus carota* L. (carrot), *Beta vulgaris* L. (beetroot) *Phyllanthus emblica* L. (amla), and *Lycopersicon esculentum* M (tomatoes) are traditionally consumed functional foods that contain a high concentration of antioxidants, ascorbic acid, polyphenols, and numerous phytochemicals. This study assessed how three distinct preparation methods affect the phenolic, flavonoid, carotenoid, and ascorbic acid contents, antioxidant level, and cytotoxicity of the combined fruit extract. The fruit samples were taken in the ratio of carrot (6): beetroot (2): tomato (1.5): amla (0.5) and processed into a lyophilized slurry (LS) extract, lyophilized juice (LJ) extract, and hot-air oven-dried (HAO) extract samples. The sample extracts were assessed for their phytoconstituent concentrations and antioxidant and cytotoxic potential. The total phenolic content in LS, LJ, and HAO extracts was 171.20 ± 0.02, 120.73 ± 0.02, and 72.05 ± 0.01 mg gallic acid equivalent/100 g, respectively and the total flavonoid content was 23.635 ± 0.003, 20.754 ± 0.005, and 18.635 ± 0.005 mg quercetin equivalent/100 g, respectively. Similarly, total ascorbic acid content, carotenoids, and antioxidant potential were higher in the LS and LJ extracts than in HAO. Overall, the LS extract had a substantially higher concentration of phytochemicals and antioxidants, as well as higher cytotoxic potential, compared to the LJ and HAO extracts. The LS extract was tested in the MKN-45 human gastric cancer cell line to demonstrate its effective antioxidant potential and cytotoxicity. Hence, lyophilization (freezing) based techniques are more effective than heat-based techniques in preserving the phytoconstituents and their antioxidant and cytotoxic potential.

Keywords: hot-air oven drying method; ethanolic fruit extracts; bioactive compounds; flavonoids; carotenoids; phenolics; lyophilization method; phytoconstituents; antioxidant; anticancer

Citation: Chauhan, M.; Garg, V.; Zia, G.; Dutt, R.; Alghamdi, B.S.; Zawawi, A.; Ashraf, G.M.; Farhana, A. Effect of Extraction Methods on the Antioxidant Potential and Cytotoxicity of the Combined Ethanolic Extracts of *Daucus carota* L., *Beta vulgaris* L., *Phyllanthus emblica* L. and *Lycopersicon esculentum* against Gastric Adenocarcinoma Cells. *Molecules* **2023**, *28*, 0. https://doi.org/

Academic Editor: Sanghamitra Nayak

Received: 10 May 2023
Revised: 25 June 2023
Accepted: 28 June 2023
Published: 31 August 2023

1. Introduction

The importance and awareness of proper food composition for a healthy life and disease prevention have grown in recent decades, leading to increased demand for functional foods. Beneficial nutrients in plant-based foods include dietary fibers, vitamins, minerals, and electrolytes. Essential phytochemicals like polyphenols, carotenoids, betalains, organosulfur compounds, alkaloids, etc., including numerous antioxidants, are also abundant in fruits and vegetables. The nutrient content of fruits and vegetables provide health benefits, while phytochemicals, especially polyphenols, have therapeutic potential [1]. Polyphenols are considered secondary metabolites that do not confer metabolic functions but are necessary for the nutritional and sensory qualities of plant foods [2–4]. The need of the hour is to use these phytonutrients as therapeutic agents against various diseases. Compared with synthetic chemotherapeutics, natural products such as anthocyanins and ginkgo biloba are proven to be less toxic to healthy cells, with fewer side effects in patients [5,6]. The literature is rife with efforts toward analyzing natural plant-based products as drugs against diseases, including cardiovascular diseases, cancers, inflammatory diseases, and autoimmune and infectious diseases [7–11]. Many phytonutrients have been in clinical trials, and some are being used in clinical settings [9,12–14].

Extraction of phytochemicals requires appropriate drying methods to preserve and retain these essential constituents from the fruits, leaves, or other parts of the plants. Numerous drying methods have been experimented with to preserve phytoconstituents. Some methods are sun drying, hot-air oven drying, freeze drying, vacuum drying, microwave drying, etc. Hot-air or conventional drying is the most popular method due to its low cost [15]. However, it has several disadvantages attributed to its long drying time and high temperatures, usually associated with undesirable effects, such as degradation of bioactive components, loss of antioxidant compounds and nutritional and sensory quality, higher shrinkage percentage, and the formation of undesirable secondary compounds [16,17]. Thus far, freeze-drying (lyophilization) is considered the best drying method that preserves dried foods' sensory and nutritional qualities with lower shrinkage percentage, higher rehydration capacity, and easy application [18,19]. According to recent findings from evaluations of the effect of different drying techniques on major antioxidants in fruits and vegetables, freeze-drying outperforms other drying methods in terms of antioxidant preservation [20–23].

Due to the presence of phytochemicals, fruits, fruit juices, and vegetables demonstrate abundant synergistic effects on human health. In this study, we chose fruits of four plants: carrots, beetroot, tomatoes, and amla. Carrot was included due to its unique combination of three flavonoids: kaempferol, quercetin, and luteolin [24–26]. Beetroot contains phenolic compounds, betalains, carotenoids, micronutrients, and macronutrients [27]. The active compounds (betalains) in beetroot are a promising alternative for supplemental therapies for multiple diseases [28]. Numerous studies demonstrate that a high intake of lycopene-rich tomatoes and tomato-based products may protect against cardiovascular disease and reduce the risk of cancers of the prostate, breast, lung, and digestive tract [29]. Amla, which contains high concentrations of ascorbic acid, aids in immune defense, fights free radicals, and protects from various chronic diseases by neutralizing oxidative stress [30]. Carrots, beetroot, tomato, and amla have flavonoids, chlorogenic acid, and vitamin C, which positively impact human health through their protective effect against oxidative stress by neutralizing free radicals [31].

Many studies have reported the effect of different drying techniques on the antioxidant activity, physicochemical properties, and active concentrations of phenolic and other nutritional compounds of carrot, beetroot, tomato, and amla individually [32]. However, thus far, no research has been conducted on combined extracts of the above four fruits and the influence of different drying methods on the antioxidant capacity, total phenolic content, flavonoids, carotenoids, and ascorbic acid content, as well as their anticancer/cytotoxic activity [33,34].

2. Results and Discussion

2.1. Effect of Drying Techniques on Crude Fiber Content

The samples were dried by the hot-air oven and lyophilization methods and mixed in the defined ratio as mentioned in the Materials and Methods section, i.e., carrot (6 mg): beetroot (2 mg): tomato (1.5 mg): amla (0.5 mg). The samples thus formed were; (A) hot-air oven-dried extract (HOA), (B) lyophilized slurry (LS) extract, and (C) lyophilized juice (LJ) extract, as shown in Figure 1.

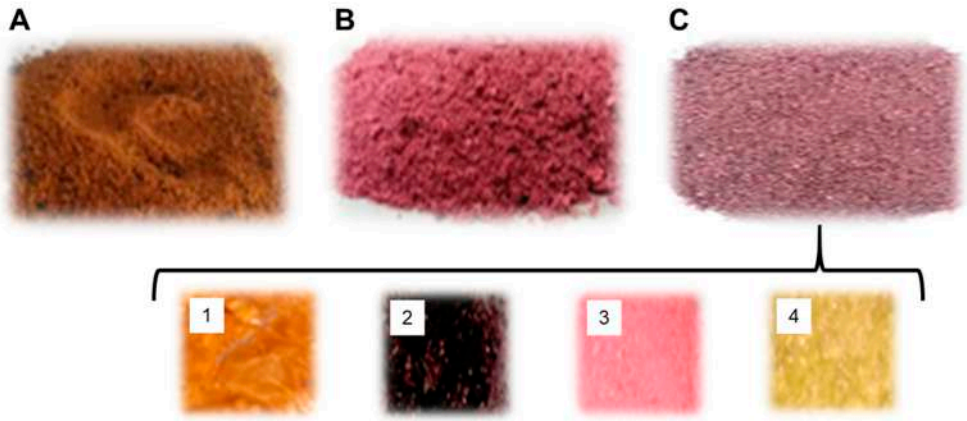

Figure 1. The dried extracts obtained from three methods used in the study. (**A**) The dried fruit sample obtained from the hot-air oven-dried (HAO) method. (**B**) The dried sample obtained from lyophilized slurry (LS). (**C**) The dried sample obtained from lyophilized fruit juices (LJ). (Inset) Examples of the dried juice extracts from each fruit (1—carrot; 2—beetroot; 3—tomato; 4—amla).

The qualitative standard was determined using the HAO extract (Figure 1A) and lyophilized extracts (LS and LJ) (Figure 1B,C). The crude fiber content in LS, LJ, and the HAO extracts was 4.2%, 1.4%, and 2%, respectively, as shown in Table 1. The lowest value for crude fiber was obtained in the hot-air oven-dried preparation (HAO), and the highest crude fiber content was present in the LS extract. This indicates that the lyophilization methods have a less adverse effect and maintain the sample extracts' fiber content. This was consistent with the findings of other, similar studies in which enzyme activities of the extracts were tested and found to be optimal with the lyophilization-based techniques [35,36]. High fiber content is beneficial in aiding the peristalsis movement of GIT, which helps bowel movement, lowers blood cholesterol, and reduces the risk of colon cancer [37].

Table 1. Effect of three drying techniques on the crude fiber content.

Dried Samples	Weight (g)	Crude Fiber (%)
Hot-air oven-dried sample (HAO)	2.0	2.0
Lyophilized slurry (LS)	2.0	4.2
Lyophilized juices (LJ)	2.0	1.4

2.2. Total Phenolic Content (TPC) and Flavonoid Content

Total phenolic and flavonoid content in the LS, LJ, and HAO extracts were determined (Table 2). The total phenolic content in the HAO extract was found to be low (72.05 ± 0.01 mg gallic acid/g) as compared to the LS extract (171.20 ± 0.02 mg gallic acid/g) and LJ extract (120.73 ± 0.02 mg gallic acid/g). Total flavonoid content in the LS extract (23.635 ± 0.003 mg quercetin/g) and LJ extract (20.754 ± 0.0005 mg quercetin/g) was found to be higher than in the HAO extract (18.635 ± 0.0005 mg quercetin/g). This indicates that the lyophilization (freezing) method is more resilient in preserving the TPC and flavonoid content than the hot-air drying method. It has been noticed in several studies

that HAO leads to a loss of phytoconstituents, which potentially diminishes the bioactive value of functional foods [38]. Hence, an effective standardized drying method must be used to harness the full potential of fruit extract for health benefits or as therapeutics.

Table 2. Total phenolic content and flavonoid content in hot-air oven-dried extract, lyophilized slurry extract and lyophilized juice extract.

Dried Samples	Total Phenolic Content (mg Gallic Acid/100 g)	Total Flavonoid Content (mg Quercetin/100 g)
Hot-air oven-dried sample (HAO)	72.05 ± 0.01	18.635 ± 0.005
Lyophilized slurry (LS)	171.20 ± 0.02	23.635 ± 0.003
Lyophilized juices (LJ)	120.73 ± 0.02	20.754 ± 0.005

2.3. Ascorbic Acid Content and Carotenoids Content

The drying method employed to generate plant extracts influences the amount of ascorbic acid and carotenoids. Ascorbic acid is a hydrophilic, heat-sensitive vitamin easily destroyed and evaporated upon heating. As a result, the vitamin C content is usually lost during heat-based sample drying methods. Low temperature-based sample processing techniques are more promising to preserve and retain vitamin C and other nutrients during drying [39,40]. In our study, the ascorbic acid and carotenoid contents were more concentrated after lyophilization drying than with the hot-air oven drying method (Table 3). The concentration of ascorbic acid obtained in the samples dried using the hot-air method was found to be 2.68 mg. In comparison, LS and LJ extracts yielded 6.51 mg and 2.99 mg of ascorbic acid, respectively. Since fruit juices are prepared by extracting the liquid component from the slurry, low amounts of ascorbic acid in fruit juices, comparable to HAO, are probably due to a loss of ascorbic acid content upon filtration to remove the slurry. Secondly, ascorbic acid is more prone to oxidation in liquid solvents than in slurries. The carotenoids content in LS and LJ extracts was 30.25 mg/100 g and 23.25 mg/100 g, respectively, and 14.00 mg/100 g in the HAO extract (Table 3).

Table 3. Determination of ascorbic acid content and carotenoids in the three extracts.

Different Dried Samples	Ascorbic Acid Content (mg)	Carotenoids Content (mg/100 g Sample)
Hot-air oven dried sample (HAO)	2.68	14.00
Lyophilized slurry (LS)	6.51	30.25
Lyophilized juices (LJ)	2.99	23.25

The nutritional profiles of plant extracts obtained after extraction limit or enhance their health benefits or their use as therapeutics. The research focus has shifted to identifying the best preparation methods that retain and enhance the bioactive contents, especially the antioxidant potential of fruit and vegetable extracts [41–43].

2.4. Antioxidant Activity

The effect of different drying techniques on the antioxidant capacity of the HAO, LS, and LJ samples was evaluated using the DPPH scavenging method and reducing power assay. Table 4 shows the antioxidant activity of the three extracts. The percentage of inhibition was found to be highest in the LS at all concentrations compared to the LJ and HAO extracts. The results are presented in Figure 2.

Table 4. Determination of antioxidant activity of the three sample extracts.

Drying Techniques	Reducing Power (Total Antioxidant Capacity) (Absorbance)
Hot-air oven-dried sample (HAO)	0.235
Lyophilized slurry (LS)	1.827
Lyophilized juices (LJ)	1.521

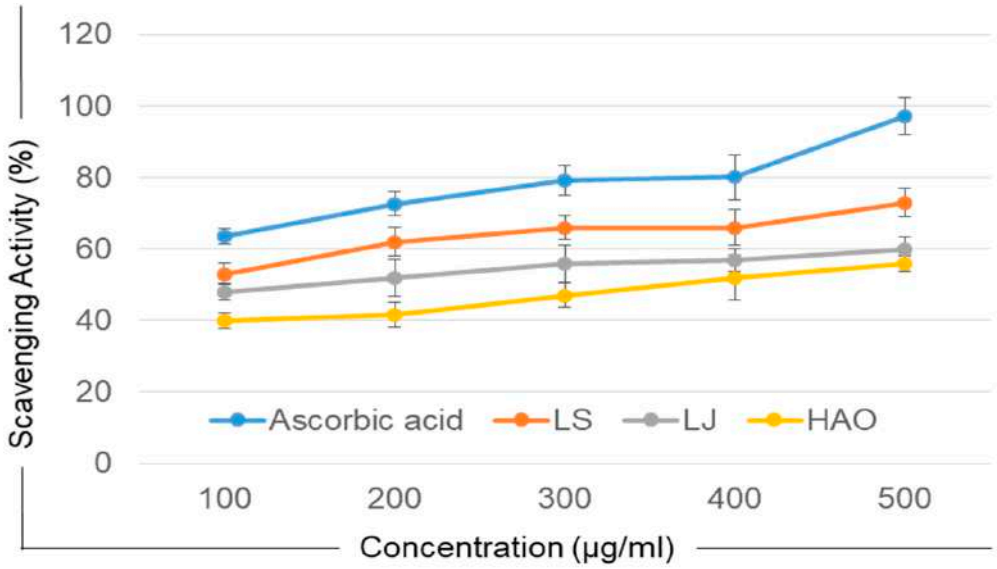

Figure 2. Free radical scavenging (antioxidant) activity using DPPH. The scavenging capacity of each dried sample extract (hot-air dried, lyophilized slurry, and lyophilized fruit juices) was assessed using DPPH. Ascorbic acid was used as a control. A high radical scavenging activity was observed in the lyophilized slurry compared to lyophilized juices or hot-air oven-dried samples.

The reducing power of all three extracts, along with ascorbic acid, is shown in Table 4. In the reducing power assay, the absorbance of the sample extracts reflected their reducing power or antioxidant capacity. The LS extract showed enhanced reducing power compared to the HAO and LJ extracts. An understanding developed from previous studies indicates that, in general, lyophilization methods provide effective preservation of chemicals and enhanced bioactivities, as compared to other methods that require dehydration-based high-temperature preservation and drying [39,44,45].

2.5. Anti-Proliferative Activity of Lyophilized Slurry (LS) Extract Assessed by MTT Assay

In vitro results indicate that the LS extract had a higher concentration of phytoconstituents and higher antioxidant activity than the HAO and LS extracts (Tables 2 and 4). Hence, we chose the LS extract to test the anti-proliferative (anticancer) activity using a gastric adenocarcinoma cancer cell line (MKN-45). Doxorubicin was taken as a control to compare the efficacy of the LS extract on MKN-45 cells. Two concentrations of doxorubicin (25 µg/mL and 100 µg/mL) and LS (10 µg/mL and 320 µg/mL) were used. As evident from the cytotoxicity assay, the LS extract showed considerable cytotoxicity with a 320 µg/mL concentration (23.33 ± 3.5% viability), which was similar to that of doxorubicin (22.11 ± 1.5% viability) at a much lower concentration of 100 µg/mL (Figure 3).

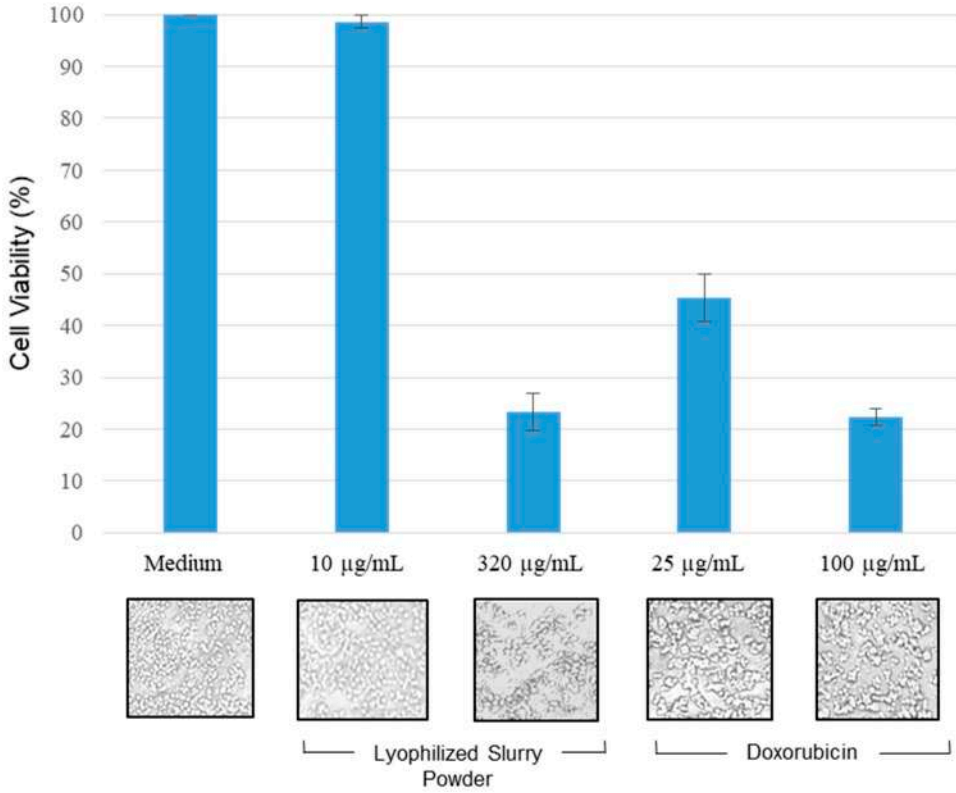

Figure 3. Inhibitory action (anti-proliferative potential) of lyophilized slurry (LS) extracts on MKN-45 cell line. MKN-45 cells were incubated for 24 h with LS at 10 µg/mL and 320 µg/mL concentrations and doxorubicin at 25 µg/mL and 100µg/mL concentrations. Percentage cell viability was tested using an MTT solution. The histograms depict the percentage viability of the MKN-45 cells treated with ethanol, LS extract, and doxorubicin after 24 h exposure to the above-mentioned concentrations. The data are presented as mean ± SD for three replicates.

Notably, previous studies demonstrated that single fruit extracts prepared by ethanol extraction had higher IC_{50} values than the combined fruit extract (LS) in our study. Ethanolic amla extract demonstrated an IC_{50} of up to 650 µg/mL tested against various cancer cell lines [46]. Similarly, Convolvulus pluricaulis leaf extract showed an IC_{50} value of approximately 1000 µg/mL in HepG2 and L-929 cell lines [47]. Nonetheless, some reports of the synergetic effect and the increased potency of combined extracts are available [48,49]. Due to the enhanced overall health benefit, recently many researchers have shifted their focus from an interest in extracted and purified single components to using combined fruit extracts for their antimicrobial, antioxidant, and anti-cancer properties [50].

Studies have also reported that mixing and blending bioactive fruits stabilizes the total phytoconstituents [51]. Recently, scientific research on combined extracts has shed light into their enhanced bioactivity. Hence, this study is among the few upcoming studies that can help to develop a better understanding of the combined fruit extracts and extraction methods aimed at enhancing the overall beneficial effects of fruits.

3. Materials and Methods

3.1. Sample Collection

Carrots, beetroot, tomatoes, and amla were collected from the local food market and authenticated by the quality assurance authority of Maharishi Dayanand University, Rohtak, Haryana, India.

3.2. Reagents

All chemicals, reagents, and solvents of analytical grade (AR) were purchased from Loba Chemicals Pvt. Ltd. Mumbai, India, Himedia (HiMedia Laboratories, Mumbai, India), CDH Fine Chemicals (Central Drug House (P) Ltd., Gujarat, India), and Merck (Branchburg, NJ, USA).

3.3. Drying Processes

Fresh ripe carrots, beetroot, amla, and tomatoes were purchased from the local food market. Each sample was washed, sliced into smaller pieces, and juiced independently before being dried through different methods. We prepared three samples, *viz.* lyophilized slurry extract, lyophilized juice extract, and hot-air oven-dried extract, using freeze-drying or heat-drying techniques. Two methods, slurry or juice, that were subsequently freeze-dried, were used. The sample collection, preparation, and testing methodology are explained in the flowchart (Figure 4).

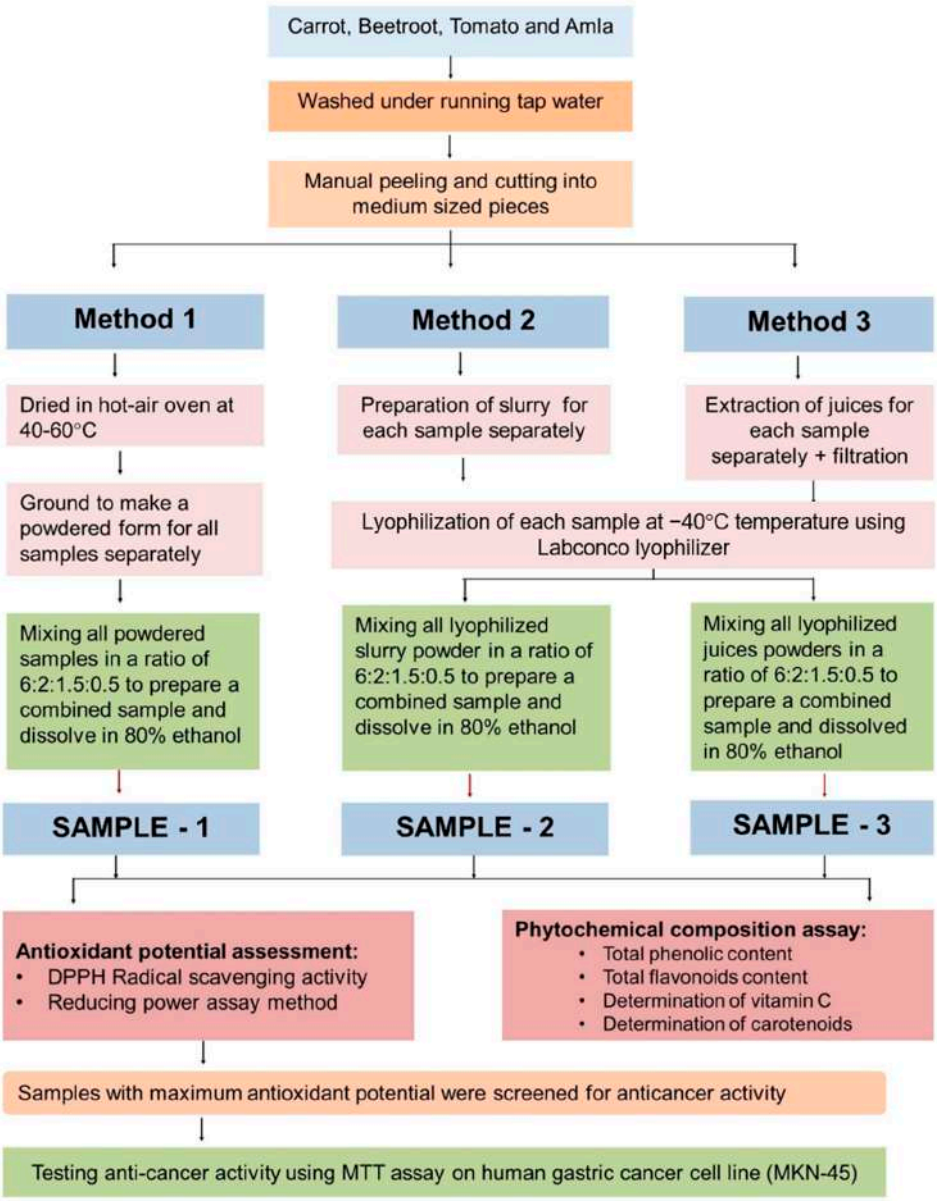

Figure 4. Flowchart of the methodology used in the study. The step-by-step methodology starting from sampling to sample drying, extract preparation, and sample testing, is shown in the flowchart.

3.4. Preparation of Samples

After adequately washing the beetroots, carrots, tomatoes, and amla under running water, the peels were removed from the fruits except for the amla and tomatoes. Amla was stripped of its seeds and chopped into medium-sized pieces.

3.4.1. Method 1 (Hot-Air Oven-Dried Powder Sample)

Each fruit sample was dried in a hot-air oven at 40–60 °C temperature, ground individually, and then mixed in a ratio of carrot (6 mg): beetroot (2 mg): tomato (1.5 mg): amla (0.5 mg). The samples were stored in an airtight container until further use (Figure 1). The temperature range for hot-air drying was set to ensure that fruits with high moisture content (tomatoes and amla) were dried completely, similarly to that of beetroots and carrots. The 40–60 °C temperature range has been optimized for drying all four fruits to a final moisture content of 0% [52]. Secondly, the ratio (carrot (6 mg): beetroot (2 mg): tomato (1.5 mg): amla (0.5 mg)) was also optimized as the highest in antioxidant potential among the three ratio combinations tested.

3.4.2. Method 2 (Lyophilized Slurry Powder Sample)

Each fruit sample was separately put into a grinder to produce a slurry. The slurries were lyophilized at −40 °C temperature using a Labconco lyophilizer. Lyophilized extracts were again mixed in the same ratio as mentioned in Method 1, i.e., carrot (6 mg): beetroot (2 mg): tomato (1.5 mg): amla (0.5 mg), and stored in an airtight container until further experimentation.

3.4.3. Method 3 (Lyophilized Juice Powder Sample)

Each fruit sample was separately put into a grinder to produce a slurry and subsequently filtered to obtain the juice. The fruit juices were lyophilized separately at −40 °C temperature using a Labconco lyophilizer. Lyophilized juice powders were mixed in the same ratio as mentioned in Method 1, i.e., carrot (6 mg): beetroot (2 mg): tomato (1.5 mg): amla (0.5 mg) and stored appropriately until further use.

3.5. Extraction

The three dried (powder) extracts—HOA extract, LS extract, and LJ extract—were prepared through an ethanol-based extraction procedure. A quantity of 100 mg of dried (powder) sample (prepared using method 1, method 2 or method 3) was soaked in 500 mL of 80% ethanol/water in tightly covered bottles and left for 24 h with occasional mixing. The ethanol extracts were centrifuged at 2500 rpm for 10 min at 25 °C and were subsequently filtered using Whatman filter paper No. 1 (Whatman International, Maidstone, UK). The samples were further evaporated to dryness at 50 °C using a rotary evaporator until concentrated extracts were obtained. Each extract was weighed and diluted with specific solvents (according to the solvent required for each experiment) to prepare different concentrations to be used for further experiments. The extracts were either used for in vitro studies immediately or stored under sterile conditions at −20 °C until further use (with slight modification from Lim, 2019).

3.6. Determination of Crude Fiber Content

Crude fiber content was determined for all three extracts using the standard procedure according to the Indian Pharmacopoeia 2018 (https://www.webofpharma.com/2022/04/indian-pharmacopoeia-2018-ip-2018-pdf.html, accessed on 7 October 2021) (Indian Pharmacopoeia, 2018) [53].

3.7. Determination of Total Phenolic Content

The phenolic contents (free and bound phenols) were analyzed by spectrophotometer using the Folin–Ciocalteu (FC) reagent method. All three dried extracts were dissolved in methanol, mixed with 125 µL of FC reagent, and left to stand for 6 min. After that,

we added 1.25 mL of 7% Na_2CO_3 solution, and the final volume was made up to 3 mL using distilled water [54]. The samples were left at room temperature for 90 min, and absorbance was noted at 760 nm using a UV/Vis spectrophotometer. The linearity reading of the standard curve was measured with gallic acid/100 g used as standard [55].

3.8. Determination of Total Flavonoid Content

A volume of 0.3 mL of 5% $NaNO_2$ solution was mixed with 1 mL ethanolic extract for all three samples separately. After 5 min of incubation, we added 0.3 mL of 10% $AlCl_3$ and incubated the mixture of samples at room temperature for 6 min. Then, we added 10 mL of 1 M NaOH solution. Finally, the reaction mixtures were left in a dark place for 15 min. We took 0.3 mL volumes from these solutions and measured the absorbance of the samples at 510 nm using a UV-Vis spectrophotometer. Quercetin was used as a control, and the results expressed in mg of quercetin equivalent (QE) per g of dried extracts [56].

3.9. Determination of Total Ascorbic Acid

A 0.005 mol L^{-1} iodine solution and 0.5% starch indicator solution were prepared separately to determine ascorbic acid in the prepared samples. A 20 mL aliquot of the sample solutions was taken into a 250 mL conical flask. Further, 150 mL of distilled water and 1 mL of starch (indicator) solution were added. The three samples were titrated using 0.005 mol L^{-1} iodine solution [57]. The endpoint of the titration was identified with the appearance of a dark blue-black color due to the formation of the starch–iodine complex [58].

The ascorbic acid concentration in the three extracts was calculated as follows:

$$\text{Ascorbic acid (mg)} = \text{M}_{\text{iodine solution}} \times \text{mL}_{\text{iodine solution}} \times 176.12\ \text{g/mol}$$

3.10. Extraction and Determination of Carotenoids

Two grams of the LS, LJ, and HAO extracts was dissolved in acetone and hexane (1:1) solution. Five milliliters of acetone was added to each 2 mL extract after specific time intervals, and this process was repeated two to three times. Each acetone-washed extract was filtered and collected separately into a beaker. The collected extracts were applied to a separating funnel, and a 10% NaCl solution in distilled water was passed over the extract through the separating funnel. The resulting solution was thoroughly stirred and kept aside for the separation of layers. Once the layers separated, the upper layer containing carotenoids was collected and treated to eliminate the water and NaCl (anhydrous) solution [59]. The absorbance of carotenoids in the obtained sample was recorded at 630 nm using a visible spectrophotometer, and the total amount of carotenoids in 100 g of each dried sample was calculated. Freshly made β-carotene standard solution of 2 mg/mL prepared in acetone was used as a standard.

3.11. 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was used to assess free radical scavenging activities of the extracts. In 0.1 mL of each ethanolic extract (LJ, LS, and HAO extracts), diluted in 0.8 mL DMSO, 0.1 mL of DPPH in methanol was added. After incubating the samples for 30 min, the absorbance was measured at 517 nm. Following this method, a lowering of the absorbance reading shows higher free radical scavenging activity. The % scavenging activity of DPPH was calculated by the following equation:

$$\%\ \text{Inhibition} = (\text{A}_{\text{control}} - \text{A}_{\text{sample}}/\text{A}_{\text{control}}) \times 100$$

where A_{sample} is absorbance of sample solution, $A_{control}$ is absorbance of control. DMSO was used as blank, ethanol was used as a control, and quercetin solutions were used as standards for the same run. The method is similar to previously established protocols [60,61].

3.12. Reducing Power Assay

One milliliter of each extract (LJ, LS and HAO) was combined with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution, followed by thorough mixing, and incubated for 20 min at 50 °C. After incubation, 2.5 mL trichloroacetic acid (TCA) was added, and the sample was centrifuged for 10 min at 3000 RPM. The supernatant was transferred into a different tube, and 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% Iron (II) chloride ($FeCl_2$). The absorbance of samples was observed at 700 nm against the blank (phosphate buffer) using a UV-Vis spectrophotometer. Ascorbic acid was taken as a reference standard, prepared similarly without the addition of a sample. An increase in the absorbance reading of the reaction mixture depicted an increase in the reducing power (Figure 2)

$$\% \text{ Reducing Power} = (1 - A_{sample}/A_{control}) \times 100$$

A_{sample} is the absorbance of the sample solution, and $A_{control}$ is the absorbance of control [18].

3.13. Evaluation of the Cytotoxic Activity of the LS Extract in Gastric Adenocarcinoma (MKN-45) Cell Lines by MTT Assay

The gastric adenocarcinoma cell line (MKN-45) was cultured under optimum conditions in DMEM media with 10% fetal bovine serum (FBS) and 5% CO_2 until a bilayer cell density was achieved. The bilayer was trypsinized using culture media without FBS, and a 5.0×10^5 cells/mL cell count was taken. One hundred microliters of diluted cell suspension (50,000 cells/well) was added to each well of the 96-well microtiter plate. After 24 h, a partial bilayer was reached. The supernatant was removed, and the bilayer was washed with culture media. The cells were then treated with 100 µL of different concentrations of the extract, diluted in DMEM medium. The plates were incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. After incubation, the sample solutions were removed from the wells, and 100 µL of MTT (5 mg/10 mL MTT in PBS) was added to each well. The plates were further incubated for 4 h. The supernatant was removed, and 100 µL of DMSO (dimethyl sulfoxide) was added to each plate well, which was gently agitated to dissolve the formazan formed by MTT. The absorbance was measured at a wavelength of 590 nm using a microplate reader [62]. The percentage growth inhibition was computed, and the dose–response curves were generated for each concentration of the extract to determine the concentration of the test sample required to inhibit cell growth by 50%. The concentration at which 50% growth inhibition was observed was considered the 50% inhibitory concentration (IC_{50}) (Figure 5).

Calculating the inhibition percentage

$$\% \text{ Inhibition} = [(\text{OD of Control} - \text{OD of Sample})/\text{OD of Control}] \times 100$$

The IC_{50} concentrations were used to test the inhibitory effect of the extract on MKN-45 cell proliferation (Figure 3).

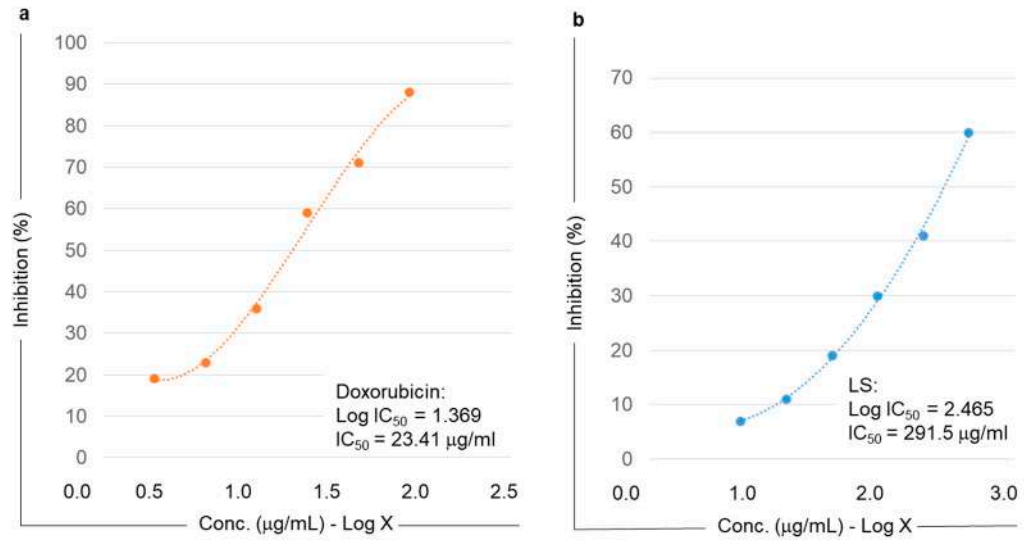

Figure 5. Calculation of IC_{50} values of the lyophilized slurry (LS) extracts. The sample extracts were tested for their cell proliferation inhibitory effect on human gastric cancer cell line MKN-45 using the MTT assay. (**a**) Doxorubicin was used as a standard and was assayed using the concentrations ranging from (0.5–2.5 µg/mL − log X), while (**b**) lyophilized slurry (LS) extract was tested in the range from 1 to 3 µg/mL (log X). The IC_{50} value was calculated using the standard formula.

4. Conclusions

The total phenolic, flavonoid, carotenoids, and ascorbic acid concentrations were much higher in the LS extract than in the LJ and HAO extracts. In the LJ extract, moderate concentrations of these antioxidants were present. The HAO extracts had very low ascorbic acid concentrations and total phenolic, flavonoid, and carotenoids contents. The LS extracts also demonstrated higher antioxidant and anti-proliferative activity, possibly attributable to higher total phenolic, flavonoid, carotenoids, and ascorbic acid contents retained during drying.

Finally, based on the findings of our study, we confirm that drying methods substantially impact the stability, activity, preservation, and concentration of antioxidant and therapeutic phytochemicals present in plant products. Hence, drying techniques should be carefully and efficiently used to maintain and protect the therapeutic capacity of dried plant extracts to obtain significant health and treatment benefits.

Author Contributions: M.C., V.G. and A.F. were responsible for composing the manuscript. V.G., G.Z., R.D. and A.F. were responsible for conceiving the experimental study design, analyzing the data, and editing the manuscript. M.C., A.F. and A.Z. analyzed the data, and produced the figures. M.C., V.G. and A.F. performed statistical analysis. B.S.A. and G.M.A. analyzed the data and edited the manuscript. All authors were involved in reviewing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research work was funded by the Institutional Fund Projects under grant no. IFPDP-83-22. The authors gratefully acknowledge technical and financial support from the Ministry of Education and Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: This study had no human subjects, and informed consent was not required.

Data Availability Statement: All data and materials used in this study are available from the corresponding author and will be provided upon reasonable request.

Conflicts of Interest: All authors declare no conflict of interest.

Sample Availability: Not applicable.

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PHARMACOGNOSTICAL ASPECTS OF *JASMINUM MULTIFLORUM*: A REVIEW

Preeti Garg[1], Vandana Garg[2*], Rohit Dutt[3]

Abstract

Jasminum multiflorum (Burm.f.) Andrews commonly known as Winter Jasmine got the top ranked position in Ashtanga Hridayama due to its incredible power of healing belongs to the family Oleaceae. It is widely distributed in subtropical parts of Himalayas and tropical parts of India. Ethnomedicinally its various parts are used to cure a wide range of ailments like ulcer, renal dysfunction, cephalagia, cardiac disorders, constipation, indigestion, inflammation, rheumatism and weakness of sight. The various secondary metabolites namely alkaloids, glycosides, flavonoids, steroids, terpenoids, irridoids, saponins, proteins and amino acids are substantiated in different parts of the plant. The pharmacological studies proved its efficacy towards antimicrobial, antioxidant, nemacitidal, antihelmintic, cardiovascular and central nervous diseases, analgesic, anti-inflammatory, cytotoxic, brochodialator activity upto now. This present work gives an eagle eye to the botanical information of plant as well as its scientific validations involving the distinct pharmacological and phytochemical benefits. Even this review highlights the lacking data and research gaps on this plant, which provides a platform for upcoming researchers for further studies on this species.

Key words:*Jasminum multiflorum*, Oleaceae, Botanical description, Phytochemistry, Pharmacological activities.

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DOI: 10.48047/ecb/2023.12.si6.695

INTRODUCTION

The treatment of ailments from ayurveda is renowned since Paleolithic time throughout the world. As the growth of the synthetic world in contemporary era is going on but still the part of modernity is unable to overcome the herbal medicines either due to their effectiveness or the spiritualness of the peoples towards them. The genus *Jasminum* is one of them, which is used by the people for their effectiveness as well by religiousness. This genera belongs to family Oleaceae possess almost 200 species. The plants of *Jasminum* genera are commonly shrubs or vines and inhabitant of tropical and warm temperate regions[1]. *J. multiflorum* commonly known as winter jasmine, which is an evergreen and twinner shrub with young branches dressed with velvety pubescence [2,3]. The plants possess white and pink flowers with sweet fragrance[4]. It is used as analgesic, relaxant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorants, diuretic, and also to treat conditions like indolent ulcers, amenorrhea, ringworm infection, leprosy, and skin diseases[5,6]. Jasmine is conspicuously valued plant used in home gardens as well as in commercial cultivation. The flowers and buds of the plant are used for making garlands, bouquets and for spiritual offerings[7]. The fragrance of the flowers attracted mushroomers of the attar industries for the production of perfumes, hair tonics and ittars[8,9]. The plant contains glycosides, irridoids,secoirridoid, secoirridoid lactones, secoirridoid glucosides, terpenoids, essential oils, flavonoids, sterols, alkaloids, tannins, carbohydrates, proteins and amino acids [5, 10,11].The biological studies reported in literature proved its potency and efficacy as antimicrobial, antioxidant, nemacitidal, antihelmintic, antimicrobial, GIT, cardiotropic, anti-inflammatory, and in central nervous diseases [10,11]. Moreover, plant still has to go through scientific screening to establish its efficacy

clinically. This review highlights medicinal and traditional uses along with phytoconstituents and pharmacological studies explored for this plant.

TAXONOMICAL CLASSIFICATION

Table No: 1 Scientific & Taxonomical classification [12,13]

Kingdom	**Plantae**
Subkingdom	Tracheobionta – Vascular plants
Division	Magnoliophyta- Flowering plants
Class	Magnoliposida-Dicots
Subclass	Asteriadae
Order	Schrophulariales
Family	Oleaceae
Genus	*Jasminum*
Species	*Jasminum multiflorum(Burn.f) Andr*
Synonyms	*Jasminum pubescens wild* *Nyctanthes multiflora (Burm.f) Andr.*

CLASSICAL NAMES

Kundah, Mahha (Sanskrit); Kundphul (Hindi); Dolor, Mogra,kundkagado (Gujrati); Kundamu, Gajari (Telgu); Malligai, Makarandam (Tamil); Kund (Bangla); Kunda (Marathi), Magimallige, Kasturimallige (Kanada); Downy Jasmine, Star jasmine, Musk jasmine (English)[14,15].

AYURVEDIC PROPERTIES

It is sara, madhura, sheeta, kashaya and beneficial in reducing kapha and pitta[16,17]

PLANT DESCRIPTION

J. multiflorum(Figure: 1); is a large, tomentose, scandent, branching, spreading evergreen plant that reaches up to a height of 3-10 ft. tall. The plant grows at an altitude of 1300 meter. It is widely cultivated in tropical and subtropical region of Southwestern, Southeastern, Asia, India, Nepal, China, Bhutan, Burma, Pakistan, Australia, USA, Philippines, Myanmar and Sri Lanka[19,20].

Figure 1: Plant of *Jasminum multiflorum(Burm.f.)*

Morphology [10,12,13,15,18]

a) Leaves

Leaves are 4-9 cm long, ovate, heart shaped, rounded base with pointed tip. They are opposite to each other on the stem. The downy pubescences are covered all over the stems and leaves which make the plant overall greyish green.

b) Flowers

Flowers are star shaped, 7-10mm in diameter, white, dense, clustered, with crowded head. The hairy calyx about 5-6 cm long with linear lobes are present in the flowers. A 1.5 cm long corolla tube possessing 7-9 lobes which are oblong having 1.5-1.8 mm in length.

CULTIVATION AND COLLECTION

J. multiflorum is cultivated in well sapped loamy soil having pH 6.0 to 7.5, but it can also be grown in black, laterite and clay soil. As the plant is highly prone to water logging a well managed drainage scheme is required during cultivation. It can be promulgated by layering, cuttings or root suckers method. The first flowering starts with in the year and harvesting is done in the months of March to May.

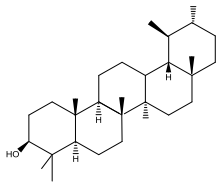
For the best yield, dead branches are removed by the end of January and bushes are pruned to almost half. After such treatment, when the temperature rises, farmyard manure is applied and irrigated to get new growths and flowering by the end of February or March[21].

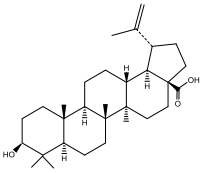
PHYTOCHEMICAL CONSTITUENTS

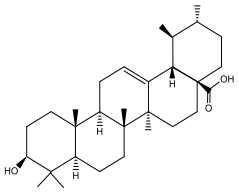
Literature revealed that the plant contains seco-irridoid, seco-irridoid lactone, seco-irridoid glucosides, and various forms of terpenoids and triterpenoids which have basic 10-hydroxyoleoside structure, derived from secologanin. The spectral analysis and chemical correlations established a novel bicyclic 2-oxo-oxepano [4, 5-] pyran ring system. The leaves contain friedelin, lupeol, botulin, α-amyrin, β-sterol, betulinic acid, ursolic acid, oleanoic acids (Figure: 2). Leaves and flowers reported to contain seco-irridoid lactones such as jasmolactones A ($C_{19}H_{22}O_8$), jasmolactones B ($C_{19}H_{22}O_9$), jasmolactones C ($C_{27}H_{32}O_{10}$), jasmolactones D ($C_{27}H_{32}O_{11}$), multiflorin, multifloroside, multifloricide, multiroside, jasmultiside, 10-hydroxyligustroside, and 10-hydroxyoleuropein [22-26].

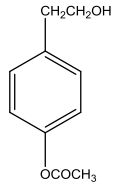
The oil from flowers contains highly aromatic compounds like eugenol,nrolidol, cadinol, jasmine, linalool, farnesol, β-farnesene, ethyl palmitate, methyl salicylate , benzyl alcohol, benzyl acetate, benzyl benzoate, hexyl benzoate, and indole [27]. A New compound, 2-p-acetoxyphenylethanol, has also been isolated from

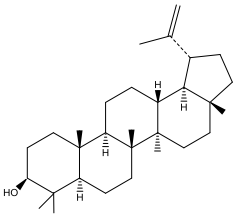
flowers of *J. multiflorum* along with long-chain saturated compounds n-tritetracontane and heptacosane [28].

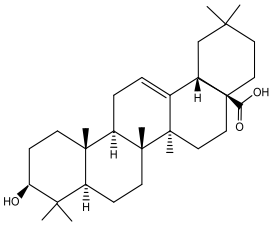

Alpha amyrin (1)

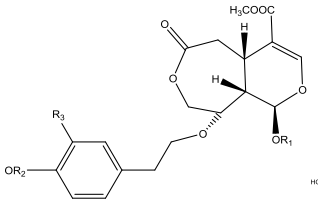

Betulinic acid (2)

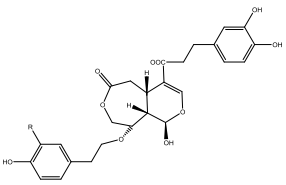

Ursolic acid (3)

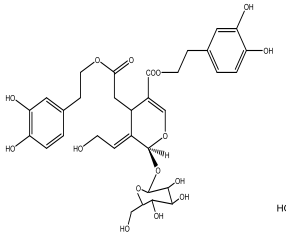

2-p-Acetoxy phenyl ethanol (4)

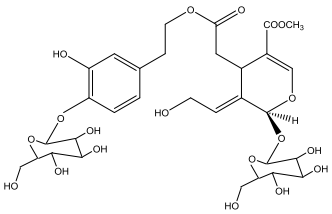

Lupeol (5)

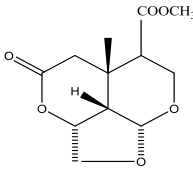

Oleanolic acid (6)

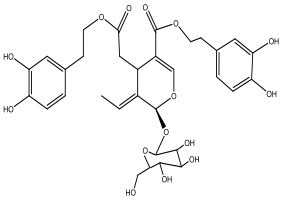

$R_1=R_2=R_3=H$; Jasmolactone A (7)
$R_1=R_2=R_3=OH$; Jasmolactone B (8)

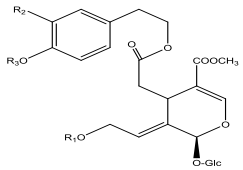

R=H; Jasmolactone C (9)
R=OH; Jasmolactone D (10)

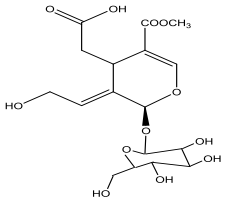

Multifloroside(11)


Multiroside (12)


Multiflorin (13)


Jasmultiside (14)


$R_1=R_3=H$, $R_2=OH$; 10-Hydroxyoleuropein (15)
$R_1=R_2=R_3=H$; 10-Hydroxyligustroside (16)


10-Hydroxyoleoside 11-methyl ester (17)

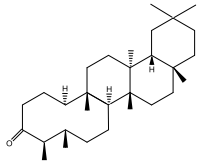

Fredelin (18)

Figure2: Chemical constituent reported from *Jasminum multiflorum*

USES OF PLANT

The whole Plant is used in healing of wounds and ulcers, constipation, flatulence, skin diseases, rheumatism, stomatitis, dysmenorrhea[12], coronary vasodilating and for cardiotropic activities[22-25].The leaves are used as antibiotics, diuretics, poultice, headache, rheumatism, skin diseases, allergy, itching, and inflammation[15], typhoid and in different types of ulcers[5,15].The flowers are acrid, alexipharmic, cardiotonic, detoxifying, digestive, emetic, laxative, and refrigerant, lactifuge, and herb of bath which fortifies the soul[29,30]. The root is used as antidote for snake bite, weakness of sight and emmenagogue (increase in menstrual flow)[2,10].Bark can be used to treat burns[17]. Also, the jasmine oils have various pharmacological properties like antioxidant, relaxant, analgesic, anxiety, antimicrobial, nemacitidal, and lactifuge[31-34]. Traditionally, it is used in cosmetic industry for making perfumes, oils and creams and also used as ornamental in form of bouquets and garlands[26].

BIOLOGICAL ACTIVITY

Antimicrobial Activity: The ethanolic fraction of the root of *J. multiflorum* at a concentration of 25mg/ml, 50 mg/ml and 100mg/ml for its antimicrobial potential by agar well diffusion method. The microorganisms selected for the estimation of antimicrobial activity were *Bacillius subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia.* Amoxicillin 100µg/ml is used as reference material.The extract showed maximum activity against *Klebsiella pneumonia* at100mg/ml with a zone of inhibition 0.5mm and for others it ranged from 0.3 to 0.4 mm[34].

The petroleum ether, chloroform methanolic and aqueous extracts of leaves of *J. multiflorum* for their antimicrobial efficacy against *Aspergillus niger, Scolorotium, Bacillus cereus, Escherichia*

coli, *Candida albican* and*Pseudomonas aeruginosa* at the concentration of 100-500 µg/ml. The result showed potent antimicrobial effect against *E. coli A. niger and C. albicans*. The assessment of antifungal activity and antibacterial activity was performed in terms of percentage of radial growth on saturated dextrose agar (SDA) and well diffusion method respectively. The methanolic extract showed a pronounced antimicrobial activity against the tested strains but less than to the standard drug Chloroamphenicol, Ampicillin and Streptomycin[35].

Antihelmintic activity:the antihelmintic potential of petroleum ether extract of aerial parts of *J. multiflorum* at a concentrarion of 5, 10, 25 mg/mL in normal saline containing 3 % Tween 80 and different extracts of roots of *Cocos nucifera* on *Pheretimac posthuma*. Piperzine citrate (15 mg/mL) and Albendazole (10 mg/ mL) were used as reference drugs. The inferences were made from the time taken to paralyses and/or death of individual worm up to 4 h of test period. The effect at 25 mg/mL concentrations was comparable with the reference standards [36].
It investigated the roots of *J. multiflorum* for anthelmentic activity. The petroleum ether extract was preapared and testedfor different concentration (5mg/ml – 50 mg/ml) against earthworm (*Pheretima posthuma*). The albendazole suspension (10mg/ml) was used as standard. The death and paralysis time were recorded and compared. Extract exhibit significant anthelmentic activity at (50mg/ml) concentration and found effective[34].

Nemacitidal activity: The aqueous extracts of flower *J. multiflorum* showed strong nematicidal activity against *Meloidogyne incognita* than *Mimosa* pudica extract on the hatching and larval stage[37].

GIT activity: Acetone, methanolic, ethanolic and xylene extracts of flowers of *Rosa indica*, *Tagetes*, *Bellisperennis*, *Gladiolus*, *P.tuberose*, *J.multiflorum* and *Gerbera* in the concentration of 50%, 75% and 100% by agar well diffusion method against different species of *Vibrio* (*V. parahemolyticus*, *V. alginolyticus*and *V. cholera*) causing gastrointestinal diseases. Ciprofloxacin 1000ppm was used as reference material. The acetone, methanolic and ethanolic extracts of flowers of all varities has higher efficacy against *Vibrio* species but less than Ciprofloxacin whereas on inhibitory effect was seen in xylene extracts[38].

Efects on Cardiovascular system: The coronary vasodilating and cardiotropic properties were exhibited by compounds like Jasmolactones A, B, C, and D, isolated from the aerial parts of plant[22].10-hydroxyoleuropein and multifloroside, which was isolated from the water soluble fraction of aerial part of the plant showed both coronary dilating and cardiotropic activities[23].

CNS Activity: The ethanolic extract of leaves of *J. multiflorum* for its antiepileptic activity by topically applied biculline (a model foracute simple partial epilepsy) and maximal electroshock(a model for generalized tonic-clonic seizure), motor coordination effect using rotarod treadmill, and anxiolytic activity by elevated plus maze methods in Sprague dawley rats. The drug was given by oral route or intraperitoneally and the standard used was diazepam (2mg/kg).The result showed that the extract has significant affect on acute partial complex epilepsy and considerable anxiolytic effect but negative response on motor co-ordination[39].

The anticonvulsant and hypnotics effects of ethanolic extracts prepared from the aerial parts of *J.multiflorum* on albino mice. The anticonvulsant activity of ethanolic extract in the doses ranging from 50, 75 and 100 mg/kg, i.p. was studied by Maximum electroshock (MES) and Pentylene tetrazole (PTZ) inducedseizure methods. In MES method, the extract was administered 0.5 h before application of electric shock.The duration oftonic hindleg extension was noted. The results revealed that the mice treated with ethanolic extract exhibited hindleg extension for 14.65 ±1.17, 18.35 ± 1.82 and 16.41 ± 1.41, respectively.
In PTZ induced seizure,the extract was administered 0.5 h prior to the administration of Pentylene tetrazole (80 mg/kg, i.p.). The onset of myoclonic spasm, incidence, nature and severity of convulsions and death/recovery were noted.Diazepam (2.0 mg/kg, i.p.) was used as a reference standard. The ethanolic extract significantly dose dependently inhibited the onset and incidence ofconvulsion. The mice treated with a dose of 50 mg/kg, i.p.exhibited seizures in 68.5%and all animals exhibiting seizures died within 0.5 h. No mortalitywas observed in the groups treated with 75 and 100 mg/kg even after 24h.
The general behavioural effects like righting reflex, pinna reflex, corneal reflex, awareness, grip strength, touch and pain responses on mice by conventional methods was studied using a dose of 25, 50, 75 mg/kg, i.p.Chlorpromazine (5 mg/kg,

i.p.) was used as a reference drug. The results showed depressed awareness and alertness, touch and pain responses, grip strength, altered righting, pinna and corneal reflexes in the mice treated with extract when compared to the control but less than standard.
The hypnotic activity was experimented by seeing the effect on sleeping time using the doses ranging from 35, 50 and70 mg/kg, i.p.The extract was administered 0.5 h prior to the administration of pentobarbitone sodium (40 mg/kg, *i.p.*),diazepam (3 mg/kg, *i.p.*) and meprobamate (100 mg/kg, *i.p.*). The sleepingtime was noted by recording the interval between the loss and regaining of righting reflex. The sleeping time was significantly increased in the extract treated animals[40].

Analgesic activity: The analgesiceffects of ethanolic extracts prepared from the aerial parts of *J. multiflorum* at a dose of 30, 40 and 50 mg/kg, i.p. by acetic acid induced writhing method. The number of abdominal constrictions (writhing) and stretching with ajerk at the hind limbs were counted between 5 and 15 min after administering acetic acid. The results were compared with acetyl salicylic acid (68 mg/kg), paracetamol (68mg/kg) andmorphine sulphate (1.15 mg/kg).
The animal treated with extracts significantly reduced the number of writhes and stretches. The percentage of protection was by 70.5 %. , 88.2 % and 100% respectively. The analgesic compounds acetyl salicylic acid (68 mg/kg), morphine sulphate(1.15 mg/kg) and paracetamol (68 mg/kg) gave 60.15, 70.12 and 61.43 %,protection, respectively [40].

Antioxidant activity: The antioxidant activity of hydromethanolic extract of leaves of *Jasminum multiflorum* usingβ-carotene-linoleic acid and Ferric reducing antioxidant power assays.The extract showed maximum inhibition activity at 75mg/ml concentration, 68.23±0.35 % inhibition and 60.30±0.60 for β-Carotene-linoleic assay and FRAP assay; respectively.The results showed that the leaves of *J. multiflorum* have antioxidant potential [41].

The antioxidant potential of methanolic extract of flowers of *J. multiflorum* by hydrogen peroxide scavenging assay. The concentration used for both the extract and standard (Ascorbic acid) was 20-60µg/ml. The results demonstrated that as the concentration increases the activity also increased. The IC_{50} was calculated and found to be 16.25[42].

Methanolic, ethanolic, ethyl acetate and aqueous extracts of leaves and flowers of *J. multiflorum* for its antioxidant activity by DPPH, ABTS and chelating potential methods. Total phenolic and flavonoid contents were also determined. The Ec_{50}results revealed that the ethanolic extracts of leaves and flowers has maximum antioxidantal potential (leaves 141.2 ±1.24 µg/ml & flowers 252.4 ±2.41 µg/ml in DPPH; leaves and 149.3 ± 2.41 µg/ml and flowers 101.4 ±2.35 µg/ml in ABTS and leaves 28.90 ±1.26 µg/ml and flowers 59.64 ± 0.98 µg/ml in chealting potential) as compared to other extracts of leaves and flowers, but more than that of ascorbic acid [43].
The total phenol and flavonoid content were found maximum (leaves 31.58 ±1.61 mg/g TPC and 13.54 ±0.69 mg/g TFC and Flowers 25.98 ±1.32 mg/g TPC and 11.89 ± 0.61 mg/g TFC) in the ethanolic extracts of leaves and flowers as compared to other extracts. But in comparison to leaves and flowers, the leaves have more potency.

The antioxidant activity of methanolic extracts of flowers. The extract was tested by DPPH free radical (2, 2-diphenyl-1-picrylhydrazylhydrate).The results showed that the oxidation was effectively inhibited by the extract with IC_{50} 81µg/ml. The positive control used was butylatedhydroxyl toluene (BHT) having IC_{50} 12.5µg/ml[44].

Anti-inflammatory activity: The anti-inflammatory activity of hydromethanolic extract of leaves of *Jasminum multiflorum*by histamine release assay.The tested extract possessed anti-inflammatory activity with IC_{50} 67.2 µg/ml[41].

Cytotoxic activity: The cytotoxic effect of hydro-methanolic extract of leaves of *Jasminum multiflorum.* Cytotoxicity was performed using a neutral red uptake assay towards breast cancer (MCF-7) and colorectal cancer (HCT 116) cell lines.*J. multiflorum* showed high cytotoxic activity with IC_{50} of 24.81 µg/ml and 11.38 µg/ml for MCF-7 and HCT 116 cell lines, respectively.

Bronchodilator activity: The bronchodilator effect of methanolic extract of leaves of *J. multiflorum* on goat tracheal tissue. The histamine (2000µg/ml) with doses of 0.1ml, 0.2ml, 0.4ml, 0.6 ml, 0.8ml and 1.6mlinduced contraction of isolated goat trachea were recorded in absence and presence of methanolic extract(100 &200µg/ml). Chlorpheniramine Maleate (10g/ml) was used a standard drug.
Similarly, the bronchodilator effect of acetylcholine (2000µg/ml) was measured on a tracheal strip with doses of 0.1ml, 0.2ml, 0.4ml, 0.8ml, and 1.6ml in the absence and presence of

methanolic extract of "*Jasminum multiflorum*" L (100 & 200µg/ml). The standard drug used was aminophylline (10g/ml). The methanolic extract showed the response in dose dependent manner [45].

CONCLUSION

Herbs are striding in the fields of medicine to treat various ailments due to their lesser undue effects. Conscientious survey of literature showed that *J.multiflorum* is one of the most popular remedy even in the ancient era due to the presence of pharmacologically active secondary metabolites. Upto now only few compounds have been substantiated from this plant by various phytoresearchers and others still awaiting. Pharmacologically a little work has been done on this plant by exploring for its antimicrobial, antioxidant, antimicrobial, antihelmintic, nemacitidal,gastrointestinal, CNS, and cardiovascular system related activities,analgesic, anti-inflammatory, cytotoxic, Brochodialator activitybut many other biological studies are yet to be explored. The assiduous literature study reveals that a detailed research is required with the aim to explore the hidden treasure of secondary metabolites owing various pharmacological activities of *J .multiflorum* which will provide a smooth path for future researcher.

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Authored by

Dr.Pinki Gupta, Associate Professor Department of Commerce, GMN College, Ambala Cantt

vol. 53 No. 4 (I)

in

Anvesak A bi-annual Journal

UGC Care Group - 1

ISSN : 0378 - 4568

January – June 2023

Impact Factor: 6.20

Editor
Anvesak
A bi-annual Journal

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ABSTRACT

Recent years have seen a surge in interest in the study of the dynamic link between savings and investments, particularly in developing nations like India. Long-term economic development has been attributed to savings and investment; two crucial macroeconomic forces with microeconomic roots that help maintain price stability and increase employment opportunities. It is very crucial to spot the investment preference of investors to estimate the future of market and economy. In the present study, we have explained the preferences of household investors from traditional investment avenues to market-based investment avenues. The results show that the household investors are now more inclined towards the market securities in comparison to the conventional investment options.

Keywords:*Investors' preferences, household investors, savings and investment, Indian investors*

INTRODUCTION

To achieve targeted economic development and dynamic capital market stability, a nation's internal resources' savings and investment propensities are crucial, but understanding aggregate propensity alone is insufficient. The structure of saves and investment, the destinations for investments, investor and saver characteristics, as well as their attitudes and motivations, are all important to understand. Since India's independence, the significance of investments and savings in accelerating its economic growth has been underlined.

The market is supported by a pool of cash supplied by households and individual investors. Household income, consumption, and distribution are therefore essential elements of any economic research. These variables affect the kind and pace of saving as well as the rate of economic growth in a particular economy. Continued study on this subject is necessary to comprehend the savings and capital creation trends in our nation. Both the 1996 Micro Impact of Macro and Adjustment Policies in India (MIMAP) study and the in-depth "Household Savings and Investment Behaviour in India in 2003" report from EPW Research Foundation and NCAER were attempts in this direction. These studies examine India's saving behaviour in great depth, yet they fall short.

An investment, in the words of K.K. Bajaj (2009), "means the purchase of money-related instruments that provide a yield that is exactly commensurate to the risk embraced over some future endeavour term." The Reserve Bank of India's data have also shown that the household makes up the largest portion of the nation's gross domestic investments. The giving up of present desires in favour of unsure future advantages is another definition of investment. It also indicates that there is a logical structure to this type of decision-making.

According to a long-held theory about the macroeconomic mechanisms behind growth, higher savings, when transformed into profitable investment, would spur economic expansion (Harrod, 1939; Domar, 1946; Lewis, 1954; Solow, 1956). Since the induced rise in income raises savings, which in turn causes an increase in investment, an increase in the savings rate improves steady-state production by more than its direct effect on investment. Endogenous growth theories have supported the notion that the basic cause of long-term economic expansion is the accumulation of physical capital since the middle of the 1980s. (Barro, 1990; Romer, 1990; Lucas, 1988).

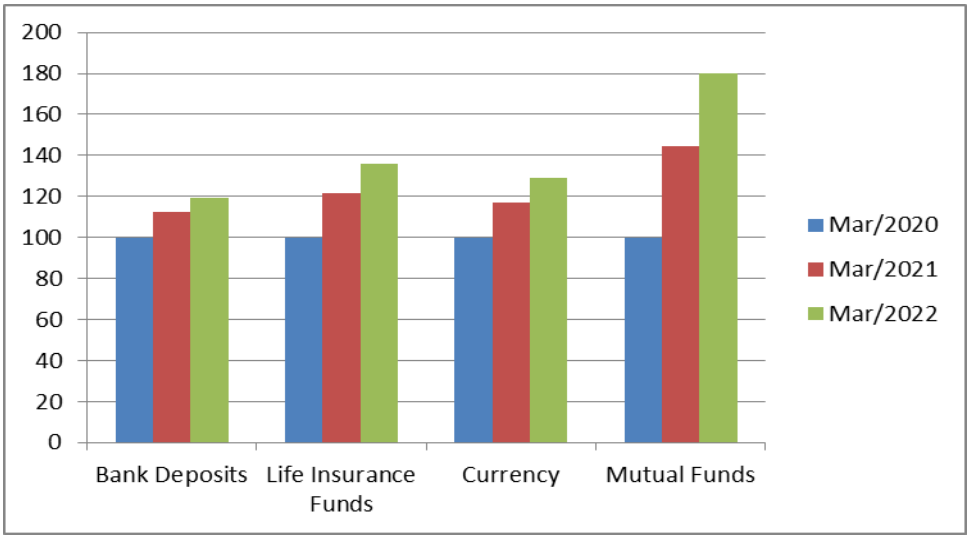
The composition of assets of household in India can be understood with the help of RBI data

Table 1: Stocks of Financial Assets of Households(Amount in Crore)

	Mar/2020	**%**	**Mar/2021**	**%**	**Mar/2022**	%
Bank Deposits	9696674	100	10916584	113	11570494	119
Life Insurance Funds	3884772	100	4718718	121	5287980	136
Currency	2232261	100	2614237	117	2883904	129
Mutual Funds	1197093	100	1730461	145	2152141	180

Source:RBI database

Figure 1: Change in the composition of assets of household in India



It is clear from figure 1 that the household investors are shifting their investments from safer investment options towards market-based investment avenues. This may be due to the increasing awareness level and ease of investment in modern options.Besides, the safer investments are treated as less earning in comparison to market-based options. However, each of these investment vehicles has its own set of advantages and disadvantages. Some of these options are liquid and simple to market, while others are not. Compared to other options, some are more dangerous than others.

THEORITICAL BACKGROUND

The average Indian household has a net worth of 5% in financial assets, 11% in gold, and 84 percent in real estate, according to a 2017 study by the RBI's Household Finance Committee. People are shifting their investments away from counterproductive assets like gold and towards other possibilities. Since November 2016, investors have started moving their money away from ineffective assets like gold and towards other options (Business Standard, 2017).

With the exception of certificates of deposit and post office savings, individuals in the high financial literacy group (HFLG) show greater levels of knowledge for all financial products. Additionally, members of the low financial literacy group (LFLG) mostly favour investing in conventional, secure financial goods rather than complicated financial products, which are more likely to include risk but have the potential to provide larger returnsMarwan(2016).

Rajarajan (1999) investigated investors in Chennai and discovered that the stage of an individual investor's life cycle is a crucial factor in influencing the quantity of financial asset investments and the proportion of risky financial assets.

Bank employees perceived insurance as an investment opportunity as opposed to a tool for risk reduction (Lalit Mohan, 2010). It has been noted that investors prefer gold to banks when looking for a secure investment, investors choose insurance investments because they provide stability (Sasi Kumar, 2010).

The SEBI - NCAER Survey (2000) was conducted to determine the number of households, the population of individual investors, their economic and demographic characteristics, the size of their portfolios, and their preferred investment strategies for both stock and other savings vehicles. Since information was gathered from 3,00,0000 geographically separated rural and urban households, this research of Indian investors is both unique and thorough.The following are a few of the study's important findings: Households choose

investments that are in line with their risk tolerance; bank deposits are popular with investors of all income levels; 43% of non-investor households, or roughly 60 million households, appear to be unaware of stock markets; and, in comparison to low income groups, higher income groups have a higher share of investments in mutual funds (MFs), indicating that MFs have yet to establish themselves as the go-to investment option for novice investors.However, the analysis indicates that household investment in MFs is projected to rise over the following two years.

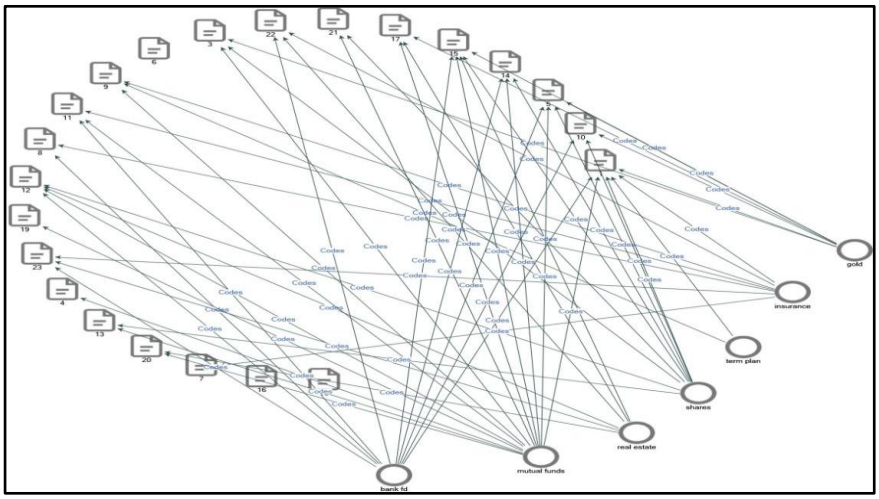
According to an investor preference survey, the fact was provided that the link between investment and the elements taken into account when making investments. In general, as investment risks grow, investors seek bigger returns to make up for the increased risk. There are several components as well as interactions between them. These factors help us to guarantee safety, liquidity, capital growth, and tax benefits in addition to rewards(Shrotriya,2019).

DISCUSSION

I have taken a sample of 650 respondents of which 40 people did not give the response while 34 responses are incomplete and 48 responses were invalid, so only 528 respondents are considered for this study. The structured questionnaire is used to collect data from respondents. Cronbach's alpha was used which gives the test value .898. I conducted pilot testing on 122 responders once the items were approved by experts.

Looking into the well-known possibilities available to investors is the first step in determining which investment vehicle is the most popular. Informal questions were posed to the respondents to gather more about their preferred investment vehicles.

Figure 2: Preferred Investment Vehicle for household invetors



The project map (Figure 2) created using NVIVO 12 Plus software displays the investment vehicles that investors choose to use when making investments. The interviews are represented by the rectangles and the interviewees are represented by the figure's circular nodes. Given that the majority of respondents are linked to the nodes for mutual funds and bank FDs, the figure implies that equity shares, insurance, bank FDs, and mutual funds are the most popular investment vehicles for investors. Given that just two and four respondents, respectively, are connected to these nodes, term plans and gold are the least desired investment option. Some of the people also have access to real estate. According to the research that are currently available, bank deposits (savings and current), insurance policies, provident funds, and securities (shares, debentures, and bonds) are India's most important investment vehicles, followed by mutual funds and derivatives. Risk, yield, tax shelter, marketability, and convenience are the five main criteria that determine an investor's selection (Skinner, 1988).

Now the research question arises

Whether all the investment options are equally popular among the investors?

We are framing an alternate hypothesis to check whether all the investment options are equally popular among the investors.

Ha: All the investment vehicles are not equally popular among household investors.

For the purpose of analysis and testing the hypothesis, Friedman’s two way analysis of Variance by ranks test is used.

Figure 3: Related samples’ Friedman’s two way analysis of Variance by ranks test

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Bank, Postoffice, PF, Insurance, Comm_paper, Govt_sec, Equity, Mutualfund, Realestate, GDR and Derivatives are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Figure 3 shows the result of Friedman’s test for two way analysis of variance. This test shows the results that the investment vehicles do not have the same preference level for the investors. As $p<.05$, null hypothesis has been rejected and it is concluded that all the investment vehicles are not equally popular among investors.

Preference Status in Different Investment Vehicles

It is observed from the above discussion that the investors are not interested to invest equally all the investment options. We can find the clearer picture with the help of the data collected from the respondents regarding their preference

Table 2: Preference status in Investment Vehicle

	Bank	%	Insurance	%	Equity	%	Mutual	%
Not at all preferred	23	4.4	28	5.3	75	14.2	69	13.1
Not preferred	40	7.6	64	12.1	74	14	81	15.3
Neutral	80	15.2	108	20.5	150	28.4	128	24.2
Somewhat preferred	197	37.3	182	34.5	127	24.1	128	24.2
Strongly preferred	188	35.6	146	27.7	102	19.3	122	23.1
Total	528	100	528	100	528	100	528	100

Table 2 depicts the household investors' preferences for various investment vehicles. With 35.6% of respondents, bank deposits are the most favoured investment vehicle, insurance policies (26.7%). With this data it can be inferred that bank deposits are the most popular conventional investment vehicle among investors. With 23.1% of respondents, mutual funds are the most favoured investment vehicle and equity shares are preferred by (19.3%) investors under study. In short, it can be concluded that mutual funds are the most popular investment vehicle among investors now a days.

Figure 4: Figure showing preference status in Real Estate and Market-based Investment Vehicles

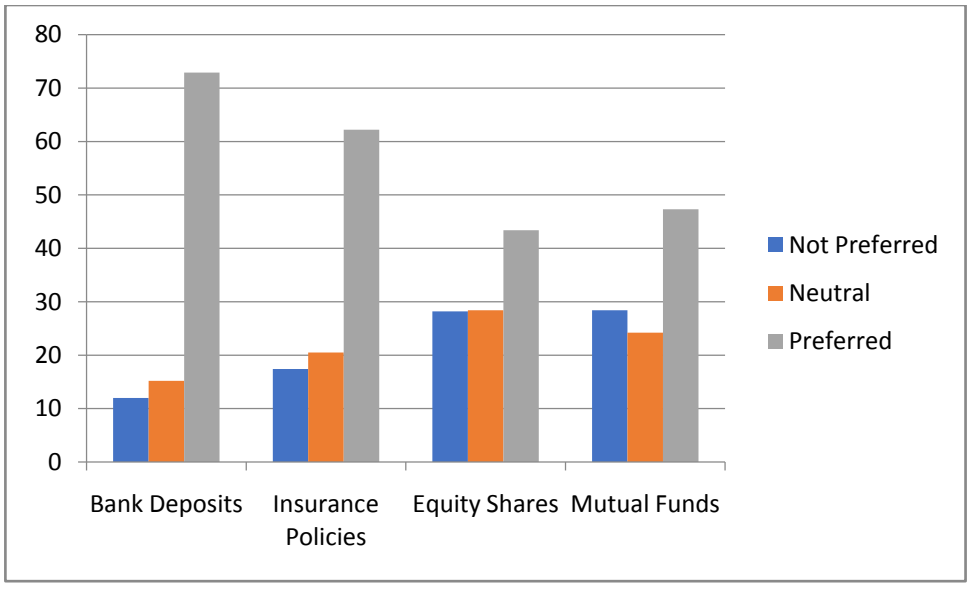


Figure 4 depicts the picture of preference of household investors in conventional investment vehicles. Bank deposits are the most preferred investment vehicle as more than 70% of the respondents are favourably responding. Insurance policies are also responded favourably by more than 60% respondents. Figure 4.13 depicts household investors' preferences. Mutual funds are the most popular investment vehicle, with more than half of the respondents favouring them.

CONCLUSION

According to the study, traditional investment vehicles like bank deposits and insurance policies are more common than market-based products like equity shares and mutual funds. The findings correspond to the literature that is currently in existence (Samudra and Burghate, 2012; Kumar and Thimmaiah, 2015). The investment instruments that investors choose to use are significantly influenced by their financial literacy. The home investors are reallocating their investment portfolios as a result of the COVID-19 outbreak changing their risk attitude. The three best investing options are insurance, mutual funds, and bank FDs. The relevant studies (Heo et al., 2020; Schroders' Global Investors research, 2020; Mushir&Suryavanshi, 2021) lend credence to the findings. In the midst of the COVID-19 epidemic, insurance plans are the most popular type of investment. The study's result with regard to insurance plans is also supported by the literature that is currently available (Riyazahmed, 2021; Mushir&Suryavanshi, 2021).

All regulatory entities should raise public awareness of investing techniques. It is important to establish small investor awareness activities and camps. It is suggested that the government take action to educate kids about money at an early age. The findings will have significant ramifications for creating curricula in the emerging subject of behavioural economics and finance as well as for defining job profiles and job descriptions in this sector.
More participants and financial products are required for the successful mobilisation of investors' funds in order to meet their needs. Investors should be aware of their psychological tendencies and financial objectives before to selecting any investment vehicle on the financial market. To measure their biases and make informed judgements, they must acquire certain impartial techniques.

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International Journal of
Multidisciplinary
Educational Research

ISSN: 2277-7881
JISRAF Impact Factor: 8.017
Index Copernicus Value: 5.16
International Scientific Indexing Value: 2.286
Published by: Sucharitha Publications
Visakhapatnam -530 003, Andhra Pradesh – India
Email: victorphilosophy@gmail.com
Mob: 09247782851

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Dr. K. VICTOR BABU
Editor-in-Chief
International Jornal of Multidisciplinary Educational Research-ISSN: 2277-7881
Visakhapatnam-530 003, A.P-India

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Social Sciences, Humanities, Commerce & Management, Engineering & Technology, Medicine, Sciences, Art & Development Studies and Law
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DOI: http://ijmer.in.doi./2023/12.03.58
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INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
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Article Received: 2nd March 2023
Publication Date:10th April 2023
Publisher: Sucharitha Publication, India
Digital Certificate of Publication: www.ijmer.in/pdf/e-CertificateofPublication-IJMER.pdf

A STUDY OF CONSUMER BUYING BEHAVIOUR IN TELECOM SERVICE PROVIDERS IN HARYANA

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ABSTRACT

Marketers' success depends on the dynamic nature of consumer behaviour as it is very critical to understand. Marketing researchers seeking data in a market area may find a wide range of quantitative and qualitative information about an individual sector market. By learning how and why consumers choose any product or service, marketers can identify which products or services people want and which they don't want. It helps the service providers to understand the consumer's needs, aspirations, expectations, problems, etc. The present research deals with the demographic profile of the consumers of wireless telecom service providers in Haryana and how it affects their buying behaviour.

Keywords: channels, education, expenditure, promotion, value-added-services, etc.

INTRODUCTION

Consumers play a vital role in the success or failure of any business. They drive revenues for the business.Consumerr data plays a vital role in the development of an effective business strategy. One can understand the background characteristics of the population under study. Demographic data of the consumers is used by the business to understand their characteristics as they affect their buying decision. It is the statistics about the population in a particular geographical area. It explains the socio-economic profile of the population. In modern times, it is used in association with behavioral data to analyze consumer dynamism and it helps in taking valuable conclusions on new market initiatives. When a new product or service is launched by any organization, demographic data analysis becomes an inevitable part of the market study. We cannot think of a market analysis

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without analyzing the demographic variables. Demographic information helps service providers to target marketing campaigns more precisely and to track how society is changing. The study of consumer behaviour in marketing helps service providers to operationalize the marketing concept, to frame production policies and price policies, shorter their product life cycle, effective market segmentation and to avoid product failure chances. It also helps in making adjustments with changing environment and also in taking decisions regarding sales promotion.

REVIEW OF LITERATURE

Gunasekaran et. al., (2007) in their study on Emerging Wireless Technologies for Developing Countries, on rural telecom in North Indian States found that a number of factors including the characteristics of the rural areas, difficult topography, low population density, and spread-out population and climatic conditions made it difficult to provide telecommunication services of acceptable quality by traditional means at an affordable price.

Eshghi Abdolreza et. al. (2008) in their research found that if a service provider builds and maintains a positive corporate reputation, it encourages service recommendations to others. Transmission quality consisting of network quality and convenience variables was also found to impact customer satisfaction, repurchase intention, and recommendation of services to others.

Venkatram Rahul et. al., (2012), analysed the professionals who worked or had worked in the telecom industry in China or India. It was found that the number of subscribers did not directly contribute to the revenue of the telecom industry. Further Revenue from the telecom industry had a direct impact on government regulation and policies in China but not in India.

Uddin Mohammed Belal et. al., (2012) in their study attempted to recognize the influencing factors of customer satisfaction and post-purchase intentions. It was found that there was a positive influence of service quality and fair price on the value perception of customers of mobile phone service operators. And as a result, perceived value also had a positive influence on customer satisfaction. Thus, perceived value performed mediating roles between service quality, fair price, and customer satisfaction.

Khan Yasser et. al. (2012) in their study investigated the buying behavior of customers toward Telecom services to determine customer preferences toward service providers. It was found that all variables including Promotion, Quality of Service, User-friendly, Relative Advantage, and

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Enjoyment had a positive relationship with consumer perception. It was also observed that Telecom services reduce overall cost, and time of operation for contacting overseas partners and provide opportunities in order to communicate with people around the world.

Acharya Neeta (2014) conducted a research work to study the factors/ dimensions of service quality that were significantly contributing to overall service quality perceptionsIt was found that the Indian cellular mobile service industry was not fully satisfying its customers. The Indian cellular mobile service industry gives less importance to customers in terms of convenience, tangibles, assurance and value-added services, etc.

Vijay P. et. Al. (2016), in their research paper examined the preference of mobile customers towards the mobile network service providers in Coimbatore city. It was found that service quality, Value added service and Customer care service was more preferred and the least preference was given to Promotional offers.

Rajasekaran Revathy et.al. (2018), in their study attempted to explore the key factors which motivated consumers to purchase and use Smart Phones. They also found the reason behind the usage of smartphones and identified the customer attitude towards smartphones. Product features, price, peer groups, and brand image were found as four key factors which influenced the usage and purchase decision of smartphones.

Kumar Anil et. al. (2020) examined the impact of trust, social commerce construct and perceived usefulness on purchase intention among Indian students. It was found that three imperative factors, i. e. trust, social commerce construct, and perceived usefulness had significantly & positively affected the purchase intention. The study proved that there was a direct and significant effect of consumers' trust in product recommendations on purchases. A Positive relationship was found between social commerce and purchase intention.

RESEARCH METHODOLOGY

The present study is descriptive and exploratory in nature. The population under study is the population of Haryana state. Information was collected by using primary as well as secondary data. The data were collected from a questionnaire that were got filled from 568 consumers in Haryana mainly from three districts namely Gurugram, Ambala, and Panchkula. Secondary data was also

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collected from the Telecom Regulatory Authority of India (TRAI) website, the work done by the researchers in the past, and various journals, research papers, newspapers, and other sources.

OBJECTIVES OF THE STUDY

To study of consumer buying behaviour in telecom sector service providers in Haryana.

DATA ANALYSIS AND INTERPRETATION

The demographic profile of the consumers in telecom service sector of mainly Airtel and Jio has been analysed in Haryana state. A number of factors have been analysed as depicted in table 1.1:

Table 1.1 Variables under study

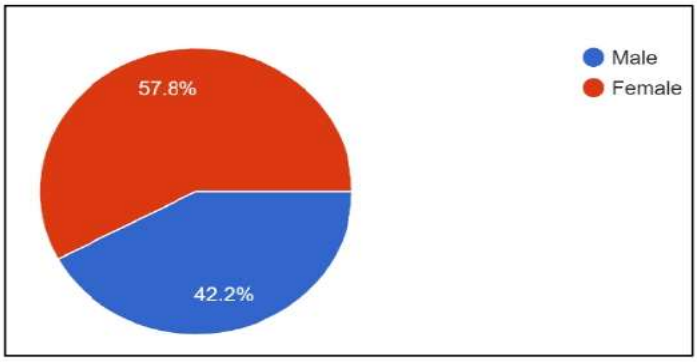
Independent	Dependent
➢ Gender ➢ Age ➢ Marital Status ➢ District ➢ Background ➢ Educational Qualification ➢ Occupation	➢ Number of Sims used:1,2,3or more ➢ Selection of a telecom service provider: Jio, Airtel, Both ➢ Time period for using the services of Telecom Service Provider ➢ Channel preferred to buy telecom ➢ Average amount of spending for recharge

PROFILE OF THE CONSUMERS

The demographic profile of the consumers based on gender, age group, marital status, district, background, educational qualification, and occupation was analyzed. The association of these demographic variables has been analysed with the dependent variables mentioned in table 1.1.

1.1 Gender Wise Telecom Users

Gender is the basic factor out of all the demographic factors that affect consumer buying behavior resulting in the promotion mix of the service providers. The perception of consuming goods and services differs with the difference of gender. Males and females tend to have different choices while



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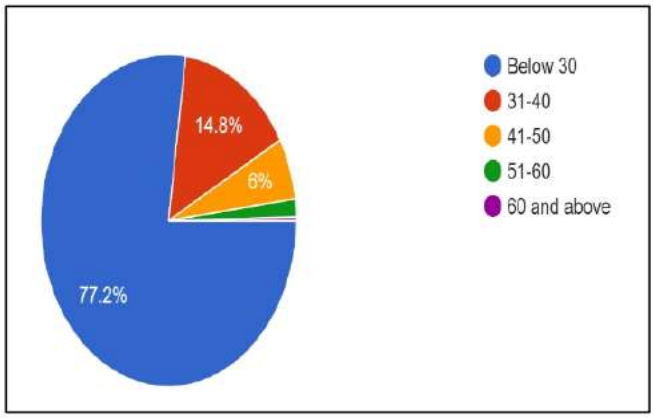
selecting the services because of their upbringing and socialization differences from both male and female consumers have been analyzed so that the results may represent the whole society.

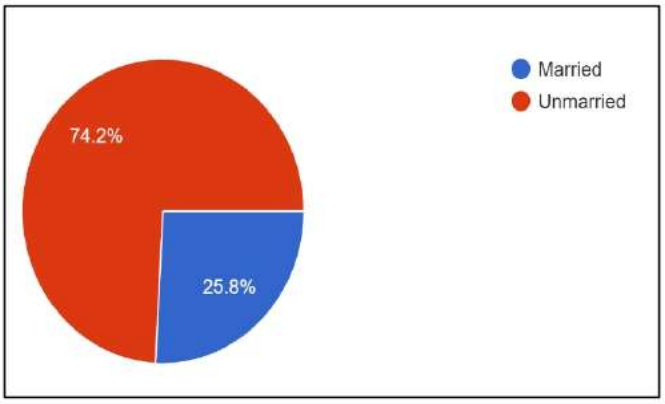
1.2 Age-Wise Telecom Users

Age plays a significant role in the selection of products and services. It creates a critical difference between consumer choice and consumer consumption habits and patterns. All the age groups take their decision based on their own experience and mindset. Every age has its state of mind, perception, and characteristics. Responses of consumers of all age groups have been taken into consideration for the present analysis.



1.3 Marital Status Wise Telecom Users

Product/ service preferences are influenced by the marital status of the consumers and their priorities. People of different marital statuses can respond differently to the various attributes of the goods and services. Single individuals' priorities are themselves and their own needs while married couples prioritize their family and their homes. So, knowing the consumers' marital status can guide not only the promotion of products and services which are marketed to them but the language and overall message of the campaigns. Considering all these facts, both married and unmarried respondents have been taken into account.



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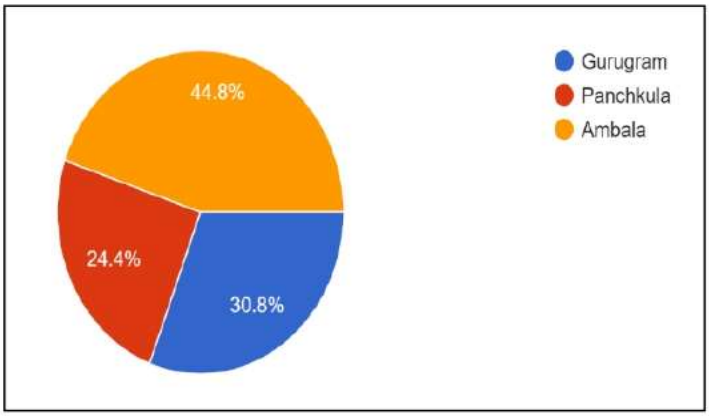
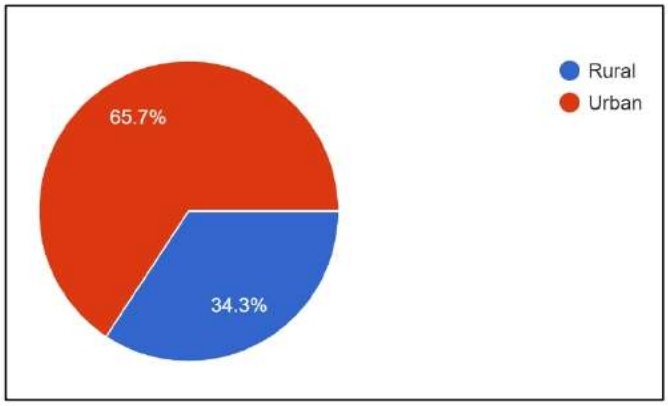
1.4 District Wise Telecom Users

Geographical mobility is an important characteristic of the population that is used in the process of the segmentation of services marketing. A change of location causes changes in the preferences of potential consumers depending on the geographical determinants of the place of their residence. Consumers of different districts experience different services due to different network towers, media coverage, and value-added services, etc. Besides these factors, consumers from other districts and states settled in these districts also have an impact on the selection of services.



1.5 Background Wise Telecom Users

The Indian rural market is larger than the urban market. People regardless of residing in a city or village purchase the products/ services and consume them. So, it becomes necessary to study both the consumers as the basis of the promotion mix for any service provider and the service providers cannot afford to ignore any of the consumers. A rural customer is very conscious of "value for money", and he does not trust outsiders easily. It is not very easy to convenience a rural customer in comparison to urban consumers. It is a challenge to introduce anything new to rural customers while urban consumers are more innovative, and accept new products easily. Rural customers are more brand loyal than urban customers. However, as the literacy level is low in rural areas, they recognize the brand more through its basic features such



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DOI: http://ijmer.in.doi./2023/12.03.58
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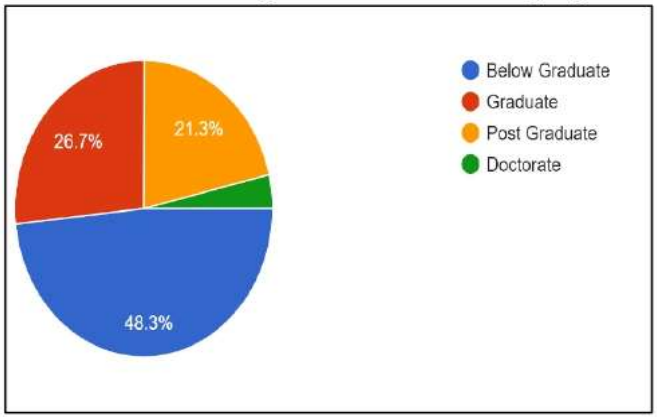
as colour, symbol, and logo. So, information from consumers of both rural and urban backgrounds has been collected.

1.6 Education Wise Telecom Users

Education is a powerful influence on the buying behavior of consumers. The level of literacy provides marketers with opportunities to sell sophisticated products and services. As the population of society gets more educated it becomes more sophisticated in its buying behavior. The relationship between educational qualification and the need for recognition of consumers would further help the service providers to understand the target group and evolve marketing strategies to make the consumers satisfied. Therefore, the opinion of consumers from different educational qualifications has also been observed.



1.7 Occupation Wise Telecom Users

The occupation of an individual influence his/her buying decision resulting in an effect on the promotion mix. An individual's designation and the nature of work influence his buying decisions. There is a direct influence of an individual's nature of job on the products and brands he/she picks for himself/herself. So, data has also been collected from the consumers having different occupations.

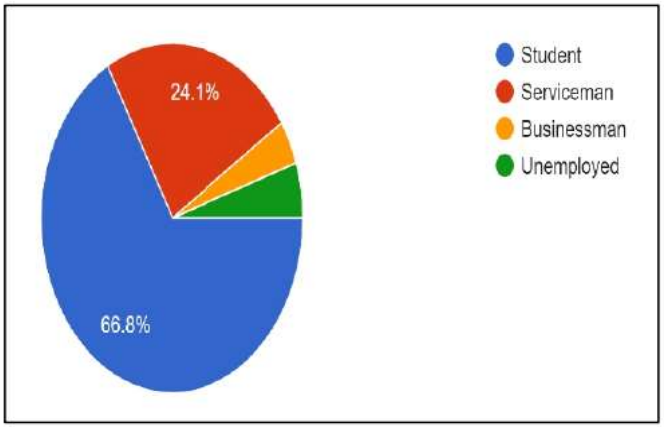


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Table 1.2 ANALYSIS OF CONSUMER BUYING BEHAVIOUR

Variable	Significant	Insignificant
Gender	• Number of sims used by them • Selection of a telecom service provider • Time of usage of services • Expenditure on monthly recharge	Channels for purchasing telecom services
Age	• Number of sims used by them • Time of usage of services • Channels for purchasing telecom services • Expenditure on monthly recharge	• Selection of a telecom service provider
Marital Status	• Channels for purchasing telecom services • Expenditure on monthly recharge	• Number of sims used by them • Selection of a telecom service provider • Time of usage of services
District	• Time of usage of services	• Number of sims used by them • Selection of a telecom service provider • Channels for purchasing telecom services • Expenditure on monthly recharge
Background	• Time of usage of services • Channels for purchasing telecom services • Expenditure on monthly recharge	• Number of sims used by them • Selection of a telecom service provider
Education	• Number of sims used by them • Selection of a telecom service provider • Time of usage of services • Channels for purchasing telecom services • Expenditure on monthly recharge	
Occupation	• Number of sims used by them • Selection of a telecom service provider • Time of usage of services • Channels for purchasing telecom services • Expenditure on monthly recharge	

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Demographic data having seven independent variables have been analyzed with the help of the Chi-Square test to find the association between the independent and dependent variables. different aspects of consumer buying behaviour including number of sims used by them, selection of a telecom service provider, time of usage of services, Channels for purchasing telecom services, and expenditure incurred on monthly recharge have been considered to finalize the results.

CONCLUSION

The demographic profile of the consumers based on gender, age group, marital status, district, background, educational qualification, and occupation was analyzed and it has been observed that there is a significant association between the gender of the respondents and several variables such as the number of sims used by them, selection of a telecom service provider, time of usage of service of their respective service provider and average monthly expenditure incurred by them for recharge. However, no significant association has been observed between gender and various channels for purchasing their telecom services.

There is a significant association between various age groups of the consumers and the variables including the number of sims used by them, time of usage of service of their respective service provider, various channels for purchasing their telecom services, and average monthly expenditure incurred by them for recharge. But there is no association of age with the selection of their service provider.

A significant association has been found between marital status and various channels for purchasing their telecom services and average monthly expenditure incurred by them for recharge while no significant association has been observed between the marital status of the consumers and the number of sims used by them, selection of a telecom service provider, time of usage of service of their respective service provider.

Districts have a significant association only with the time of usage of services of telecom service providers while there is no significant association between the number of sims used by them, selection of a telecom service provider, various channels for purchasing their telecom services, and money spent by them on monthly recharge.

If the background-wise association is observed, it was found that there is a significant association between the background of the consumers and the time of usage of service of their respective

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service provider, channels used for purchasing their telecom services, and the average expenditure incurred by them for monthly recharge. However, no significant association has been depicted between the background of the respondents and variables such as the number of sims used by them and the selection of their respective service providers.

A significant association has been observed between both education level and occupation of the consumers with all five variables including the number of sims used by them, selection of their service provider, duration of using services, selection of a channel for purchasing the services, and average monthly expenditure for recharge.

In a nutshell, it can be summarized that the demographic profile of the consumers affects the buying decision of consumers to a large extent.

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Authored By

Ms.Pinki Gupta, Associate Professor Department of Commerce, GMN College, Ambala Cantt

Published in Vol. 17, No.1, Jan-March 2023
JOURNAL OF MANAGEMENT AND ENTREPRENEURSHIP with ISSN : 2229-5348
UGC-CARE List Group I
Impact Factor: 6.257

UGC Care Approved, Peer Reviewed and Referred Journal

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Significance of Relationship between Demographic factors and investment behaviour in different investment categories

Dr.Payal Rani, Associate Professor Department of Commerce, Dronacharya Govt. college, Gurugram

Ms.Pinki Gupta, Associate Professor Department of Commerce, GMN College, AmbalaCantt.

ABSTRACT

Understanding investing behaviour and getting to know clients are never easy. Despite expressing their requirements and desires, investors do not follow through on them. They might not be aware of their true motives. They could abruptly alter their beliefs in response to outside forces. On the other side, marketers need to do research on the preferences, views, and investment behaviours of their target investors. The tastes of investors are greatly impacted by demographic factors. In this research I try to find whether the selection of investment vehicles across categories of investment vehicles depend similarly on all demographic factors.

Keywords: Investment preference, demographic variables, investment behaviour

INTRODUCTION

It is never simple to understand investment behaviour and get to know clients. Investors may express their needs and wants, but they do not act on them. They may be unaware of their genuine motivation. They may react to external influences by changing their opinions at the last minute. Marketers, on the other hand, must do research about their target investor's desires, perceptions, preferences, and investing behaviour. Investors' investing preferences are heavily influenced by demographic considerations.

Demographic variables will mostly influence the vehicles used by investors to commit their idle cash. The first stage in the investing process is to establish the investment objectives in terms of time frame and to develop an investment policy based on personal needs. Age, gender, marital status, income, and employment all have an influence on an individual's investment behaviour.Each individual is distinct from all others in some way, and therefore his decision is as well. The purpose of investment is to meet and satisfy the target client's wants and wishes. Investor behaviour is the study of how individuals, groups, and organisations select, purchase, utilise, and dispose of goods, services, ideas, or experiences to suit their wants and aspirations.

Age has an essential part in financial decisions since a person may take more risks while he is younger, but as he gets older, he becomes more risk adverse and utilises his expertise to invest more. Furthermore, his investing motivation may alter as he ages. Studies found that gender has a significant influence on investors' preferences for various investment vehicles (Bhushan&Medury, 2013; Patel &Modi, 2017; Ferreira &Dickason, 2018). Previous research has suggested that women investors are more conservative than males.

According to the findings of the survey, women are not financially independent. Women have a cautious mindset, thus they choose to invest in less hazardous assets (Praba, 2016). Women's engagement in investment choices has expanded as the environment has changed and women play a vital role in every area. With increased access to technology, resources, and knowledge, women are growing more confident in making autonomous judgements and investing in market assets. . According to a study of over 35,000 families, gender influences financial decisions since males choose more riskier assets in their investment portfolios and prefer trading in the stock market than women (Barber & Oden, 2001). Females, according to the study, are more risk averse than males. Young and educated persons are more likely to pursue new hazardous investment possibilities due to limited resources, a lack of investment alternatives, and a lack of investing trends (Bashir et al., 2013).Various studies imply that, despite having more information about investing, older individuals' investment skills degrade as they age due to the detrimental effects of cognitive ageing (Korniotis, 2011). We know that banks are increasing the interest rates on fixed-term deposits for older folks.

If a person earns a consistent income from his work and there is less fluctuation and risk associated with his future income, he may invest in an investment vehicle with more risk and return, whereas a person with an unpredictable income may prefer to engage in a somewhat safer investment alternative. Mutual fund investment is closely related to occupation and financial knowledge (Kaur&Bharucha, 2021). Furthermore, there is a distinction in the investment patterns of public and private sector personnel (Kalra, Dhameeja&Arora, 2012).Financial illiteracy has been studied for its affects and implications. It is also stated that in order to comprehend retirement plans, individuals must be educated (Lusardiet.al., 2014). With the emergence of online transactions of investments in the securities market, online banking, and other services, investing has become inextricably linked with not just literacy but also computer literacy.

It has been shown that a household investor's odds of entering the stock market in the next five years are 30% greater if their parents or children did so in the previous five years (Li G., 2009).Factors to evaluate include the investor's marital status and the number of dependents in his household. (Leff, N. H., 1969) discovered that the birth of a kid in the family has a significant impact on an individual's investing pattern. According to research, solo investors are less risk averse than those with dependents (Roszkowski et. al.1993).If a person earns a steady and substantial income, he may invest in more hazardous assets because of the income cushion, but if he earns less and saves with difficulty, he will choose to invest in safe investment alternatives (Bhushan&Medury, 2013; Sadiq&Ishaq, 2014). If a person is in a high tax bracket, he will want to invest in a vehicle that would allow him to delay his tax due and so enhance his net income.

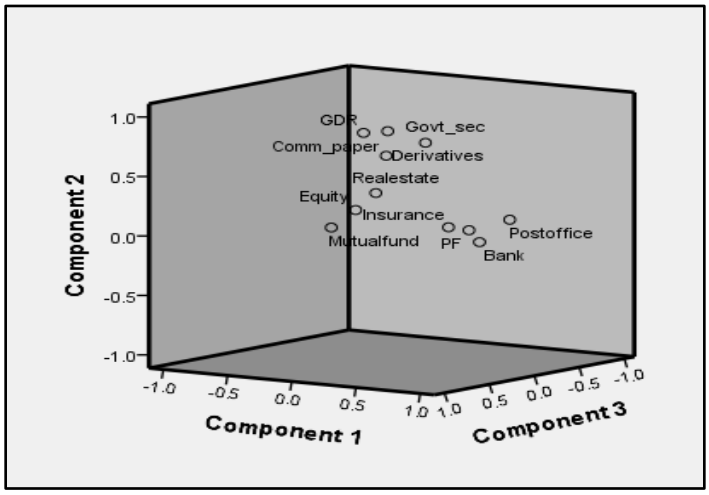
From the above discussion a research question comes to my mind:

Whether all demographic factors have equally significant relationship with the choice of investment vehicles in different categories of investment vehicles?

DISCUSSION AND RESULTS:

For categorisation of investment vehicles, I have used factor analysis through which three components of the investment vehicles have been found named: conventional investment vehicles, real estate and market-based investment vehicles. Thus it is an important question whether there is any significant relationship between the socio economic background and investment pattern/attitude towards different types of investment vehicles. It can be measured by Spearman's rank-order correlation.

Figure 1: Component plot on rotated space



Source: The Author

Using Factor analysis I found three components for preference in investment vehicles. Bank deposits, post office deposits, provident funds and insurance policies are grouped into one component on the basis of their factor loadings. These investment vehicles are ‘conventional investment vehicles’ associated with low risk. Commercial papers, government securities, global depository receipts and derivatives have been recognised as the four factors grouped for second component. All of these factors are related to debts instruments or the derivatives. Thus the second component may be named ‘debts and derivatives’. Equity shares, mutual funds and market securities are the factors grouped into third category. These factors belong to the ownership based market securities with real estate in addition. Thus the third component may be named as ‘real estate and market-based investment vehicles’. The components derived from factor analysis may be seen in figure 1. The three components can be seen as clusters. According to the findings of the study, only two components are mostly availed/used for investment i.e..‘Conventional investment vehicles’ and ‘real estate and market-based investment vehicles’. Thus I wish to find whether the socio-economic variables are equally significant for both types of investment vehicles or not.

Spearman’s Correlation for Test of Association between Demographic Variables and Choice of Investment Vehicles

A Spearman's rank-order correlation was run to determine the relationship between demographic factors and preference for investments in conventional investment vehicles.

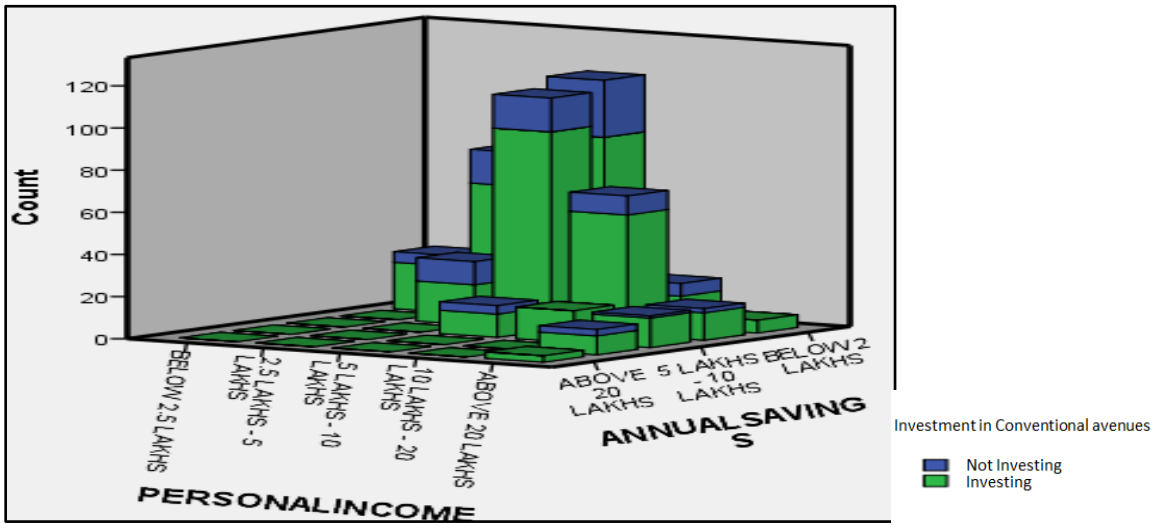
Association between Demographic Variables and choice of Conventional Investment Vehicles

Table 1: Spearman's rho between demographic factors and Investment in Conventional investment vehicles and Real Estate and Market-based investment vehicles		
Factors		**Conventional investment vehicles**
Educational Qualification	**Correlation Coefficient**	-0.015
	Sig. (2-tailed)	0.727
	N	528
Gender	**Correlation Coefficient**	0.017
	Sig. (2-tailed)	0.698
	N	528
Work Sector	**Correlation Coefficient**	-.096*
	Sig. (2-tailed)	0.027
	N	528
Marrital Status	**Correlation Coefficient**	0.036
	Sig. (2-tailed)	0.409
	N	528
Personal Income	**Correlation Coefficient**	.123**
	Sig. (2-tailed)	0.005
	N	528
Age	**Correlation Coefficient**	-.090*
	Sig. (2-tailed)	0.04
	N	528

Correlation coefficient between the investment in conventional investment vehicles and age is -.090 having p= .040. There was a negative correlation between conventional investment vehicles and age of the respondents, which was statistically significant (r_s = -.090, p<0.05).As is shown in table 1, correlation coefficient between the investment in conventional investment vehicles and educational qualification is -.015 having p = .727. There was a negative correlation between conventional investment vehicles and educational qualification, which was statistically not significant (r_s = -.015, p>0.05).Correlation coefficient between the investment in conventional

investment vehicles and gender is .017 having p = .698. There was a positive correlation between conventional investment vehicles and gender, which was statistically not significant (r_s = .017, p>0.05).Correlation coefficient between the investment in conventional investment vehicles and work sector is -.096 having p = .027. There was a negative correlation between conventional investment vehicles and work sector, which was statistically significant (r_s = -.096, p<0.05).Correlation coefficient between the investment in conventional investment vehicles and marital status is .036 having p= .409. There was a positive correlation between conventional investment vehicles and marital status, which was statistically not significant (r_s = .036, p>0.05).Correlation coefficient between the investment in conventional investment vehicles and personal income is .123 having p= .005. There was a positive correlation between conventional investment vehicles and personal income, which was statistically significant (r_s = .123, p<0.05).

Figure 2: Figure showing the relationship between demographic factors and preference regarding investment in conventional investment vehicles



Source:The Author

Figure 2 shows that as the personal income are increasing, the investors are investing in conventional investment vehicles more or less equally for each income and saving group. We can see that most of the respondents are investing in these vehicles as the non-investing proportion is comparatively very low as compared to the investing ones.

Association between Demographic Variables and choice of Real Estate and Market-based investment vehicles:

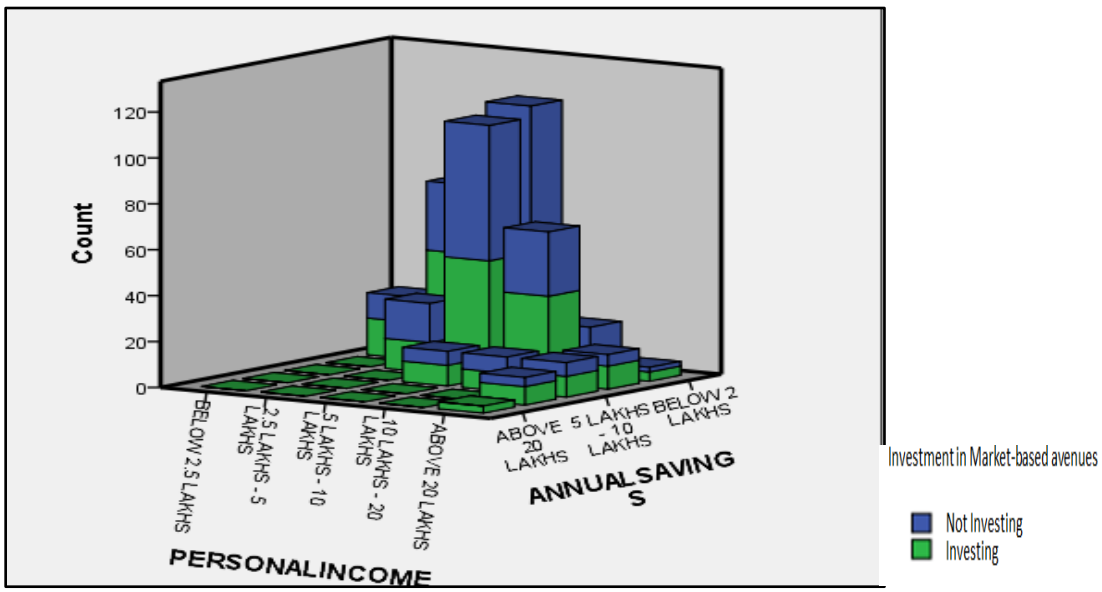
Table 2: Spearman's rho between demographic factors and Investment in Real Estate and Market-based investment vehicles

Factors		**Real Estate and Market-based investment vehicles**
Educational Qualification	**Correlation Coefficient**	0.027
	Sig. (2-tailed)	0.537
	N	528
Gender	**Correlation Coefficient**	-0.032
	Sig. (2-tailed)	0.062
	N	528
Work Sector	**Correlation Coefficient**	.105*
	Sig. (2-tailed)	0.015
	N	528
Marrital Status	**Correlation Coefficient**	-0.007
	Sig. (2-tailed)	0.867
	N	528
Personal Income	**Correlation Coefficient**	.162**
	Sig. (2-tailed)	0
	N	528
Age	**Correlation Coefficient**	-.124**
	Sig. (2-tailed)	0.004
	N	528

Correlation coefficient between the investment in Real Estate and Market-based investment vehicles and age is -.124 having p= .004. There was a negative correlation between in Real Estate and Market-based investment vehiclesand age of the respondents, which was statistically significant (r_s = -.124, p<0.004).As is shown in table 2, correlation coefficient between the investment in Real Estate and Market-based investment vehicles and educational qualification is

0.027 having p = 0.537. There was a positive correlation between Real Estate and Market-based investment vehicles and educational qualification, which was statistically not significant (r_s = 0.027, p>0.05).Correlation coefficient between the investment in Real Estate and Market-based investment vehicles and gender is -0.032 having p = 0.062. There was a negative correlation between Real Estate and Market-based investment vehicles and gender, which was statistically not significant (r_s = -0.032, p>0.05).Correlation coefficient between the investment in Real Estate and Market-based investment vehicles and work sector is 0.105having p = 0.015. There was a positive correlation between Real Estate and Market-based investment vehicles and work sector, which was statistically significant (r_s = 0.105, p<0.05). Correlation coefficient between the investment in real estate and market-based securities and marital status is -0.007 having p= 0.867. There was a positive correlation between real estate and market-based securities and marital status, which was statistically not significant (r_s = -.007, p>0.05).Correlation coefficient between the investment in real estate and market-based securities and personal income is .162 having p= *0*. There was a positive correlation between real estate and market-based securities and personal income, which was statistically significant (r_s = .162, p<0.05).

Figure 3: Figure showing the relationship between personal income, annual savings and preference regarding investment in real estate and market-based securities



Source: The Author

Figure 3 illustrates that when personal income rises, investors invest more or less evenly in real estate and market-based investment vehicles for each income and saving category. We can see

that the respondents are investing in these vehicles as the non-investing proportion is more or less equal to the investing ones.

CONCLUSION

It can be concluded from the above discussion that there is significant association between conventional investment vehicles and perceived risk and awareness while perceived return does not have significant association with conventional investment vehicles. It is concluded that all the factors are not equally significant in choice of conventional investment vehicles.When comparing conventional and real estate & market-based investment vehicles, it is discovered that individuals are more attracted towards real estate and market-based investing vehicles for the same group of income and savings.

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मध्य भारती
मानविकी एवं समाजविज्ञान की द्विभाषी शोध-पत्रिका

MADHYA BHARTI
(UGC CARE Group-1, Multi disciplinary)

CERTIFICATE OF PUBLICATION

This is to certify that the article entitled

PROMOTIONAL STRATEGIES OF TELECOM SERVICE PROVIDERS AND THEIR CHALLENGES: A STUDY OF AIRTEL AND JIO

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Published in
Madhya Bharti -Humanities and Social Sciences
(मध्य भारती) मानविकी एवं समाज विज्ञान की द्विभाषी शोध-पत्रिका
: ISSN **0974-0066** with IF=6.28
Vol. 83, No. 21, January - June : 2023
UGC Care Approved, Group I, Peer Reviewed, Bilingual, Biannual,
Multi-disciplinary Referred Journal

Chief Editor
प्रो. अभिवादन शर्मा

PROMOTIONAL STRATEGIES OF TELECOM SERVICE PROVIDERS AND THEIR CHALLENGES: A STUDY OF AIRTEL AND JIO

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Abstract
The telecommunication sector in India is one of the fastest-growing business segments of the country. The cellular industry is growing at a very fast speed because it has become a necessity. As a result, there is an increase in potential buyers as well as its market size. Due to exponential growth in the Indian telecom sector, old marketing practices are becoming out of context. Changing paradigms have put every telecom player under tremendous pressure to outperform the competition and meet customer expectations. It has become very difficult to attract new customers as well as retain and delight existing consumers of different telecom service providers on a sustainable level. They are also facing the challenge of attracting their potential subscribers by offering them a bundle of services. This paper deals with the development of an understanding of the challenges faced by telecom service providers in India while implementing promotional strategies.

Keywords: Mergers and acquisitions, customer feedback, provoking publicity, environment, competition etc.

INTRODUCTION

An organization can concentrate its resources on the optimal opportunities with the process of marketing strategy that can help it in achieving its goals of increasing sales and attaining a sustainable competitive advantage. Marketing strategy is treated as a competitive advantage. It includes all basic &long-term activities which come under the field of marketing dealing with the analysis of the strategic initial situation of a company. It also helps with the formulation, evaluation, and selection of market-oriented strategies. Therefore, it contributes to the goals of the company & its marketing objectives.

The promotion mix is the blend of different methods and tools of communication any organization uses in presenting the company, products, or services to target its customers. Effective promotion is a key component of the marketing mix, as it is the element with the help of which we can attract customers, persuade them to buy, and generate loyalty.

Promotion is one of the four main strategic components of the marketing mix, generally known as the "4 P's." The role of the promotion mix is to create synergy with the other three elements product, place, and pricing so that the organization can optimize the company's customer and profit potential. Promotion brings together the strengths of the product, its availability, and the fairness of its benefits at a particular price. The marketing communication mix also called as promotion mix consists of the following major promotional tools: Advertising, Direct Marketing, Interactive/ Internet marketing, Sales Promotion, Publicity/ Public Relations and Personal Selling.

REVIEW OF LITERATURE

Varma Aparna J et. al. (2007) in Mysuru studied the effectiveness of the marketing strategies of BSNL and Airtel. The areas that were to be improved for BSNL in comparison to Airtel were also identified. It was identified that the formulation and execution of marketing strategies were significantly different between BSNL and Airtel service providers. There was ample scope for BSNL services providers to further improvement of their services marketing mix other than their pricing strategy. **Chaudhary Bharat Bhushan** (2012) in a comparative aimed to study the process of providing Cellular Mobile Telephone Service (CMTS) and service quality standards set up by the telecom regulatory authority of India (TRAI). The quality of customer service offered by GSM service providers was found better than CDMA providers. The price and quality of the network offered by CMSP influenced the perceptions of the customer to a great extent. The customers were

looking for mobile service which was reliable and innovative. **Palaniappan. G et. al.** (2015) analyzed the perception level of the customers of BSNL mobile service providers. It was identified that the service provided by BSNL is at a satisfactory level. It was further observed that BSNL should focus on promotional measures equal to the private service providers to enhance their service activity to satisfy their customers. **Singh Surabhi** (2016), studied the perception of customers about the Jio network. It was identified that nine factors are the basis of a strong consumer attitude towards Reliance Jio. The reliability, performance expectancy, and other factors had enabled the presence of Reliance Jio in the mind of customers. **Chinthala Gowthami et. al.** (2017) in a study attempted to know the customer satisfaction level with the services provided by JIO telecommunication service providers and further analyze the determinants of customer satisfaction. It was found that the majority of the respondents were satisfied with the internet service provided by the JIO telecommunication service provider but not satisfied with customer care service. **Srikumar Hema et. al.** (2017) analyzed the impact of service quality dimensions on customer satisfaction towards Airtel and Vodafone services in Nilgiris and Coimbatore districts. It was found that the service quality dimensions of tangibles, assurance, reliability, responsibility, and Empathy played a major role in the recommendation of Airtel and Vodafone. **Singh Rajbinder**, (2017) examined the impacts of Reliance Jio on the Indian telecom industry. It was observed that the company would have generated a significant market share in the next 3-5 years. In the end reliance, Jio has successfully hit the target market and has deep pockets to invest in but has a threat to competition and technology today also. **Singh Sukhvir et. al.** (2017) compared Vodafone and Airtel based on ten measures of brand equity. It was found that Airtel was in a leadership position in five brand equity measures including satisfaction/loyalty, perceived quality, leadership & popularity, organizational associations, and market share as compared to Vodafone. The other three measures i. e. brand personality, brand awareness, market price, and distribution coverage had been shared by both Airtel and Vodafone. The study concluded that the Brand Equity of Airtel was higher than that of Vodafone. **Haq Noorul** (2019), took a research study to examine the impact of Reliance JIO on the Indian telecom industry. It was observed that after the entry of Reliance Jio Info comm into the telecommunication industry, the economic structure of the market was still the same but the level of competition had grown unexpectedly. It was revealed that the earlier losses shown by all telecom operators were only due to inefficiency in the market. If Reliance Jio Info comm would work at the same pace and follow the same method of business and survive with any support from associated firms, it would redefine competition in the telecom sector. Then the best and honest would survive and vice-versa has to shut down their business. **Jasrotia Sahil Singh et.al.,** (2019), in a research study on disruptions in the Indian Telecom Sector, analyzed the impact of R Jio (Reliance Jio) on the Indian Telecom Industry and examined the factors influencing customer churn from other telecom operators towards Reliance Jio. Reliance Jio brought a change by providing high-speed internet data at cheaper and affordable rates, which was not a trend. Telecom operators used to charge more prices to have access to high-speed data and initially they used PM Narendra Modi in their promotions which was a major influential factor to customers. **Sahai Surpreet**(2020), attempted to give an insight into the telecom sector and the steps being taken by them to improve their relationship with their customers. The customer's perceptions and spending patterns specifically related to the telecom study were assessed. The most important determinant found for a consumer was price and sacrifice perception including monetary and non-monetary sacrifice. In today's competitive environment, customers are quick to abandon services that do not meet their expectations. Her research provided customized as well as tailored solutions to the telecom industry in terms of planning long-term strategies related to marketing and other operating divisions.

Srikumar Hema et. al. (2017) analyzed the impact of service quality dimensions on customer satisfaction towards Airtel and Vodafone services in Nilgiris and Coimbatore districts. An association between overall customer satisfaction and service quality dimension was found. There was a significant difference between the recommendation and service quality dimensions of the

respondents of Airtel and Vodafone users. Customer care service was found very important and was producing a direct impact on customer satisfaction. Further, the service quality dimensions of tangibles, assurance, reliability, responsibility, and Empathy played a major role in the recommendation of Airtel and Vodafone. But the study was limited to only Airtel and Vodafone customers from Walk-in Stores and Universities located in Coimbatore and Nilgiris.

OBJECTIVE OF THE STUDY

To study and compare challenges faced by both telecom companies in the light of promotional strategies.

HYPOTHESIS

Null Hypothesis (H0): There is no significant difference between the challenges faced by both telecom companies in the light of promotional strategies.

RESEARCH METHODOLOGY

The study is descriptive and exploratory in nature. The population under study is the population of Haryana state in India. Information was collected by using primary as well as secondary data. Three districts of Haryana having the highest qualification rate namely Gurugram, Ambala, and Panchkula were selected and the data were collected from a questionnaire that was got filled out by 568 consumers from these districts. Secondary data was also collected from the Telecom Regulatory Authority of India (TRAI) website, the work done by the researchers in the past, various journals, research papers, newspapers, and other sources.

ANALYSIS OF CHALLENGES FACED BY JIO AND AIRTEL

The business world is full of challenges. The telecom sector is considered the fastest and most popular sector. Jio and Airtel are the two largest telecommunication service providers in India. Both service providers use different types of promotional tools at different periods of time. Thus, it implies several challenges in terms of network, infrastructure, education, mergers, competitions and feedback as well. An analysis has been made to identify and compare the challenges faced by both the service.

Table1: Z-Test for significant difference between challenges faced by Jio and Airtel

Variables	Service Provider	N	Mean	Std. Deviation	Z	p-value
Social and cultural values	Jio	228	2.08	1.170	.032	.975
	Airtel	251	2.08	1.050		
Aggressive provoking publicity	Jio	228	2.35	1.168	-.562	.574
	Airtel	251	2.41	1.147		
Unusual creative publicity	Jio	228	2.35	1.195	.347	.728
	Airtel	251	2.31	1.070		
Personalised services	Jio	228	2.20	1.146	-.330	.742
	Airtel	251	2.23	1.089		
Resolving service issues	Jio	228	2.22	1.097	.370	.712
	Airtel	251	2.18	1.035		
Demand for IoT	Jio	228	2.14	1.137	.372	.710
	Airtel	251	2.10	1.015		
Mergers and Acquisitions	Jio	228	2.28	1.224	-.614	.540
	Airtel	251	2.35	1.115		
Customer Feedback	Jio	228	2.12	1.164	.259	.796
	Airtel	251	2.09	1.101		
Unfriendly Regulatory Environment	Jio	228	2.33	1.203	-.338	.736
	Airtel	251	2.37	1.204		

Source: data collected through a questionnaire

It has been exhibited from the perusal of table 1 that there is no significant difference in the challenges of both the telecom service providers i. e. Jio and Airtel in the light of promotional strategies. The p-value of z-test of all the challenges i. e. maintenance of Indian social and cultural values, aggressive provoking publicity, unusual creative publicity, personalized services, resolving service issues, demand for Internet of Things, mergers and acquisitions, proper customer feedback, unfriendly regulatory environment are more than .05. Thus, there is no significant difference in the challenges faced by Jio and Airtel in terms of promotional strategies.

Null Hypothesis (H0): There is no significant difference between the challenges faced by both telecom companies in the light of promotional strategies.

So, the **null hypothesis is accepted and the alternate hypothesis is rejected.**

CONCLUSION

The increasing cost of acquiring new customers drives service providers to discover new ways to acquire, retain and increase their subscriber base on a regular basis. Thus, it is very crucial for these service providers to retain the existing customer for the industry and this is possible only by providing quality services to them to gain their loyalty. Effective marketing practices through appropriate marketing mix elements, the creation of a unique and distinctive positive image in the mind of the customers is a must, in order to long run endurance. In this direction promotion mix of the company proves to be the backbone of its marketing mix as it is the only way to implement its marketing mix in an effective way. Keeping in view of this only concept, the challenges which create hurdle in the implementation of the promotion mix of any organization has been analyzed, and found that the challenges faced by two leading service providers i. e. Airtel and Jio are not significantly different. They should understand the customer expectations and concentrate on their optimum marketing mix. The service providers should know how to respond their customers effectively and on time.

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CERTIFICATE OF PUBLICATION

Article Entitled

A STUDY OF VALUE-ADDING PROMOTIONS OF TELECOM SERVICE PROVIDERS IN INDIA: A SPECIAL REFERENCE TO HARYANA STATE

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Published in
SOUTH INDIA JOURNAL OF SOCIAL SCIENCES ; ISSN: 0972 – 8945
Vol. XXI, No.1, January–June : 2023, IF: 6.876
UGC CARE Group I, Bi-Annual Journal

SOUTH INDIA JOURNAL OF SOCIAL SCIENCES
ISSN: 0972 – 8945

A STUDY OF VALUE-ADDING PROMOTIONS OF TELECOM SERVICE PROVIDERS IN INDIA: A SPECIAL REFERENCE TO HARYANA STATE

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ABSTRACT

Promotion is the key to the success of every business whether it is producing goods or services. To market its products promotion, and marketing both are important for any organization. Service quality has been considered as a vital component by the company. As mentioned, the number of mobile phone users is increasing day by day. The competition among service providers is also increasing day by day. The pressure of competition encourages the competitors to increase the product/service quality as well as reduce the price. The paper concentrates on the various value-added promotions done by telecom service providers and their relationship with consumer response. Value-adding promotions have a positive relationship with each other and with consumer response also. The service providers should take care of them as the value-added services have an immense impact that can help the organization retain its consumer successfully. ***Keywords: potential consumers, demonstrations, membership programs, marketers, etc.***

INTRODUCTION

In the age of huge competition, every telecom company tries to attract new customers and retain their existing ones. Promotions lead to the development of knowledge of the brand for all the promoted products and brands. However, promotions of repeated sales like reduction in price for a short time, discount, or coupon may avert the attempts of the brand because these may be a low- quality signal or an obsolete kind. Repeated price promotions may lead to confusion for the customers since they are not able to understand why high-quality products are promoted and offered at a special price. According to self-perception theory, customers who choose a product based on price promotions which is an external reason rather than a positive attitude towards the product which is an internal reason generally change their choice and choose other products whenever the external reason is eliminated.

Skills and experience are required to develop a promotion mix in marketing. Marketers should gather lots of data about the consumers to come up with an effective promotion mix and work out a budget that they can afford for a promotion, and decide the most efficient marketing channels for their audience. It helps in improvement in the effectiveness of promotional campaigns, helps to segment the audience, improves communication with clients, informs subscribers, and stands out from the crowd.

The sales promotion strategy may be classified into two parts: cost-down and value-added promotions. They act as a means of attracting more customers and delivering value by providing a greater benefit. The value-added service provides ample possibilities that will help to retain customers for them. Value-added services (VAS) provided by telecom companies do not involve basic services including SMS, and voicemail, but they include other amenities such as e- commerce, sports, entertainment, astrology, and much more. These are either free or chargeable. VAS cannot be activated by any telecom service providers without prior consent from any customer. The telecom provider has to deactivate the subscription if the customer decides to unsubscribe from VAS within 24 hours of activation of services and refund the money. A toll-free number is also available for those who want to deactivate the services, and who receive them without a subscription.

REVIEW OF LITERATURE

Gunasekaran et. al., (2007) did their research on rural telecom in the north Indian states. It was found that rural areas, difficult topographical, low population density, spread-out population, and climatic conditions made it difficult to provide telecommunication services to acceptable quality by traditional means at an affordable price. So, it was recommended that villages near a larger town could take advantage of the fiber backbone as a remote village can be connected via a VSAT link. From the fiber backbone, a point-to-point or point-to-multipoint Wi-MAX link could be used to connect one or more villages near the town. **Varma Aparna J et. al. (2007)** found that the formulation and execution of marketing strategies significantly differ between BSNL and Airtel service providers. There is ample scope for BSNL services providers to further improvement in their services in the form of a marketing mix. It was identified that the formulation and execution of marketing strategies were significantly different between BSNL and Airtel service providers. There was ample scope for BSNL services providers to further improvement of their services

marketing mix other than their pricing strategy. **Wang Chengchang et. al.** (2009), took comparative research on the marketing strategies of the Telecommunication Industry. This research was an attempt to compare the marketing strategies of the telecommunication industry ofIndia and China. Through comparison, it was found that the niche market had been taken very seriously by the Indian telecommunication industry and they were focusing more and more on it. But the Chinese telecom industry had not taken the niche market so seriously. It was revealed that the two countries were different in marketing strategies mainly because government policies were different in the two countries. **Mathew P. T.** (2009) in a research study observed that there was high price sensitivity in the market, and perceived differences in the brands are many. According to him, BSNL should focus on the quality of service and be ready to respond to competition, service improvements, and some additives such as new value-added services and attractive call rates, etc. The BSNL should improve the quality of the network. And the same should be communicated to the customers. BSNL should come up with more attractive customer-specific tariff plans which would help the company to survive in the competition and help in increasing thecustomer base, market share, minutes of usage, and hence the total revenue. **Nasit Alpesh A** (2011), analyzed 1200 telecom service consumers in Gujarat state, India. The study aimed to study the existing marketing strategies adopted by telecom industries, to work out the growing challenges faced by the telecom sector, to analyze the role of various marketing techniques in the telecom sector, and to study the customer response to various marketing mix adopted by the telecom sector companies. As a result, it was observed that usage of Information base services and service providers were dependent. Information Base services, and GPRS/Internet services availability were the same for all the companies. Usage of Call Diverting services and service providers was not found independent as well as usage of News update services and service providers was also not independent. **Catoiu, I., & Gardan, D. A. (2010)** conducted research on the perception of Romanian consumers toward Mobile Marketing Campaigns. The research was undertaken to find future trends in mobile marketing and mobile media. It was observed that the perception of Romanian consumers towards specific mobile marketing techniques was influenced primarily by perceptions toward mobile services in general not specifically by their marketing campaigns. **Obasan et. al.** (2012) in a research study assessed the effectiveness of promotion as amarketing management tool in the Nigerian Telecommunication Industry. The study was

an attempt to know about the effect of Promotion on marketing management in the Nigerian

telecommunication industry. The study depicted that promotion had a significant effect on marketing management in the Nigerian telecommunication industry. **Gautam Abhinanda** (2013) in a research study analyzed (below the line) promotion strategies of the telecom industry in the western (Up) circle (India). The study was with a special reference to Reliance Communications for which responses from Meerut, Bulandshahr, Muzaffarnagar, Baghpat, and Bijnore were collected. He attempted to study various factors which were effective in boosting sales such as consumer schemes, roadshows, event sponsoring, distributor's/retailer's schemes, attractive margins, association with other brands, group schemes, and discount offers offered by Reliance Communication. It was found that promotion possessed a significant key role in determining profitability and market success. Further brand building, through 'Above the Line' medium and mass media were the effective advertising strategies that were being followed as promotional tools. **Jaimini H Dave** (2013), took research to know the opportunities, specific problems, and limitations of the Indian Telecommunication Industry. In the process of finding the opportunities, the Indian Telecom market was found as one of the fastest-growing markets in the world with

787.29 million Telephone connections as of 31st December 2010. Furthermore, it was the second-largest network in the world after China. It was the second-largest wireless network in the world. About over 18 million connections were being added every month. Wireless telephones were increasing at a faster rate. **Baruah Papori et. al.** (2014), in research discussed the growth of Telephones, teledensity, public versus private telecom sector, wireless versus wire-line segments, different service providers and their market share, etc. In this regard, the telecom sector would give special focus on unreserved areas in the North-Eastern region and backward states of the country. It was concluded that the growth and development of the Telecom sector of India had made it a key contributor to India's economic and social up-gradation. **Suthar B.K. et. al**. (2014) analyzed the impact of the marketing mix and consumer perception on brand loyalty. The study examined the relationship between marketing mix and customer perception of brand loyalty especially in the context of Bharat Sanchar Nigam Limited, Vadodara. A positive relationship between different dimensions of the marketing mix and customer perception was revealed by the collected data on brand

loyalty of Bharat Sanchar Nigam Limited as one of the Cellular Service Providers (CSPs) in Vadodara Telecom in the District of Gujarat. **Zafar F. et. al**. (2014) made a comparison between two countries that is UK and Germany that were dealing with the innovationand new technology of mobile. Strategies adopted by mobile phone companies were analyzed to attract customers to buy new models of mobile. The study investigated various factors determiningcustomer satisfaction and advertising of mobile phones in the UK and Germany. Smartphones were identified as the most demanding among teenagers. Not only this but the customer value could be revealed completely in a long-term relationship with customers. **Choy Lai Chi (2015)** studied consumers' perception of mobile phones of China brands in Ipoh. The significant impact of price, perceived quality, brand awareness, etc. on the purchase intention was also observed. It was found that males were more exposed to and familiar with technology products. There was a significant relationship between price and purchase intention; perceived quality and purchase intention; brand awareness and purchase intention as well as between perceived features and purchase intention. Thus, there might be more significant independent variables that would be ableto explain the buying intention. **Abedin Md. Zainal and Ferdous Laboni (2015)** studied promotional strategies of Telecommunication industries and customers' perception of Airtel Bangladesh Limited. A sample of 44 Bangladeshi consumers was taken into consideration. The purpose of the study was to determine the impact of the promotional activities of Airtel BangladeshLimited. It was observed that customers were not satisfied with Internet Service, SMS, and advertisements of Airtel. Airtel was not an active participant in charity events to establish a good corporate image. **Chinthala Gowthami et. al. (2017)** took research work on Customer Satisfaction Towards Telecommunication Service Providers – A study on Reliance JIO. Respondents were found highly satisfied with the Internet facilities provided by Jio but not that much satisfied with the customer care services provided by Jio. The company needs to focus more on customer care services, responding to the customer's issues and concerns, and providing solutions. **Chandra Sayandeep et. al.** (2018), took research to study the impact of sales promotional tools on consumers' buying behavior. They accessed how the customer behaved and purchased when they were exposed to different promotional schemes floated by the apparel retail stores. The promotional offers were aimed to increase sales, attracting new customers, and keeping old customers. The impact of the promotional offers (buy one get one,

coupons, price discount) on the consumers' behavior related to apparel buying was also examined. Loyal consumers were availed with monthly discount coupons which motivated them to revisit. **Kalra Siddharth et. al.** (2018),took research to study the impact of Jio on the Indian Telecom Industry through their marketing strategies. This study aimed to find out how the marketing strategies of Jio had impacted the customers of the Indian telecom industry and what were the main strategies that the customers

preferred and attracted them to this network. It was observed that the majority of the increasing customer base of Jio was primarily because of the impressive marketing strategies tackled by Jio. It was also interpreted that Jio had put a lot of effort and research into its marketing strategies by capturing those areas where they knew consumers were lacking from other service providers. **Niyarta Khushboo** (2019) analyzed the marketing strategies of Bharti Airtel Limited, Vodafone, Bharat Sanchar Nigam Limited (BSNL), and Reliance Jio Infocomm Ltd. A comparison was madeamong the marketing strategies of Airtel, Reliance Jio, Vodafone, and BSNL related to the fourth-generation mobile telecommunication services in India, and their impact on customer satisfaction and loyalty of telecom consumers was also observed. It was found that there was a significant difference among the strategies adopted by different telecom service providers; product, price, place, promotion, people, physical evidence, and process. **Bharti K. & Nigam S. (2020)** studied the effect of sales promotional tools on consumer buying behavior. These marketing activities could easily enhance the profits of a company through sales promotion activities. analyzed the effect of sales promotional tools on consumer buying behavior. It was observed that Buying decision was mainly influenced by its prior purchase evaluation. However, sales promotion could raise short-term sales and not create loyal consumers.

STATEMENT OF THE PROBLEM

"Consumer response towards value adding promotions of telecom service providers with referenceto Haryana state"

RESEARCH METHODOLOGY

The present study is exploratory and descriptive in nature. The population under study is the population of Haryana state. To achieve the objectives, information was collected by using secondary as well as primary data. The information was collected through a questionnaire filled from 568 consumers in Haryana state mainly from three districts namely Gurugram, Ambala, andPanchkula. Secondary data was collected from the Telecom Regulatory Authority of India (TRAI) website, the work done by the researchers in the past, and various journals, research papers, newspapers, and other sources.

OBJECTIVES OF THE STUDY

- To study the consumer response towards value-adding promotions of the telecom service providers.
- To suggest measures that may help wireless telecom operators in implementing promotionmix in the future.

HYPOTHESIS

Null Hypothesis (H0): (Null Hypothesis): There is no significant relationship between the consumer response and value adding promotions of the telecom service providers.

DATA ANALYSIS AND INTERPRETATION

The relationship between consumer response and value-added services offered by the telecom service providers in Haryana has been analyzed. For this zero-order correlation matrix has been applied to find out the relationship between consumer response and value-added services. Different types of value-added promotions are used by various telecommunication service providers to attract them and keep them satisfied. A number of value-added promotions offered by Airtel and Jio have been taken into consideration such as free samples and free trial of product, brochures/ mailings showing the discounted products, discount coupons/ price off promotions/ free data withthe purchase of another product, at the counter display promotions/ demonstrations, membership programs, brand Ambassador’s image; and online promotional offers.

Table 1 Relationship between different value-adding promotions offered by telecom serviceproviders and consumer response: Zero Order Correlation Matrix

Value Added Promotions	1	2	3	4	5	6	7	8	9	10
Sample	1									
Brochure	.721 (**)	1								
Lottery	.650 (**)	.645 (**)	1							
Cash Back	.744 (**)	.725 (**)	.667 (**)	1						
Discount	.736 (**)	.692 (**)	.665 (**)	.766 (**)	1					
Demonstration	.645 (**)	.703 (**)	.629 (**)	.734 (**)	.747 (**)	1				
Membership Programmes	.638 (**)	.682 (**)	.619 (**)	.702 (**)	.724 (**)	.773 (**)	1			
Image of Brand Ambassador	.657 (**)	.683 (**)	.611 (**)	.665 (**)	.709 (**)	.746 (**)	.753 (**)	1		
Online Promotion	.677 (**)	.701 (**)	.614 (**)	.690 (**)	.756 (**)	.746 (**)	.759 (**)	.771 (**)	1	
Repeat Purchase	.035	.048	-.005	.100 (*)	.081	.098 (*)	.061	.027	.057	1

**** Correlation is significant at the 0.01 level (2-tailed).**

***** Correlation is significant at the 0.05 level (2-tailed). Source: Data collected through a questionnaire**

By applying zero-order correlation, relationships among various aspects of consumer buying behavior influenced by various value-added promotions offered by the telecom service providers have been depicted in the above table. All the telecom service providers offer all or a blend of some of value-added promotional schemes as per the market situations and competition level. By analysing different aspects of value-added promotions are evaluated such as distribution of free samples, brochures, and emails of discounted products, prize or lottery offers, buy one get one freeor cash back promotions, discount coupons, demonstrations, membership programs, the image of brand ambassador and online promotions on account of the effect on consumer response, the relationship among the various factors have been established. The value of all the evaluated value- added promotions exhibited a significant relationship at a 1 percent level of significance. It has been observed that the most significant

relationship is between various discount coupons or price-off promotions or free data with the purchase of another product and online promotions done by the service providers. It implies that the a number of value added promotions are also inter related which has been observed from the table as price related value added promotions has shown highestcorrelation.

As far as consumer response and value-added services are concerned, there seems to be a low degree of correlation between them. The table reveals the fact that there is a moderate degree of correlation between various value-added promotions done by the service providers but a strong relationship has not been observed between the consumer's response with the various value-addedpromotions done by Jio and Airtel.

Table 2 Testing of Hypothesis at a Glance

H0(Null Hypothesis)	**Significant/ Insignificant**	**Decision**
There is no significant relationship between the consumer response and value adding promotions of the telecom service providers.	Significant	H0 rejected

Thus, the null hypothesis is rejected and the alternate hypothesis is accepted.

CONCLUSIONS AND SUGGESTIONS

Different aspects of consumer behavior about value-added services of both the telecom service providers i. e. Jio and Airtel have been analyzed. Several value-added promotions run by them including samples, discounts, lotteries, demonstrations, etc. have been taken into consideration to assess the relationship between consumer response towards them. It was observed that all the value-added promotions are correlated with each other but when their relationship with the consumer's response was analyzed, it was found that there was a low degree of relationship between the consumer's response and various value-added promotions done by the service providers.

Thus, the study reveals the fact that there is a positive relationship between the consumer responseand value-added promotions offered by telecom service providers. Perhaps, that is the reason why value-added promotions attain maximum attention while formulating any marketing strategy. Thisimplies digital transformation is the demand of the hour to adapt to the changing needs and expectations of consumers. Therefore, various discount coupons, price-off promotions, and free data offers with the purchase of the company's other products

should be associated with their onlinepromotions. With an efficient blend of core services and value-added services consumers can be influenced by their product or service and the same holds good for the telecom sector too. It is important in today's fiercely competitive scenario; a service provider should effectively use a proper mix of services that are to be provided to the consumers to realize maximum returns. In thisdirection value-added services contributes a lot and the service providers must consider them as they have a positive impact on consumer response in terms of repeat purchase.

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Shodhak : A Journal of Historical Research
ISSN : **0302-9832**
Volume: **53,** Issue: **02,** No: 14**,** May - August : **2023**

ENTREPRENEURSHIP AND INNOVATION: PROPELLERS OF AN ECONOMIC GROWTH

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Abstract

The two concepts of entrepreneurship and innovation are considered interrelated and mutually dependent. There is a greater need for innovation and entrepreneurship due to globalization, technological progression and development, exaggerated competitiveness and global recession. If the developing countries want to accelerate growth, they should emphasis on entrepreneurship and innovation. This article analyzes the relationship between entrepreneurship and innovation and the implications of this relationship for individuals, and economy growth of a country. In this article factors affecting the innovation have also been discussed. The link between the universities and the industries is very important to achieve a more effective entrepreneurial activity. Industrial firms can reduce their costs of innovation by outsourcing their research by collaborating with educational institutions. Indian economy is one of the fastest growing economies in this modern globalization world. India's vision of becoming a $ 5 trillion economy is complexly connected with an innovation-oriented approach to economic expansion. India has mounted two places and has been ranked 46th by the World Intellectual Property Organization in the Global Innovation Index 2021 rankings. This article mainly presents the scope and elaborate the roles of innovation and entrepreneurship in economy growth.

Keywords: Entrepreneurship, Innovation, Economy.

Introduction

The 21st century is the age of knowledge and information, the age characterized by the capacity to regenerate, develop, employ and protect new and innovative ideas, which particularly comes into play with those industries that base their competitive advantages on advanced technologies. Knowledge transfer attainable today by innovation transfer is exceptionally significant for overcoming the development and technology gap. The development of electronic technology shifts the knowledge gravity centre from manufacturing processes and products to management, information processing and to the development of artificial intelligence.

Entrepreneurship is the process of seeking and utilizing opportunities available in the business environment or those opportunities realized through innovation to create value to business organizations. The compatibility between entrepreneurship and innovation create marketing strategies that are characterized by entrepreneurship and innovation, such strategies involve innovative methods that transform existing products and services into new ones with high quality that adds value to existing businesses. Entrepreneurship is the willingness of an individual / group to take risks and develop, organize and manage a business venture in todays competitive marketplace that is constantly evolving (Techaloo.com, 2017).

Innovation is applying your creativity to come up with a unique idea or solution. It is technological invention, which lets parts do what they could not previously do. You have an engine, wheels and axles and you put them together to invent a car. Progress depends on innovation and great innovators can get rich (Davison, 2015).

The two concepts of entrepreneurship and innovation are considered interrelated and interdependent. There is no doubt that creative innovation has a great impact on entrepreneurship when it comes to designing and executing any business. Innovations can be new products/business

models/customer relationship/any form of new dream/idea. Innovation, however, does not have to mean the creation of something totally new. Improvements and Iterations upon an existing idea/product/service are also innovations. Innovation is not just the idea – innovation is only achieved when the idea has been transferred into an outcome which has value.

Entrepreneurship, by contrast, is applying the innovation to bring the ideas to life. It is social invention, which lets people do what they could not previously do. Entrepreneurs grasp the opportunity to cash in on the innovation. They build businesses and propel the innovation forward. Without innovation, the productions of enterprise become obsolete. According to Akbar Fadaee (2014) entrepreneurs combine creative idea with existing market opportunities and actively pursuing an entrepreneurial opportunity to reality with launch of the business. In short, entrepreneurs have to contribute to accelerating change. Entrepreneurs are pioneers, innovators, leaders and inventors. They are at the forefront of technological, economic and social movements – in their fields, in their forward thinking, in their desire to push forward.

Defining Entrepreneurship and Entrepreneurs:

Entrepreneurship is the process of starting a business or other organization. It involves identifying an opportunity, developing a business plan, and launching the venture. Hundreds of business startups fail after taking a risk to begin entrepreneurship. They have failed because of their lack in innovation. It's easy for these entrepreneurs to see the opportunity, but most don't know how to build it. Hunter (2012) explains it best in that very little innovation is generated by start-up ventures.

Entrepreneurs are persons who convert great ideas into business opportunities. It involves a lot of risk-taking hence most entrepreneurs are risk-averse persons. Most entrepreneurs are innovative or take advantage of an innovation that is currently in the market.

The major characteristics of entrepreneurs that have been listed by many include the following:

1. Hard work, energy and Independent-mindedness
2. A risk taker
3. Self-confident, resilient. and multi-skilled;
4. Innovative skills and determined;
5. Results-orientated and multi-tasking;
6. Self-motivation and total commitment.

Innovative entrepreneurs have something called creative intelligence, which enables discovery yet differs from other types of intelligence (Dyer et al., 2009). It is more than the cognitive skill of being right-brained.

Who are Innovators?

Innovators are persons who introduce something new in terms of a product, service, process, model or an idea into the markets. Innovation is an essential component in all sectors as it adds value and creates change in an existing service or product, while also leading to the creation of new products. Innovation is derived due to technological changes, new markets, new knowledge, and even economic changes. Some of the skills required for innovators include creative thinking and the need for carrying out experiments.

Innovation can take several forms:

1. *Innovation in process*: Process is the combination of facilities, skills, and technologies used to produce, deliver, and support a product or provide a service. The most famous and groundbreaking examples of process innovation is Henry Ford's invention of the world's first moving assembly line. This process change not only simplified vehicle assembly but shortened the time necessary to produce a single vehicle from 12 hours to 90 minutes.

2. *Innovation in products*: Product innovation can come in three different forms.

a) The development of a new product, such as the Fitbit or Amazon's Kindle.

b) An improvement of the performance of the existing product, such as an increase in the digital camera resolution of the iPhone 11.
c) A new feature to an existing product, such as power windows to a car.
3. *Business Model Innovation*: Business Model Innovation is the way in which a product is brought to the market. Examples include introducing subscription-based services, creating new pricing models, or developing new ways to deliver products and services. This form of innovation is almost always radical, risky, and transformative.
4**.** *Social Innovation*: This type of innovation involves creating solutions to social problems. Examples include developing new technologies to address climate change, creating programs to reduce poverty, or developing new ways to improve access to healthcare.

True innovators must behave like entrepreneurs in order to be successful. This means that they must be able to identify potential opportunities in the market, develop strategies for taking advantage of them, and manage the risks associated with launching a new venture.

Distinction between Innovation and Entrepreneurship

Innovation and entrepreneurship are two closely related words used in today's global village. Some may think they mean the same, but using them interchangeably would be making a big mistake. While they revolve around the same idea, it is crucial to understand the difference. Kennard (2021) states that "entrepreneurs are innovators and innovators can be entrepreneurs". Entrepreneurship refers to the "examination of how, by whom, and with what effects opportunities to create future goods and services are discovered, evaluated, and exploited" (Shane & Venkataraman, 2000). This definition subsumes entrepreneurial operations, such as "production innovation, start-ups, new ventures, corporate venture spin-outs, and spin-ins; and, more importantly, multiple levels of analysis from founder-focused, individual and dyadic levels up to the field and international environment contexts" (Jennings et al., 2013)

The main difference between innovation and entrepreneurship is that innovation is the process of creating something new or improving upon an existing idea, while entrepreneurship is the process of turning an innovative idea into a successful business venture. Innovation involves coming up with new ideas and solutions, while entrepreneurship involves taking those ideas and turning them into a viable business. Innovation is essential for businesses to stay competitive in the marketplace, as it allows them to create unique products and services that differentiate them from their competitors. Entrepreneurs must also be able to identify opportunities in the market and develop strategies for taking advantage of them.

Innovation is applying your creativity to come up with a unique idea or solution. Innovators are people who come up with new ideas and inventions. They are creative thinkers who have the ability to think outside the box and come up with unique solutions to problems. Innovators often work in research and development departments of companies or in universities. It is technological invention, which lets parts do what they could not previously do.

Entrepreneurs, on the other hand, are people who take the ideas of innovators and turn them into successful businesses. The combination between entrepreneurship and innovation is a crucial factor to the long-term sustainability of such strategies, because Entrepreneurship and innovation are positively related to each other and interact to help an organization to flourish, both are complementary and a combination of the two is vital to the organizational success and sustainability in today's dynamic environment. Entrepreneurship and innovation are dynamic and holistic processes in entrepreneurial and innovative organizations. Table 1 summarizes distinction between Innovation and Entrepreneurship.

TABLE 1: Distinguishing between Innovation and Entrepreneurship

Innovation	Entrepreneurship
"The process that generates value through the creation, development, and implementation of new ideas, technologies, products, services, and business models" (Kennard, 2021)	The "scholarly examination of how, by whom, and with what effects opportunities to create future goods and services are discovered, valuated, and exploited" (Shane & Venkataraman, 2000)
"Leveraging resources to develop innovation is central to exploiting market opportunities" (Carnes et al., 2022)	The overall challenge of an entrepreneur is how to make innovation, flexibility, and creativity operational (Kennard, 2021)
"At the heart of any innovation process lies a fundamental practice: the way people create ideas and solve problems" (Verganti et al., 2020)	It is influenced by the environment or wider community. It is dynamic and responsive to external changes. May be comprised of an entrepreneurial team (Bruyat & Julien, 2000; Cunningham & Lischeron, 1991)
Innovation can lead to the implementation of not only new products but also new processes, methods, or practices (Gault, 2018; Knight, 1987)	'A process by which individuals, either on their own or inside organizations, pursue opportunities without regard to the resources they currently control' (Stevenson and Jarillo, 1990). The entrepreneurial individual/organizational focus is on the opportunity, rather than the resources
Innovation is more than invention. Invention becomes an innovation only when it is put to productive use	Entrepreneurs recognize, foresee, and can act on opportunities that others do not see or act on. Hence, the entrepreneur is an innovator, responsible for the process of value creation (Bruyat & Julien, 2000)
Not all managers or business owners are entrepreneurs. One can run a business without trying new ways of "doing" business. This attempt at new ideas and new production methods separates these entrepreneurs from the venture of innovation (McDaniel, 2000)	Entrepreneurs use innovation to identify and exploit new opportunities (Grilli, 2022). Entrepreneurs use innovation to create change. In doing so, they often disrupt markets

Social Entrepreneurship

There is a significant demand for entrepreneurs with a new vision to cope with global, national and local problems that the market and the state have failed to solve (OECD, 2010). These opportunities emerge as new social enterprises, which can be defined as organizations driven by social or cultural values as opposed to financial gain or profit. Therefore, social entrepreneurs create value but they are not motivated by the appropriation of this value (European Commission, 2013). On the one hand, a social enterprise seeks to respond to the social needs of its target publics, and for that it needs to be financially sustainable. On the other hand, an enterprise aiming to be profitable needs to serve its stakeholders, namely the shareholders, in order to achieve that goal. Thus, while social entrepreneurship views stakeholders as the "ends" and the social enterprise as the "means" of entrepreneurship, economic entrepreneurship has the opposite perspective and treats the stakeholders as a mean to accumulate financial capital (Ridley-Duff & Bull, 2013). Hence, with different leading purposes, all the organizations must satisfy the economic, social, ecological and psychological demands of their stakeholders through a process of value

creation. However, social value creation is considered the distinctive feature of social entrepreneurship (Sullivan-Mort, Weerawardena, & Carnegie, 2003).

Factors affecting Innovation in the Indian Scenario

Innovation cannot be fully understood without comprehending the opportunities and challenges that it entails. Thus, it becomes imperative to deep dive into innovation with reference to Indian context and identify where our opportunities and challenges lie. Following are the factors that affect innovation:

1) **Research and development (R&D)**

In India, R&D investment has been relatively low. In the past few years, R&D investment in the country has declined from 0.8 per cent of the GDP in 2008–09 to 0.7 per cent in 2017-18. Reason for the low spending on R&D in developing countries like India is that investments in R&D take time to produce results. In a country like India where there are bigger issues such as hunger, disease control, and raising the quality of life to contend with, resources are often diverted towards tackling them. Low private participation is one of the key hindrances in India's overall low R&D expenditure.

2) **Firm Size**

India is a country where the manufacturing sector is peculiarly structured, with either very small-sized firms or a very large-sized firms and a clear 'missing middle'. Large-sized firms have a greater tendency to reap the benefits of innovation than their small-sized counterparts. This could be due to economies of scale, higher costs incurred, etc. With more than 6.5 crore MSMEs contributing roughly 30 per cent to the GDP, there is no doubt that a transition to a mid-sized or large-sized firm and a shift from informal to formal R&D would further enhance the role of MSMEs and innovation in India.

3) Labour market

It is sometimes believed that adopting new and innovative technologies would displace labour from the market. In India, this belief can cause great concern due to the country's massive labour force. However, this is not always true as innovation has the capacity to generate new jobs as well. Analysing data for pharma, transport, ferrous metals, and textiles for the period 2000–01 to 2013–14, showed that there exists a positive relationship between innovation and employment. In fact, it suggested that innovation is the factor that would drive long-term growth and employment.Moreover, it is not just labour availability and the quality of labour but also labour legislations that affect innovation. A right balance between labour laws that doesn't compromise on labour standards which also promotes an environment that is conducive to nurture innovation is something that should be strived for.

4) Risk Factor

There is always an element of risk involved in innovation. But most Indians tend to be risk-averse, which is tied to a fear of and intolerance for failure, making it difficult to generate innovative ideas or promote existing ones. In the absence of adequate support moral, financial, and other our youth migrate to other countries rather than taking risk working in our own country.

5) **Market demand**

Research should also be aligned to the demands of the industry. It is observed that there is a mismatch between what is taught at the university level and what is required at the industrial level. To resolve this, we need a working model between the two. Working with the academia and research institutions can allow firms to gain early access to research outputs and influence the research agenda of these institutions. Subsequently, industrial firms can reduce their costs by outsourcing their research and collaborating with educational institutions.

6) **Venture capital**

Mostly, lending institutes show unwillingness to extend credit to newly established enterprises due to lack of collaterals and a high risk of default. Although microfinance provides capital for businesses, its limited scope and amount curtail the innovative capacity of an undertaking.

7) **Delay in acquiring patents**

Innovation leads to the creation of economically-useful knowledge, in the form of intangible assets like patents and trademarks. In India, such intangible assets filing process are complex and face procedural delays.

Role of Higher Education in Innovation and Entrepreneurship

According to Kahn (2022) most of the students studying innovation and entrepreneurial courses in the universities hardly commence start-ups. But Palladino (2022) contradicts this statement and to him most of the graduates have gone on to develop their own start-ups, join innovation labs or work in the entrepreneurship eco-system. To know this fact, a controlled study to track the students' outcomes needs to be conducted. This would make for an insightful research project.

One approach has investigated the influence of intrinsic personality traits, characteristics, and behaviors to become an entrepreneur. It has been proposed that entrepreneurs "have a high degree of risk tolerance, a high need for achievement, a desire for autonomy, a belief that they can control the achievement of goals, and higher-than-normal levels of creativity, intuition, and opportunism" (Kennard, 2021). Other work has investigated the influence of extrinsic factors such as education, gender, ethnicity, societal norms, generational entrepreneurship, or catalytic events (e.g., job loss) (Dyer et al., 2009). These factors may instigate the entry into higher education to acquire the requisite skills and networks needed to innovate or engage in the process of entrepreneurship by bringing an innovation to market.

Van Looy et al. (2011) show that the scientific productivity is positively associated with entrepreneurial effectiveness, through patent activity and contract research. Thus, the link between the universities and the industries is very important to achieve a more effective entrepreneurial activity. Industrial firms can reduce their costs of innovation by outsourcing their research by collaborating with educational institutions.

Educational institutions can create conditions favorable for entrepreneurship to be established, and this can be supported by government resourcing and policy. There is clearly an increased pressure to emphasize entrepreneurship education to stimulate levels of economic activity (O'Connor, 2013). Entrepreneurship courses will produce aspiring managers who are aware of effective ways to pursue opportunities and manage resources.

Preparing students for entrepreneurship has become a key strategic objective in the knowledge-based economy to assist in the sustained growth of successful start-ups and SMEs. Entrepreneurs need to have the skills to keep up with market conditions that include rapid changes to innovation. Idea generation remains core to successful innovation and essential for student training.

In a learning environment, subject design should be determined by learning objectives. If the subject aims to teach students only about how to develop a new product, innovation is the focus. If, however, they wish to extend this to how a student would go to market and raise capital to do so, then entrepreneurship too becomes relevant.

According to the Economic Survey 2021-22 of India, the average pendency for final decision in acquiring patents in India is 42 months as of 2020. This is much higher than 20.8, 20, 15.8 and 15 months respectively for the USA, China, Korea and Japan.

Economic conceptions of Innovations and Entrepreneurship

Economic conceptions of innovation and entrepreneurship focus on the economic benefits that can be gained from creating new products and services. Innovation is seen as a way to create value for customers, while entrepreneurship is seen as a way to capture that value. Innovative entrepreneurs must be able to identify potential opportunities in the market, develop strategies for taking advantage of them, and manage the risks associated with launching a new venture. By combining innovation and entrepreneurship, businesses can create new products and services that will help them to succeed in the

marketplace. Economists view entrepreneurship as an important factor in economic growth, as it can create jobs and stimulate economic activity by development of new technologies.

Entrepreneurs are also important for creating a competitive environment in the economy, which can lead to increased efficiency and better quality products. Among the reasons that prompt people to entrepreneurship are the raising of the economic level, the actualizing of new products and ideas, the obtaining of self-assurance and social status (Akın, 2010).

There is a greater need for innovation and entrepreneurship today due to globalisation, technological progression and development, exaggerated competitiveness and global recession. Also, it will continually impact consumers and economic development in the future (Hisrich et. al., 2014). So, innovation and entrepreneurship is the important subject for almost small, medium or large businesses in every sector. Moreover, even as companies abstain from how innovative they are to be, the conditions of today constantly impose on the companies on continuous and sustainable economic and social innovations. As much as it takes place in the global innovation economy; it has become extremely important to be able to manage the innovation that will guide the companies and institutions which will be in the activities of innovation and to create the climate that produces new entrepreneurs in this aim (Büyükuslu, 2012).

If the developing countries want to accelerate growth, they should emphasis on entrepreneurship and innovation. In the past, entrepreneurs failed to achieve sustainable success because they were missing some aspects of management; or achieve success in the long-term. In this context, the importance of management shows up in approaches about entrepreneurship.

Entrepreneurial Innovation and Economic Growth

The recent history seems to show us that innovation is one of the most important factors for economic growth. It aids companies in overcoming growth barriers by coming up with fresh solutions for prospective issues. There is a positive relationship between economic growth and innovation according to an International Monetary Fund study. The impact of electricity and railroad technology on boosting economies in the late 19th century and the communications and computer revolutions of the 1990s are good examples. Spending on innovation has many advantages as each tech job supports three jobs in other parts of the economy. Moreover, in some countries spending on digital infrastructure is becoming more important than spending on physical infrastructure projects such as bridges, dams, and highways. Digital transformation and technological innovation boost many sectors such as education, infrastructure, manufacturing, agriculture, and health with spillover effects happening through technology transfers and knowledge sharing.

The Fourth Industrial Revolution will drive the next spurt of economic growth. Innovation is vital to fight off climate change through decarbonization, renewable energy and carbon capture technology. A greener economy will have positive effects for the global economy with the International labour organisation estimating the creation of 100 million new jobs by 2030. The increasing use of artificial intelligence to replace monotonous work can drive human capital towards work that requires cognitive skills which in turn will increase productivity. Fintech is driving financial inclusion and access to finance notably among women. Blockchain technology and cloud computing are vital in proliferating digital enhancement. Taking account of all these factors, innovation can once again drive economic expansion and ease fiscal policy making in the future. (Tall Rafi , Development Economist). GDP is the measurement of a countries output and in order to increase the GDP there are two ways: (1) by increasing the number of inputs that we use into the production process. (2) By increasing the productivity of inputs. Productivity can be increased by innovating new products or by innovating new production processes.

Innovation and Economic Growth in Indian Context

Indian economy is one of the fastest growing economies in this modern globalization world. India's vision of becoming a $ 5 trillion economy is complexly connected with an innovation-oriented approach to economic expansion. India has mounted two places and has been ranked 46th by the World Intellectual Property Organization in the Global Innovation Index 2021 rankings. India has been on a rising course over the past several years in the Global Innovation Index (GII) from a rank of 81 in 2015 to 46 in 2021. Filing of intellectual property (IP) patents in India rose 30% in the last five years while the number of patents granted during the same period almost tripled, according to the Economic Survey 2021-22. The survey noted that 58,502 patents were filed in India in 2020-21, up from 45,444 in 2016-17. Similarly, 28,391 patents were granted in India in 2020-21, up from 9,847 in 2016-17 and 7,509 in 2010-11. Though patents filed in India have grown considerably, as per the World Intellectual Property Organization (WIPO), the number is still a fraction of the 5.30 lakh patents granted in China and 3.52 lakh patents granted in the USA, according to the Economic Survey (Invest India: webpage). We can utilize our demographic dividend to foster innovation and drive the nation towards becoming a knowledge economy.

India is comprehensively prepared to steer in a new age of creation and expansion in the current scenario. Compelled by 'Atmanirbhar Bharat' and 'Make in India' endeavours, there is a solid stimulation to empower the regional manufacturing sectors that would, in turn, contribute to the production of innovative products at economical rates. Along with improving the production scale, India has also strived to enhance its study capabilities by introducing new Science, Technology, and Innovation Policy 2020. These efforts play a critical part in promoting the country's innovative power.

The government's Atal Innovation Mission, DST-NIDHI's PRAYAS, Digital India and Startup India has also uplifted the entrepreneurial spirit. These initiatives have facilitated access to the essential resources to harness the youthful vibrancy of the country. The current innovation ecosystem has been made a part of India's policy plan to expand, bolster and drive a positive impact. One of the major objectives of Digital India is to achieve **"Faceless, Paperless, Cashless"** status. The promotion of digital payments has been accorded the highest priority by the Government of India to bring each and every segment of our country under the formal fold of digital payment services. The vision is to provide the facility of seamless digital payment to all citizens of India in a convenient, easy, affordable, quick and secured manner.

During the last three years, digital payment transactions have registered unprecedented growth in India. Easy and convenient modes of digital payment, such as Bharat Interface for Money-Unified Payments Interface (BHIM-UPI); Immediate Payment Service (IMPS); pre-paid payment instruments (PPIs) and National Electronic Toll Collection (NETC) system have registered substantial growth and have transformed digital payment ecosystem by increasing Person-to-Person (P2P) as well as Person-to-Merchant (P2M) payments. At the same time, pre-existing payment modes such as debit cards, credit cards, National Electronic Funds Transfer (NEFT) and Real-Time Gross Settlement (RTGS) have also grown at a fast pace. **BHIM-UPI has emerged as the preferred payment mode of users.** The Government of India also launched the digital payment solution **e-RUPI,** a cashless and contactless instrument for digital payment which is expected to play a huge role in making Direct Benefit Transfer (DBT) more effective in digital transactions in the country. All these facilities together have created a robust ecosystem for a digital finance economy. UPI has gone a long way in making digital payments a habit, and in firmly placing India on the track toward a cashless economy (Press information Bureau Government of India: webpage). Innovation cannot be fully understood without comprehending the opportunities and challenges that it entails. Thus, it becomes imperative to deep dive into innovation with reference to Indian context and identify where our opportunities and challenges lie.

Conclusion

Innovation and entrepreneurship are key to a any countries economic growth. Promotion and allocation funds towards education and research & development can enhance long term sustainable economic growth of a country. India can be a leader county instead of a follower country by increasing the innovation. To achieve this goal primary focus should be more on education and R&D spending that will lead to the financial productivity of India in future. This article concludes the effectiveness of innovation and entrepreneurship in the economic growth of a country.

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Vol. VIII, No. 01(XXI) : 2022
ISSN - 2277-7067

राष्ट्रहिताय संस्कृतम्

Kavikulaguru Kalidas Sanskrit University
Ramtek, Dist. Nagpur, Maharashtra

Peer Reviewed

Journal of Fundamental & Comparative Research

UGC CARE Listed Journal

शोधसंहिता

New Research Frontiers

Research Journal
ISSN No. 2277-7067

Journal of Fundamental & Comparative Research

शोधसंहिता

A Peer Reviewed Bi-annual Interdisciplinary Research Journal of the University

UGC CARE Listed Journal
New Research Frontiers

राष्ट्रहिताय संस्कृतम्

KAVIKULAGURU KALIDAS SANSKRIT UNIVERSITY
RAMTEK

Shodhasamhita : Journal of Fundamental & Comparative Research
Vol. VIII, No. 1(XXI) : 2022
ISSN: 2277-7067

बाल मजदूरी : कारण एवं निवारण

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सार–संक्षेप

मनुष्य एक सामाजिक प्राणी है। मनुष्य के विकास से ही समाज की उन्नति संभव होती है। स्वस्थ समाज और राष्ट्र का निर्माण तब ही संभव है, जब प्रत्येक व्यक्ति को जीवन विकास के पर्याप्त साधन उपलब्ध हों। इसलिए प्रत्येक व्यक्ति को उचित साधन प्रदान किये जाए ताकि वह अपने परिवार का भरण–पोषण कर सके। उसके बच्चे शिक्षित हों और उचित रोजगार प्राप्त कर अपने जीवन के वास्तविक लक्ष्यों और उद्देश्यों की प्राप्ति कर सकें। इसलिए प्रत्येक राष्ट्र की यह नैतिक जिम्मेदारी है कि वह अपने नागरिकों को विशेषकर बच्चों को उचित शिक्षा प्रदान करें, क्योंकि बालक राष्ट्र की धरोहर होते हैं। बालकों के विकास से ही राष्ट्रों का विकास संभव है। परन्तु यह तब संभव हो पाएगा, जब बच्चों का उचित मानसिक और शारीरिक विकास होगा। परन्तु वास्तविकता तो यह है कि प्रत्येक बच्चे को उचित शिक्षा और साधन नहीं मिल पाते जिनके अभाव के कारण वह अपने जीवन के लक्ष्यों और उद्देश्यों की पूर्ति नहीं कर सकता। अतः उसे मजदूरी करके अपनी आवश्यकताओं की पूर्ति करनी पड़ती है, जिससे वह अपने बचपन, शिक्षा और नैतिक मूल्यों से दूर चला जाता है। इस श्रेणी के बालकों को अपनी मूलभूत आवश्यकताओं की पूर्ति के लिए भी संघर्ष करना पड़ता है। इससे बच्चों, परिवार और समाज पर प्रतिकूल प्रभाव पड़ता है। वास्तव में इस प्रकार की बाल–मजदूरी से पूरा समाज और राष्ट्र आहत होता है। बाल–मजदूरी के अनेकों ऐसे कारण है, जिससे इसमें बढोतरी हो रही है। सरकार द्वारा बाल–मजदूरी रोकने के अनेकों सार्थक प्रयास किये है, जिसमें कुछ सफलता भी प्राप्त हुई है, लेकिन भविष्य में इस दिशा में और अधिक प्रयास करने की परम आवश्यकता है।

मुख्य शब्द : विकास, मजदूरी, शिक्षा, नैतिक, संघर्ष, सार्थक, लक्ष्य, उचित।

बाल मजदूरी : कारण एवं निवारण :

भारत में प्राचीन समय में संयुक्त परिवार प्रणाली बहुत ही सुदृढ थी। सभी लोग आपस में मिलजुल कर जीवन व्यतीत करते थे। आपसी सौहार्द का वातावरण था। सभी लोग आत्मनिर्भर और स्वावलम्बी थे। लेकिन जैसे–जैसे समय परिवर्तित होता गया, संयुक्त परिवारों का रूप खंडित होने लगा। भारत में पिछले तीन दशकों में यह स्तर बहुत ही व्यापक हो गया और आपसी संबंध बिगडने लगे, जिससे एकांकी परिवारों की संरचना उभरने लगी। इससे सामाजिक परिदृश्य बदल गया और लोगों के आय के साधन और जीवन निर्वाह में चुनौतियां सामने आने लगी। जिससे परिवार के अधिकतर सदस्य छोटे–छोटे कार्य करने लगे, और उन्हें मेहनताना प्राप्त होने लगा और इसी कार्य ने बाल मजदूरी को जन्म दिया। परिवार को सहयोग देने की इच्छा ने उन्हें मजदूर बना दिया। धीरे–धीरे यह प्रवृति व्यापक स्तर पर उभरने लगी। जिससे अधिकतर बच्चे कार्य करने लगे, और पैसों के आकर्षण ने उन्हें मजदूर बना दिया।

साधारण शब्दों में बाल मजदूरी भारतीय समाज और संस्कृति पर एक व्यंग्य है, जिसने सामाजिक परिकाष्ठा और परिवारों के स्वरूप को बहुत प्रभावित किया है। बाल मजदूरी किसी भी क्षेत्र में बच्चों द्वारा अपने बचपन में दी गई सेवा बाल मजदूरी है। इसे गैर जिम्मेदार अभिभावकों के कारण, या कम लागत में निवेश पर अपने लाभ को बढ़ाने के लिए मालिकों द्वारा जबरदस्ती बनाए गए दबाव के कारण या जीवन जीने के लिए जरूरी संसाधनों की कमी के चलते बच्चों द्वारा स्वेच्छा से किया जाता है। लेकिन इन सभी के कारण अर्थहीन हो जाते है, क्योंकि इन सभी कार्यों के करने से बच्चों का बचपन दयनीय बन जाता है। हमारे देश में बाल मजदूरी एक व्यापक रूप धारणा करती जा रही है।

भारतीय संविधान के अनुसार वह व्यक्ति जिसकी आयु 14 वर्ष से कम हैं, और पैसा कमाने के लिए काम कर रहा है, बाल मजदूर कहलाता है। बाल मजदूरी से बच्चों को मानसिक और शारिरिक विकास के अवसर नहीं मिलते और परिणाम यह होता है कि उनके जीवन के विभिन्न अवसर कम हो जाते हैं। जिससे वे अपने जीवन के वास्तविक लक्ष्यों और उद्देश्यों की प्राप्ति नहीं कर पाते। भारत में मजदूरी करने वाले बच्चों में एक बड़ी संख्या ग्रामीण क्षेत्रो से संबंधित है। एक अनुमान के अनुसार लगभग 80 प्रतिशत बाल मजदूरी की जड़े और संबंध ग्रामीण क्षेत्रों से है। देश में ऐसे पांच राज्य है, जिनमें बाल मजदूरों की संख्या सबसे ज्यादा पाई जाती है। इन राज्यों में उत्तर प्रदेश, बिहार, राजस्थान, महाराष्ट्र और मध्यप्रदेश में है, यहां बाल मजदूरों की संख्या लगभग 55 प्रतिशत है। इन पाँच राज्यों में से उत्तर प्रदेश और बिहार में बाल मजदूरों की संख्या सबसे अधिक है।

बाल मजदूरी केवल भारत में ही व्याप्त नहीं है बल्कि यह वैश्विक स्तर पर विद्यमान है। विश्व में बाल मजदूरी की सबसे ज्यादा संख्या अफ्रीका में विद्यमान है। यहां 7.21 करोड़ बच्चे बाल श्रम की कैद में है। यहां तक कि दुनियां के सबसे विकसित कहे जाने देश अमेरिका में बाल मजदूरों की संख्या एक करोड से भी अधिक है। यही नहीं यदि पूरे विश्व का आंकलन करें तो लगभग 152 मिलियन बच्चे बाल मजदूरी में ग्रस्त है। जिनमें से 73 मिलियन बच्चे अपनी आजीविका के लिए खतरनाक उद्योगों में कार्यरत हैं। भारत में बाल मजदूरों की संख्या लगभग 47 लाख है। जोकि कुल

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Journal of Fundamental & Comparative Research

This is to certify that the article entitled

बाल मजदूरी : कारण एवं निवारण

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Published in

Shodhasamhita : Journal of Fundamental & Comparative Research
Vol. VIII, No. 1(XXI) : 2022
ISSN: 2277-7067
UGC Care Approved, Peer Reviewed and Referred Journal
Kavikulaguru Kalidas Sanskrit University, Ramtek

ज्ञान-विज्ञान विमुक्तये
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