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Original Article

Optimization of Green Synthesized Black Tea Nanoparticles using Central Composite Design

Sarika Nikam*, Shilpa Chaudhari

Department of Pharmaceutics, Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune, Maharashtra, INDIA.

ABSTRACT

Objectives: Traditional method of optimization is lengthy and time consuming while response surface methodology evaluates the effects of multiple factors and their interactions on one or more response variables with fewer experiments. The aim of present study is optimization of green synthesized black tea nanoparticles using central composite design. **Materials and Methods:** 5, 15, and 25% black tea concentrations were reacted with 5, 10, and 15 mM Silver nitrate ($AgNO_3$). An optimization study was carried out to optimize the levels of the independent factors like concentration of extract, solution of Silver nitrate, stirring speed, and stirring time. 30 numbers of experiments were performed and evaluated for responses like particle size and % yield. Characterization of silver nanoparticle was done by UV-visible spectroscopy, zeta sizer, XRD, FTIR, FESEM, etc. **Results:** Nanoparticles formation was revealed by color transformation from light yellow to brown. Prepared particles were monodisperse with Z-Average: 137.8 nm, polydispersity index 0.278, and Zeta potentials 22.7 mV. Electrophoretic Mobility Mean was 0.000176 cm 22/Vs, indicating the stability of silver nanoparticle suspension. **Conclusion:** Optimized parameters offered by Central Composite Design were 10mM $AgNO_3$, 10% extract of black 150 min, and 700 rpm. 3D plots revealed that the metal salt concentrations and stirring rate showed a direct relationship whereas extract concentration and stirring time showed indirect relationship with particle size. % yield was highest with mid level of solution of metal salt (A) and concentration of extract (B) Stirring time (C) and stirring speed had no impact on % yield.

Keywords: Black tea, Silver nanoparticle, Green synthesis, Central composite design, Design expert, Response surface method.

Correspondence

Ms. Sarika Ankushrao Nikam
Department of Pharmaceutics,
Dr. D. Y. Patil College of Pharmacy, Akurdi,
Pune, Maharashtra, INDIA.
Email id: sarikadeshmukh1986@gmail.com

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INTRODUCTION

Green synthesis of nanoparticle is one of the tactics to develop metal nanoparticles. Plant extract and biological micro-organisms are used in green synthesis for development of nanoparticles. Green synthesis of metal nanoparticles is cost effective, easy to scale up and nature friendly method.[1] In metal nanoparticles synthesis reducing agents reduces metal ions and stabilizing and capping agents offers stability to the preparation.[2] Silver nanoparticles show strong biocidal effect against different micro-organisms, also used in prevention and treatment of many diseases and infections.[3] Apart from therapeutic applications, silver nanoparticles are increasingly employed in apparel, food, implant coatings, and other applications.[4] *Camellia sinensis* is an original Chinese plant, which provides the leaves for the tea brew.[5] Due to its wide usage and cost-effectiveness, black tea infusion is being used in this study, with this trying to estimate the effectiveness of black tea in the development of AgNPs. The major component present in tea is polyphenols, which make up 20–35% of its dry weight.[6] The fermentation process causes changes in the amount of polyphenol in leaves which gives the dark red color to black tea.[7] The polyphenols are separated into two different primary component types. The first is catechins and the second group is phenolic acids like Gallic acid.[8] Polysaccharides, alkaloids, caffeine, amino acids, and saponins are other compounds present in the tea infusion.[9,10] The amount of various phytochemicals in the leaves is highly influenced by the processing used during manufacture, as well as the age and origin of the plant.[11] In the present study, silver nanoparticle structures were successfully generated by using black tea extract.

MATERIALS AND METHODS

Materials

Black Tea powder was procured from the local market of Pune. The analytical grade silver nitrate ($AgNO_3$) and all other chemicals used in the experiment were acquired from Research Lab Fine Chemicals Ltd., Mumbai, India, and were used exactly as received. Freshly prepared double distilled water was used in

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all experimental procedures. Preparations were stored in dark to minimize photochemical reactions.

Methods

Extraction of maximum phenolic compounds from Black tea

Heat application increases plant cell permeability by disrupting the cells and also breaks the interaction between polyphenol and lipoproteins, which increases polyphenol solubility in water. The heat extraction method can extract the most polyphenol content possible. 10 gm black tea powder, 100 ml of distilled water were combined in a conical flask, and stirred for 4 hr at 40°C. The liquid extract was vacuum filtered through Whatman filter paper no. 1 and then stored at 4°C for later use.[12]

Synthesis of silver nanoparticles using black tea extract

Black tea extract and Silver nitrate mixed and Stirred at 700 RPM for 2 hr. The color of the solution turned yellowish to reddish-brown. Silver nanoparticle suspension was centrifuged and washed with double distilled water 3-4 times. Silver nanoparticle suspension was lyophilized and stored in at cool, dry and dark place.[13]

Preliminary screening for synthesis of nanoparticles

The selection of metal salt and plant extract was done by performing preliminary screening. 5, 10, 15% concentration of metal salt were treated with 5, 15, 25% of black tea extract. Various batches were performed and examined for UV spectroscopy to confirm the effect concentration on the synthesis of nanoparticles. Upper and lower limits of the concentration of metal sat as well as plant extract were selected for further study of experimental design and optimization.[12]

Optimization by Design of Experiment

5% to 20% concentrations of plant extract were selected for optimization study. Central Composite Design was applied for the optimization of independent factors and their relationships were investigated by Response Surface Method. Design Expert 13 Trial version was used for the study. Independent factors selected for the study were the solutions of metal salt (A), the concentration of extract (B), stirring time (C), and stirring speed (D).[13] Values used for experimental design are listed in coded form in Table 1. According to CCD, a total of 30 runs of experiments were performed as shown in Table 2.

Table 1: Experimental variables in coded form.

Independent Factors	Symbol	High	Low
Solution of metal salt (mM)	A	-1	+1
Conc. of Extract (%)	B	-1	+1
Stirring Time (Min)	C	-1	+1
Stirring Speed (RPM)	D	-1	+1

Table 2: Experiments.

	Factor 1	Factor 2	Factor 3	Factor 4
Run	Solution of metal salt (mM)	Conc. of Extract (%)	Stirring Time (Min)	Stirring Speed (RPM)
1	0	0	0	0
2	1	1	1	1
3	-1	1	-1	-1
4	-1	1	1	-1
5	0	-2	0	0
6	-1	-1	-1	1
7	-1	-1	-1	-1
8	1	1	-1	1
9	1	-1	-1	1
10	0	0	2	0
11	1	-1	1	1
12	1	1	-1	-1
13	0	0	0	2
14	-1	1	1	1
15	-1	1	-1	1
16	1	-1	-1	-1
17	0	0	0	0
18	0	0	0	0
19	0	0	-2	0
20	0	0	0	-2
21	0	0	0	0
22	-1	-1	1	-1
23	0	0	0	0
24	-1	-1	1	1
25	1	1	1	-1
26	1	-1	1	-1
27	0	0	0	0
28	-2	0	0	0
29	0	2	0	0
30	2	0	0	0

Analysis of silver nanoparticles

UV-vis spectroscopy

Color changes in the solution were noted by visual observation at time intervals of 30, 60, 90, and 120 min. Absorbance was measured using UV–visible spectrophotometer with 1cm quartz cells (UV-1700 Shimadzu) at time intervals of 30, 60, 90, and 120 min. The wavelength range selected for the study was 200–800 nm at room temperature.[12-15]

Zeta potential and size of particles

Silver nanoparticle suspension was diluted in deionized water. Particle size and polydispersities were determined by Particle size Nano Particle Analyzer (Horiba Scientific and S2-100).[12-15]

Fourier Emission Scanning Electron Microscopy (FESEM)

Morphological features of nanoparticles were studied by using FEI Nova Nano SEM 450. A freeze-dried sample of nanoparticles was used for analysis. A smear of Sonicated freeze-dried nanoparticles was made on the platinum grid, dried overnight under a vacuum, and performed a FESEM study.[12-15]

Powder X-ray diffraction

A powder X-ray diffraction (PXRD) study was done on an X-ray diffractometer (PW 1729 Philips, Netherland) by using freeze-dried samples of Black tea extract and silver nanoparticles.

FTIR

FTIR study was done to determine major functional groups in Black tea extract as well as AgNPs. It was done by FTIR (Perkin Elmer, Spectrum BX) using limits of 4000 to 280 cm^{-1}.[12-15]

Total phenolic content in Black tea extract

The amount of total phenol was ascertained using the Folin-Ciocalteu (FC) reagent method. The extract was reduced with FC reagent, resulting in the formation of a blue colour. The calibration curve of gallic acid was used to estimate the phenolic content concentration. Gallic acid solution was combined with FC reagent (2.5 ml) and sodium carbonate (75 g/L) to create calibration curves at concentrations of 50, 100, 150, 200, 250, and 300 g/ml (2.5 ml). The solution was incubated for 30 min. UV-vis spectrophotometer was used to measure absorbance at 765 nm. By individually mixing 1 ml of Black tea extract with 2.5 ml of FC reagent and 2.5 ml of sodium carbonate solution, the absorbance was calculated. Gallic acid equivalent mg/gm of solution was used to represent the total phenolic content.[16]

Phenolic compounds profile in AgNPs

By using HPLC (Waters Alliance 2695 HPLC with PDA) with a separating column of Zobrax SB C_{18} 1504.6 mm 5 m and a flow rate of 1.0 ml/min, phenolic compounds present in the extract and AgNPs were identified. Water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B) were the mobile phases used in the process. Using the programme Empower 2, chromatograms were captured at 280 nm, phenolic compounds were calculated.

Antibacterial activity

Black tea AgNPs were tested for their ability to inhibit the growth of gram-positive *Staphylococcus aureus* gram-negative *Pseudomonas aeruginosa* bacteria. Bacterial strains were acquired from the Dr. D. Y. Patil Medical College, Hospital and Research Center's Laboratory, Pimpri, Pune. To regenerate the collected strains, 200 µL of each culture was transferred into separate tubes that already contained 10 mL of nutrient broth. On nutrient agar plates, diluted cultures were spread using a sterile cotton stick to create a lawn of bacterial culture. A 6 mm diameter borer was used to create the wells. 50 µL of 1 mg/mL AgNPs dispersion and standard was poured into wells. At 37 2°C, plates were incubated for 24 hr. The clear zone around the well in the test strain lawn was regarded as an antimicrobial activity of AgNPs. The diameter of the inhibition zone (in mm) was measured. The results of the triplicate study were presented as mean SEM.

RESULTS

Synthesis of silver nanoparticles

Preliminary screening for synthesis of nanoparticles

Black tea extract in concentrations of 5, 15, and 25% was used to successfully create silver nanoparticles. Figure 1 shows the UV-Vis spectrum of synthesized nanoparticles. To design and optimize the experiment, 5, 25% concentrations were chosen as the upper and lower concentrations of the Black tea extract. It was observed that the synthesis of AgNPs was plunged after using more than 15% Black tea extract, as seen by the diminished intensity of UV peaks. With an increase in concentration from 5 to 15%, both the yield percentage and peak intensity rise. The increased availability of reducing agents from black tea extract could be the cause of the higher production at low extract concentrations.

Explanation of regression analysis

The effect of independent factors (concentration of silver nitrate, the concentration of plant extract, stirring time, and stirring speed) on the size of particle and production yield was checked. For given levels of each element response predictions were done in terms of coded factors. In coded equations, factor coefficient comparison is useful to identify the relative impact of factors.

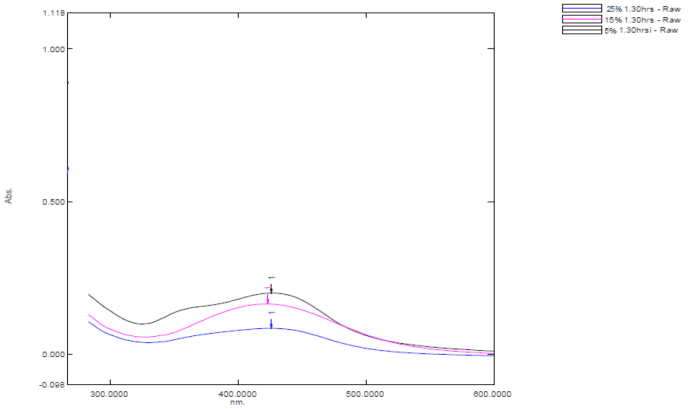

Figure 1: UV–Vis spectral analysis of silver nanoparticles synthesized using 5, 15, 25% concentrations of plant extract.

Table 3: ANOVA: Particle size (Quadratic model Response).

Source	d_f	Mean Square	*F*-value	*p*-value	
Model	14	21799.23	11.60	< 0.0001	Significant
A-Solution of metal salt	1	85994.88	45.77	< 0.0001	
B-Conc of extract	1	3582.42	1.91	0.1876	
C-Time for stirring	1	1.460E+05	77.69	< 0.0001	
D-Speed of stirring	1	19129.78	10.18	0.0061	
AB	1	956.36	0.5090	0.4865	
AC	1	389.67	0.2074	0.6553	
AD	1	3672.36	1.95	0.1824	
BC	1	322.92	0.1719	0.6843	
BD	1	1774.09	0.9442	0.3466	
CD	1	343.18	0.1826	0.6752	
A^2	1	15.12	0.0080	0.9297	
B^2	1	41734.90	22.21	0.0003	
C^2	1	620.81	0.3304	0.5739	
D^2	1	1507.58	0.8024	0.3845	
Residual	15	1878.94			
Lack of Fit	10	2043.35	1.32	0.4008	Not significant
Pure Error	5	1550.11			
Cor Total	29				

d_f is the degree of freedom

Table 4: Particle size Fit Statistics.

Standard deviation	43.35	R^2	0.9155
Mean	325.72	R^2 Adjusted	0.8366
C.V. %	13.31	R^2 Predicted	0.6135
		Adeq Precision	11.6341

Table 5: ANOVA: % yield (Quadratic model Response).

Source	d_f	Mean Square	*F*-Value	*p*-Value	
Model	14	1050.88	13.16	< 0.0001	Significant
A-Solution of metal salt	1	833.44	10.43	0.0056	
B-Conc of extract	1	5.91	0.0740	0.7893	
C-Time for stirring	1	33.25	0.4163	0.5285	
D-Speed of stirring	1	132.78	1.66	0.2168	
AB	1	8.90	0.1114	0.7432	
AC	1	54.50	0.6823	0.4217	
AD	1	7.47	0.0935	0.7640	
BC	1	2.30	0.0288	0.8674	
BD	1	6.36	0.0797	0.7816	
CD	1	291.81	3.65	0.0753	
A^2	1	1529.47	19.15	0.0005	
B^2	1	10750.27	134.58	< 0.0001	
C^2	1	269.59	3.38	0.0861	
D^2	1	167.69	2.10	0.1679	
Residual	15	79.88			
Lack of Fit	10	93.71	1.79	0.2692	not significant
Pure Error	5	52.21			
Cor Total	29				

d_f is the degree of freedom

Table 6: % yield Fit Statistics.

Standard deviation	8.94	R^2	0.9247
Mean	77.42	R^2 Adjusted	0.8544
C.V. %	11.54	R^2 Predicted	0.6371
		Adeq Precision	15.0444

Equations generated for particle size and production yields are as follows.

Equation 1

$$\text{Particle size} = +285.37 + 59.86 \times A + 12.22 \times B - 77.99 \times C - 28.23 \times D$$

Equation 2

$$\text{Production yield} = +94.75 + 5.89 \times A + 0.4964 \times B + 1.18 \times C + 2.35 \times D$$

Table 3 and Table 4 shows the outline of an analysis of variance and fit statistics (ANOVA) for particle size respectively. Table 5 and Table 6 shows the outline of an analysis of variance and fit statistics (ANOVA) for % Yield respectively.

Interpretation of 3D response surface graphs

3D Response surface plots show the effect of independent factors on values of particle size and % yield. As shown in Figure 2 Stirring time (C) and concentration of metal salt (A) are both significant factors in the biosynthesis of nanoparticles. Concentrations of metal salt (A) showed an direct effect while stirring time (C) showed indirect effect on particle size. Increased stirring speed (D) caused decreased in the size of particles. Extract concentration (B) also showed a significant effect on the size of particles; extract concentration increased particle size also increased % yield was highest with mid level of solution of metal salt (A) and concentration of extract (B). Stirring time(C) and stirring speed had no impact on % yield.

Analysis Results of synthesized silver nanoparticles

Visual observation: Color change in solution was noted by visual observation, which was an indication of the formation of silver nanoparticles. As shown in Figure 3 color of the silver nitrate and tea extract mixture was changed from yellowish to dark brown and this color change indicates the formation of silver nanoparticles.

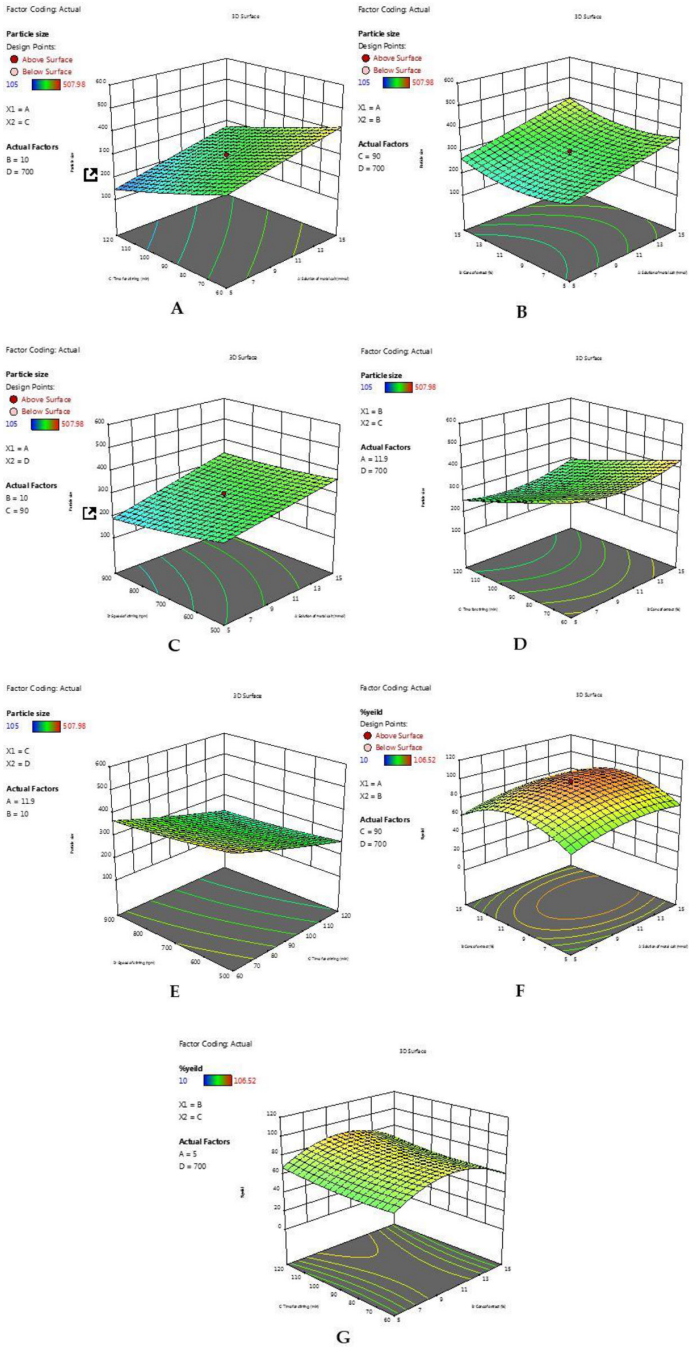

Figure 2: 3D surface response plots of particle size and % yield. A: time for stirring, solution of metal salt, B: concentration of extract, solution of metal salt, C: speed of stirring, solution of metal salt, D: Time of stirring concentration of extract, E: Speed of stirring, time for stirring, F: concentration of extract, solution of metal salt, G: time for stirring, concentration of extract.

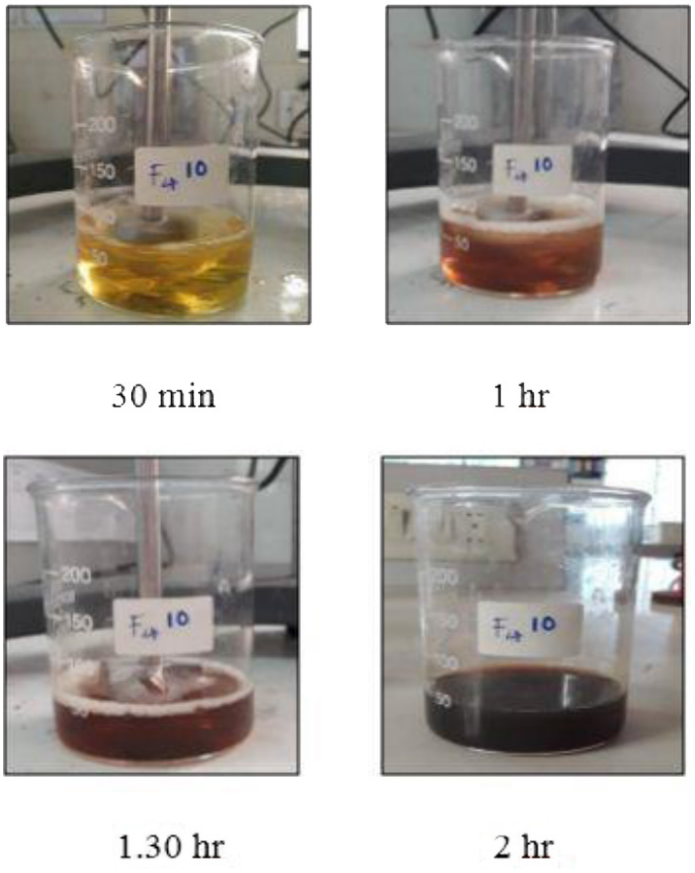

Figure 3: Color changes in solution.

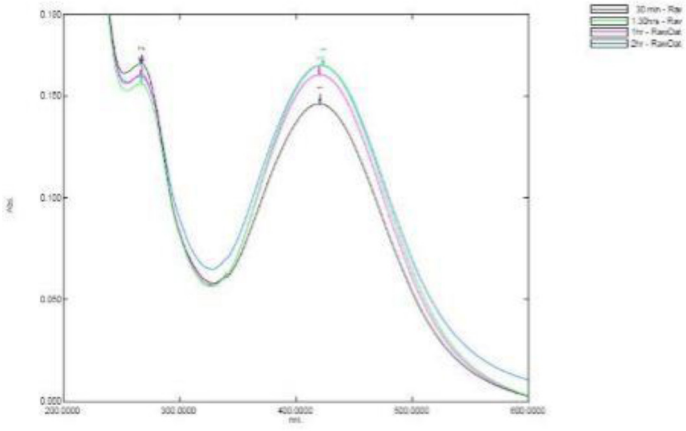

Figure 4: Absorption peak for silver nanoparticles at 30, 60, 90, 120 min.

UV–vis spectrophotometry

One of the popular methods for the characterization of the formation of silver nanoparticles is UV–vis spectrophotometer. The formation of nanoparticles in solution was shown by the appearance of the characteristic peak from 400- 470 nm. The absorption maximum for the sample was obtained at 444 nm. Absorption due to reducing agent that is polyphenol from the extract is observed around 278 nm.

UV spectra for synthesized nanoparticles were run at 30, 60, 90, and 120 min. After 30 min of stirring sample was withdrawn and analyzed by UV-vis spectroscopy. As shown in Figure 4B, the SPR peak intensity increased with reaction time.

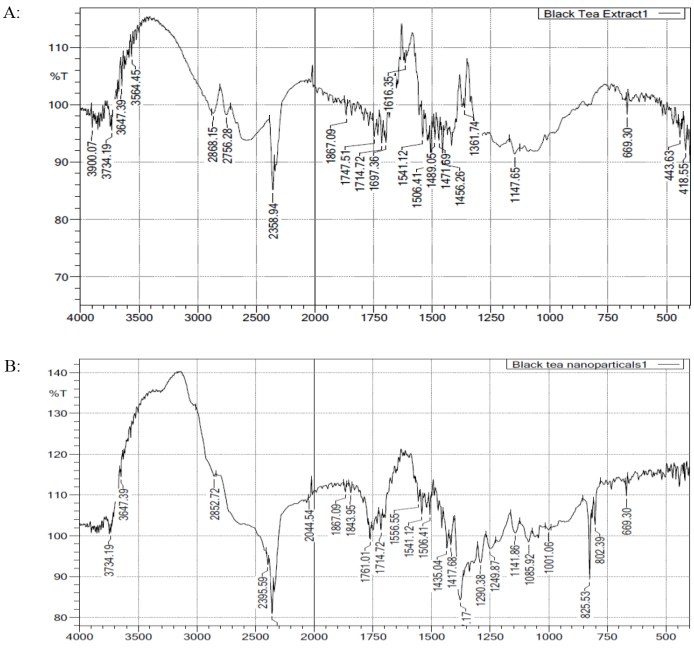

Figure 5: IR SPECTRA A: Nanoparticles, B: Black Tea Extract.

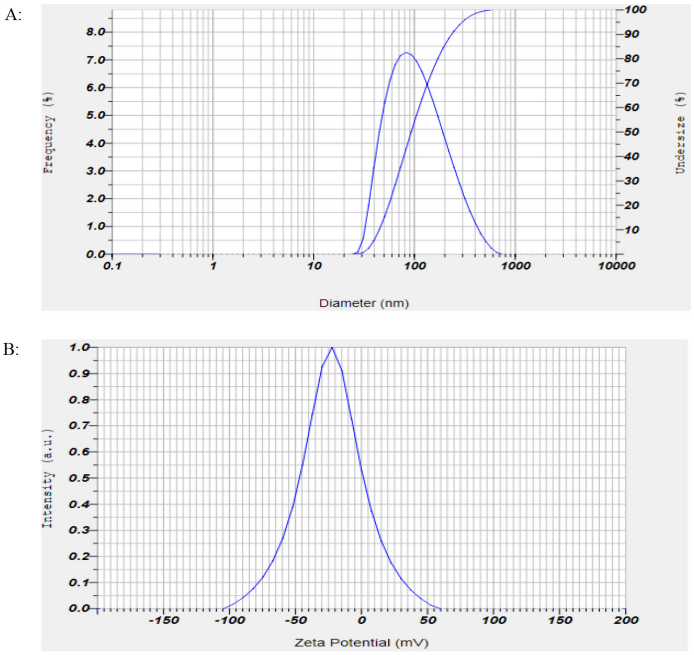

Figure 6: A: Particle size, B: Zeta potential.

FTIR

The chemical composition of silver nanoparticles was identified using FTIR analysis. The FTIR spectrum of prepared nanoparticles and black tea extract are shown in Figure 5A and B respectively. The shift of peak from 2868-2852 is due to N-H stretching, 1747-1761 due to O=H stretching, 1361-1377 (characteristic peak for OH, phenolic hydroxyl group), 1141-1147 (due to C-O stretching). Additional peaks in nanoparticles were observed at 1085 (strong OH stretching).

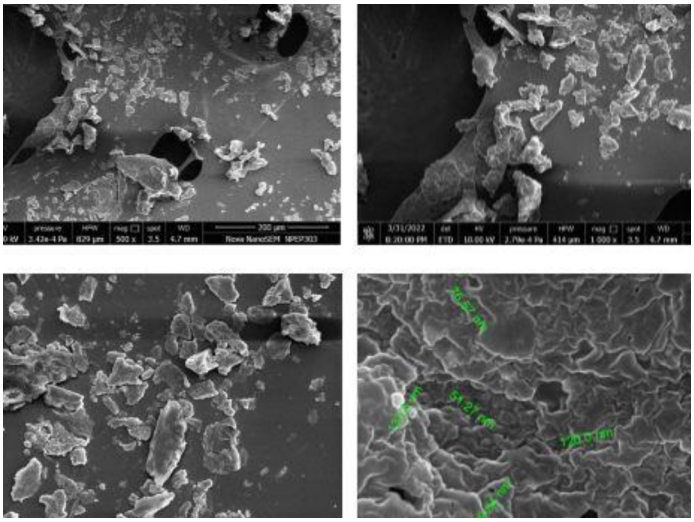

Figure 7: FESEM images of silver nanoparticle.

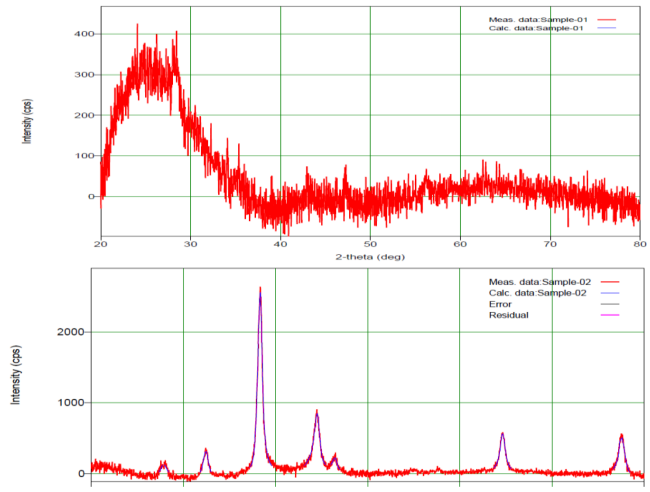

Figure 8: XRD Reports A: black tea extract, B: Black Tea Silver Nanoparticles.

Zeta potential and size of particles

In the case of suspension if the zeta potential is less than 20mV then the suspension is unstable and there will be precipitation of suspended particles but if the zeta potential is higher than 20mV then the suspension will be stable. In this study, the observed zeta potential was 22.7mV with Electrophoretic Mobility Mean 0.000176 cm 22 /Vs, indicating the stability of silver nanoparticle suspension. The particle size study as shown in Figure 6A indicates that particles were monodisperse with Z-Average: 137.8 nm and a polydispersity index of 0.278.

FESEM

Synthesized nanoparticles were visible in the FESEM images as shown in Figure 7. Nanoparticles were observed as aggregates as well as individuals. The synthesized AgNPs had a diameter of range 50-120 nm.[15]

XRD

X-ray crystallography confirms the crystallinity of nanoparticles. The X-Ray Diffraction (XRD) graph of lyophilized Black tea extract is shown in Figure 8A as a control. Figure 8B is showing XRD of Silver nanoparticles prepared from black tea extract. Figure 8A (Black tea extract graph) is not showing any intense peaks but Figure 8B (silver nanoparticles prepared from black tea extract graph) is showing 9 intense peaks of 2θ values ranging from 20 – 80. The facets of the face-centered cubic crystal structure of silver can be indexed as 27.86°, 32.43°, 38.34°, 44.51°, 46.44°, 54.85°, 57.56°, 64.60°, and 77.40°. The interplanar spacing (d-calculated) values are 3.200, 2.759, 2.3456, 2.0337, 1.9538, 1.672, 1.5999, 1.4415, and 1.231 Å. The Debye-Scherrer formula, $D=k\lambda/\beta Cos\theta$, is used to compute the average crystalline size.

Where D is nanoparticle size, k is the geometric factor (0.9), λ is X-ray radiation source wavelength and β is FWHM (full-width at half maximum) of the XRD peak at the diffraction angle θ. According to the study average crystallites of silver nanoparticles were observed at 133.7 nm.[17]

Phenolic content in extract

Using the calibration curve for gallic acid shown in Figure 9, the total phenolic content was calculated and expressed as mg/gm of solution, the gallic acid equivalent. There were found to be 78 mg/gm of total phenolic content (Gallic acid equivalent) in the extract.[16]

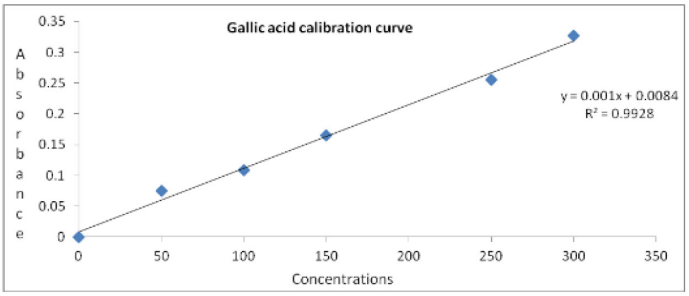

Figure 9: Calibration curve of gallic acid.

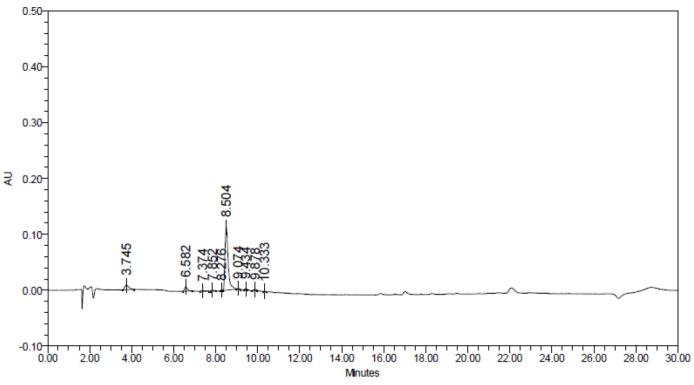

Figure 10: Phenol content profile by HPLC.

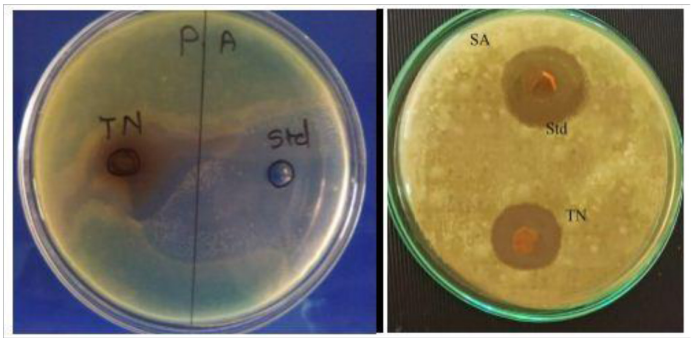

Figure 11: Zone of inhibition by Black tea silver nanoparticles.

Table 7: Zone of Inhibition by AgNPs along with Standard error mean.

	Bacteria	Zone of inhibition mm ± SEM		
Black tea AgNPs	*Staphylococcus aureus*	18.5	±	0.5
	Pseudomonas aeruginosa	14.8333	±	0.44096

SEM = Standard error mean

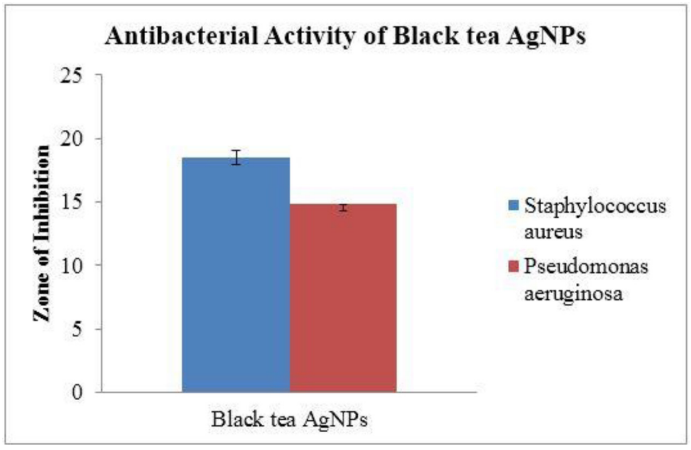

Figure 12: Antibacterial activityof Black tea silver nanoparticles.

Phenolic compounds profile in AgNPs

Gallic acid, catechins, and ellagic acid were the phenolic compounds found in the black tea extract and silver nanoparticles, as shown in Figure 10, with retention times of 3.647, 8.383, and 10.149, respectively.[3]

Antibacterial activity

After incubation, the plates were checked to see if an inhibition zone was present. The disc diffusion test revealed that the AgNPs had a clear zone surrounding them, indicating that they had antibacterial activity that could stop the growth of Gram-positive and Gram-negative pathogens. The zone of inhibition (Figure 11), which corresponds to that of the reference standard, was used to demonstrate AgNP's antibacterial activity. The diameter of the inhibition zone (in mm) was measured. The results of the triplicate study were presented as mean SEM (Table 7 Figure 12).

DISCUSSION

It has been suggested that biologically produced AgNPs are potential therapeutic molecules. Although many nanoparticles have been successfully synthesised using micro-organisms and plants, the search for new nanoparticles with precise biological, physical, and chemical properties is still at the forefront of nano science research.[18] The average crystallite sizes of the AgNPs here increased along with the concentrations of silver nitrate. The average nanoparticle size and the initial concentrations of metal ions are directly correlated, with increasing initial concentrations of metal ions producing larger nanoparticles.[19] The relationship between stirring time and AgNps sizes can be explained by the fact that longer stirring causes deposition of particles, which leads to the formation of bigger nanoparticles. More stirring simply causes more particle dispersion, which leads to smaller particle sizes.[20] Silver nanoparticles with the smallest sizes were found to have lower extract concentrations, whereas higher extract concentrations may increase the likelihood of particle agglomeration and, as a result, increase nanoparticle size. Furthermore, a lower extract concentration allows for the provision of an adequate amount of antioxidants to synthesise silver nanoparticles of an acceptable size, whereas a higher extract concentration may cause the reaction to continue and increase the size.[21] When polyphenolic rich extract was exposed to silver nitrate, silver ions were reduced to silver particles, and the color of the reaction mixture changed from yellowish to dark brown. The reddish-brown shade of the solution was due to the SPR of the silver nanoparticles formed. The Surface Plasmon Resonance (SPR) is a technique that uses a certain wavelength of incident light to cause oscillations in surface electron nanoparticles. Excitation of Surface Plasmon vibrations in metal nanoparticles causes changes in the color of the solution. Effect of time on formation of AgNPs was recorded. The broadening of the peak in the UV-Vis spectrum indicated that the particles are polydispersed. Figure 4 shows that the SPR peak intensity increased with reaction time, indicating that the silver ions continued to be reduced, and that the concentration of AgNPs is rising as a result of an increase in absorbance. At 30, 60, 90, and 120 min, UV spectra of synthesised nanoparticles were run. After 30 min of stirring, the sample was removed and put under UV-vis spectroscopy analysis. The SPR peak intensity increased with reaction time, as shown in Figure 4, indicating that plant extract was continuously reducing the metal ion. A greater concentration of silver nanoparticles in the reacting mixture was indicated by increased absorption with a longer reaction time. The presence of polyphenols in the plant extract caused the second peak to be seen at 278 nm. The second peak's intensity decreased as the reaction time increased, indicating the use of polyphenols in reducing metal ions. Absorption peak branding began after two hours, indicating an increase in particle size.[14] FTIR was used to find potential functional groups that could be involved in the biosynthesis, stabilisation, and reduction of AgNPs. Tea extract showed a peak at 3564 due to alcohol/ phenol O-H stretch which disappeared in nanoparticle graphs indicating that phenolic content present in the extract used for the synthesis of AgNPs. The synthesised AgNPs' spectra revealed distinct peaks at 669, 1085, 1556, 1761, 1867, 2659, 3053, and 3523 cm1. The spectrum of the FTIR peak at 3523 contained the strong stretching vibration O-H functional group, which was identified.[22] The findings demonstrate that phenolic compounds in black tea extract play a role in the stabilisation of nanoparticle size and shape as well as the reduction of silver ions.[23] Zeta potential of AgNPs was observed 22.7mV, with a single peak indicating the presence of repulsion between the synthesised nanoparticles.[24] The FESEM analysis of the image of the biosynthesized AgNPs revealed that their primary shape was spherical with some aggregates and irregular shapes.[25] A thorough XRD analysis showed that the silver nanoparticles created from the reduction of silver ions in Baliospermum extract were crystalline. Sharp peaks that correspond to the diffraction from the planes of Ag with the FCC lattice were visible in the XRD graphs of the biosynthesized Ag nanoparticles at positions 27.86°, 32.43°, 38.34°, 44.51°, 46.44°, 54.85°, 57.56°, 64.60°, and 77.40°.[26] When silver nitrate is reduced to AgNPs, phenolic contents are a key reducing agent. Phenolic content has been cited as a key phytochemical that contributes to antioxidant capacity. Different oxidative response inhibition patterns are displayed by polyphenols. Because of this, black tea extract's higher phenolic content is a definite indication of its higher antioxidant capacity. It is implied that the AgNPs biosynthesized from the baliospermum extract have high antioxidant activity, suggesting their potential for therapeutic use or use as a natural, affordable, and renewable bioreducing agent.[27]

CONCLUSION

Silver nanoparticles were synthesized from black tea nanoparticles and the synthesis process was optimized by central composite design by RSM. AgNPs mainly contained gallic acid, catechins, and ellagic acid. The presence of phenolic compounds in the reduction, stabilisation, and biosynthesis of AgNPs was strongly supported by the total phenolic content, phenolic compound profile by HPLC, and FTIR data. The traditional method of optimization is lengthy and time-consuming. Optimized factors suggested by Central composite design were, 10 mM $AgNO_3$, 10% extract of black tea, 150 min, and 700 rpm. 3D plots revealed that the metal salt concentrations and stirring rate showed a direct relationship with particle size, whereas extract concentration and stirring time showed indirect relationship. % yield was highest with mid level of solution of metal salt (A) and concentration of extract (B). Stirring time(C) and stirring speed had no impact on % yield. The size of prepared silver nanoparticles was in the range of 105 to 507.98 nm. Analysis of AgNPs was done by UV-visible spectroscopy, zeta sizer, XRD, FTIR analysis, FESEM, etc. A particle size study has shown that particles were monodisperse with Z-Average: 137.8 nm and a polydispersity

index of 0.278. Zeta potentials of silver nanoparticles were 22.7 mV with Electrophoretic Mobility Mean:-0.000176 cm 22/Vs, indicating the stability of silver nanoparticle suspension.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CCD: Central composite design; **AgNPs:** Silver nanoparticles; **RPM:** Revolution per minute; **XRD:** X-ray Diffraction; **RSM:** Response surface methodology; **$AgNO_3$:** Silver nitrate.

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