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Original Article

Biosynthesis of Silver Nanoparticles from Polyphenolic Extract of *Baliospermun solanifolium* using Central Composite Design

Sarika Ankushrao Nikam*, Shilpa Pravin Chaudhari

ABSTRACT

Background: Plant extracts contain a considerable amount of polyphenols. These polyphenols act as reducing, capping, and stabilizing agents in the formation of silver nanoparticles. **Objectives:** The objective of the current study is biosynthesis, optimization, and evaluation of silver nanoparticles from the polyphenolic extract of *Baliospermum solanifolium*. **Materials and Methods:** Central composite design (CCD) was used to synthesize silver nanoparticles (AgNPs) from the polyphenolic extract of Baliospermum. Preliminary screening was done to find out the upper and lower limits for the optimization study. Four independent variables like Silver nitrate concentrations (mM), Baliospermum extract (%), stirring time (min), and stirring rate (RPM) were employed. As per the design expert, 30 experiments were performed and their effects on dependent variables were listed. Analysis of synthesized AgNPs was done by visual observation, UV-vis Spectroscopy, Zeta potential, X-ray Diffraction (XRD), FTIR, FESEM, etc. **Results:** The development of Baliospermum silver nanoparticles was confirmed by a color change from pale yellow to dark brown. Characteristic peak of silver nanoparticles observed at 432 nm. −15 mV Zeta potentials confirmed the stability of silver nanoparticles. The sizes of the produced nanoparticles were measured using a FESEM and ranged from 70 to 140 nm. The crystalline nature of nanoparticles was confirmed with the help of X-ray crystallography. FTIR data strongly revealed the presence of phenolic compounds in the reduction, stabilization, and biosynthesis of AgNPs. **Conclusion:** Major Optimized factors offered by the Central composite design were 10mM $AgNO_3$, 10 % Baliospermum extract, 150 min, and 700 rpm. As per the data collected from FTIR and HPLC, silver nanoparticles could be capped with major phenolic groups like ellagic acid, catechins, and quercetin.

Keywords: Baliospermum, Central composite design, Design expert, Green synthesis, Silver nanoparticles.

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INTRODUCTION

Baliospermum solanifolium (Burm.) Suresh (Family: Euphorbiaceae), popularly known as Danti, is a plant whose phenolic extract is the basis of the current study's attempt to create silver nanoparticles. It is a small shrub that grows up to the height of 1-2m.[1-4] For the generation of nanoparticles, green synthesis uses either biological microbes or plant extracts. It is a low-cost, environment-friendly approach that can readily be scaled up for large-scale synthesis.[2] This study used a green synthesis strategy that includes the use of Baliospermum extract as a reducing and stabilizing agent to successfully create silver nanoparticle structures. Plant extract contains a considerable amount of polyphenols. Polyphenols are secondary metabolites that protect plants from a variety of stresses as well as different bacterial and fungal infections. These polyphenols act as reducing, capping, and stabilizing agents in the formation of silver nanoparticles.[3]

MATERIALS AND METHODS

Materials

Baliospermum solanifolium leaves were collected from rural areas of Nashik district, Maharashtra, India, and authenticated from Agharkar Research Institute, Pune, India. Leaves were collected, washed with distilled water, dried, and stored for further use. Silver nitrate and all other ingredients used in the experimental procedure were procured from Research Lab Fine Chemical, Mumbai, India, and used as received. All the solutions were prepared freshly and stored in the dark to avoid photochemical reactions for the experiments.

Extraction of phenolic compounds from Baliospermum aqueous extracts

Application heat causes increased permeability of plant cells by cell disruption and also breaks the interaction between polyphenol and lipoproteins which causes increasing solubility of polyphenols in water. The maximum amount of polyphenol

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content can get by the heat extraction method. Baliospermum leaves were collected, washed, dried, and ground in a mixer.10 gm of powdered leaves were added in 100 ml distilled water and stirred for 4 h at 40 ºC in a conical flask. Vacuum filtered the liquid extract through Whatman filter paper no. 1 and stored at 4ºC for further use.[3-5]

Silver nanoparticles synthesis by using Baliospermum extract

Phenolic extract of Baliospermum leaves were mixed with Silver nitrate solution and stirred at 700 RPM for 2 hrs. Color of the solution changed from yellowish to reddish-brown. Silver nanoparticle suspension was centrifuged and washed with double distilled water 3 to 4 times. Lyophilized and stored in a cool and dark place.[3,6]

Preliminary Screening

5, 15, and 25% Baliospermum extract were treated with silver nitrate solution and the effect was recorded with the help of UV spectroscopy. From the result, upper and lower Baliospermum extract concentrations were selected for experimental design and optimization.[7]

Experimental design and optimization by Response Surface Methodology

Silver nitrate concentrations (mM), Baliospermum extract (%), stirring time (min), and stirring rate (RPM) were selected as independent experimental variables and were coded as A, B, C, and D respectively. They were evaluated at +1, and -1 levels. The effect of these independent variables on particle size and production yield was investigated. The surface response method and central composite design (CCD) were selected for the present study. 30 runs were performed.[6,7]

AgNPs Characterization

The first indication of the creation of silver nanoparticles in the reaction medium is the color change. Visual observation was done at 30, 60, 90, and 120 min intervals.[8,9] UV- Vis spectra of the reaction mixture were recorded at different time intervals at the 200 - 800 nm scanning range (UV-1700 Shimadzu).[10] A nanoparticle analyzer (Horiba Scientific and S2-100) was used for the determination of polydispersity index and particle size of synthesized nanoparticles.[10,11] FEI Nova SEM 450 was used to study the morphology of AgNPs. A smear of a Freeze-dried sample of AgNPs was produced on the platinum grid and it was covered with a thin palladium coating before the FESEM study.[11,12] X-ray diffraction patterns (XRD) for Baliospermum extract and nanoparticles were studied (PW 1729 Philips, Netherland). Freeze-dried samples were used for getting XRD patterns.[11-13] FTIR (FTIR, Perkin Elmer, Spectrum BX) study was performed to determine functional groups present in both Baliospermum extracts as well as silver nanoparticles.[10,11]

Total phenolic content in Baliospermum extract

Folin-Ciocalteu (FC) reagent method was used to determine the total phenolic content in the Baliospermum extract. FC reagent was used to reduce the extract which results in blue color formation. Phenolic content concentration was estimated from the calibration curve of gallic acid. For calibration curves 50, 100, 150, 200, 250, and 300µg/ml Gallic acid solution was mixed with FC reagent (2.5 ml) and sodium carbonate of 75g/L (2.5 ml). The solution was incubated for 30 min and absorbance was taken at 765nm by UV-vis Spectrophotometer. The absorbance was calculated by individually combining 1 ml of Baliospermum extract with 2.5 ml of FC reagent and 2.5 ml of sodium carbonate solution. Total phenolic content was expressed as gallic acid equivalent mg/ml of solution.[3,14]

Phenolic compounds profile in Baliospermum AgNPs

Phenolic compounds present in Baliospermum extract and AgNPs were determined by HPLC (waters alliance 2695 HPLC with PDA) with separating column Zobrax SB C_{18} 150×4.6mm×5µm with 1.0ml/minute flow rate. Mobile phases used for the process were water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). Chromatograms were recorded using software empower 2. Phenolic compounds were estimated at 280nm.

RESULTS

Silver nanoparticles were successfully synthesized with 5, 15, and 25% Baliospermum extract. UV-Vis spectrum of synthesized nanoparticles was recorded in Figure 1. From the result, 5, 25% concentrations were selected as upper and lower Baliospermum extract concentrations for experimental design and optimization. It was observed that the synthesis of AgNPs was plunged after using more than 15% Baliospermum extract, as seen by the diminished intensity of UV peaks. The % yield and intensity of peak increase with the increase in concentration from 5 to 15%. Higher production at low extract concentration could be due to the increased availability of reducing agents from Baliospermum extract. (Figure 1).

Results for Experimental design and optimization by Response Surface Methodology

The regression equation was developed to investigate the interrelated factors by identifying the critical factors that contribute to the regression model and figuring out the optimal values for the most significant independent factors. On the basis of their *F*-value, *p*-value, determination coefficient (R^2), standard deviation, and PRESS values, various models, including linear, 2F1, quadratic, and cubic, were used to try to improve the biosynthesis of silver nanoparticles. The quadratic regression model was found to stand out among the many models that were examined. The following polynomial equation was discovered by using multiple regression analysis on the trial data to explain silver nanoparticle biosynthesis.

Equations derived for particle size and % yield as follows

Equation 1

Particle size = +227.07+12.82×A+28.04×B -57.57×C-61.90×D

Equation 2

% yield = +74.53+8.82×A+3.03×B +2.82×C-0.0583×D

Table 1, Table 2 shows the outline of an analysis of variance and fit statistics (ANOVA) for particle size respectively.

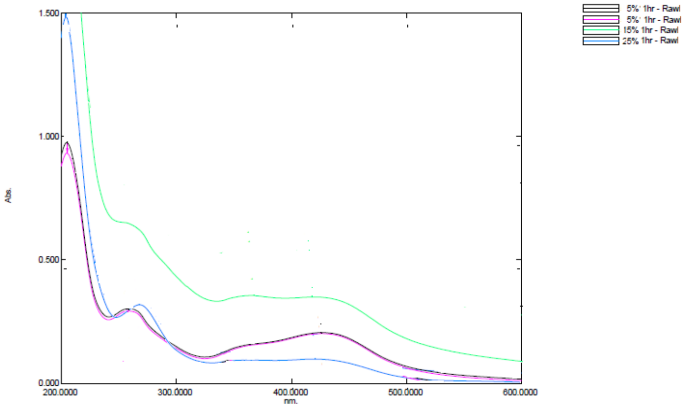

Figure 1: UV peak for 5, 10, 15% Baliospermum extracts.

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

3D surface plots Interpretation

The effects of the four factors studied, as well as the combined effects of each independent component on nanoparticle biogenesis in terms of particle size and % yield, are shown by the regression model, which is shown in the Figure of three-dimensional surface plots. As shown in Figure 2 stirring time, stirring speed and concentration of extract has a significant impact on particle size, but the presence of metal salt solution has no significant effect. Increased stirring time and stirring speed causes a decrease in particle size. The concentration of extract shows a direct effect on particle size; extract concentration increased particle size also increased. % yield of silver nanoparticles was increased with increased concentrations of metal salt, plant extract, and time of stirring, while there was no significant effect of stirring speed.

AgNPs Characterization

The color of the silver nitrate and Baliospermum extract solution slowly changed from pale yellow to dark brown (Figure 3).

The ultraviolet (UV)-visible spectra provided evidence of the presence of Ag nanoparticles. UV absorption maxima for silver nanoparticles were observed at 432 nm. The absorption spectra were investigated at varied time intervals (30, 60, 90, and 120 min). AgNPs were produced after 30 min of churning and showed a UV-vis absorption peak. The intensity

Table 1: ANOVA: Particle size.

Source	*F*-value	*p*-value	
Model	16.89	< 0.0001	Significant
A-Solution of metal salt	2.95	0.1062	
B-Conc of extract	14.113	0.0019	
C-Time for stirring	59.58	< 0.0001	
D-Speed of stirring	68.88	< 0.0001	
AB	0.2235	0.6432	
AC	1.47	0.2434	
AD	0.0229	0.8818	
BC	0.1923	0.6672	
BD	12.78	0.0028	
CD	34.00	< 0.0001	
A^2	9.54	0.0075	
B^2	34.33	< 0.0001	
C^2	0.4051	0.5341	
D^2	0.2301	0.6384	
Residual			
Lack of Fit	4.31	0.0603	Not Significant
Pure Error			
Cor Total			

Table 2: Fit Statistics: Particle size.

Std. Dev.	36.54	R^2	0.9404
Mean	277.89	**Adjusted R^2**	0.8847
C.V. %	13.15	**Predicted R^2**	0.6833
		Adeq Precision	17.4601

Table 3: ANOVA: % Yield.

Source	*F*-value	*p*-value	
Model	18.73	< 0.0001	Significant
A-Solution of metal salt	73.71	< 0.0001	
B-Conc of extract	8.66	0.0101	
C-Time for stirring	7.51	0.0152	
D-Speed of stirring	0.0032	0.9555	
AB	1.80	0.2001	
AC	0.0947	0.7625	
AD	0.0001	0.9922	
BC	0.4294	0.5222	
BD	3.63	0.0759	
CD	0.0035	0.9533	
A^2	98.21	< 0.0001	
B^2	79.38	< 0.0001	
C^2	1.63	0.2206	
D^2	0.6220	0.4426	
Residual			
Lack of Fit	4.16	0.0646	not significant
Pure Error			
Cor Total			

Table 4: Fit Statistics: % yield

Std. Dev.	5.04	R^2	0.9459
Mean	59.68	**Adjusted R^2**	0.8954
C.V. %	8.44	**Predicted R^2**	0.7134
		Adeq Precision	16.5456

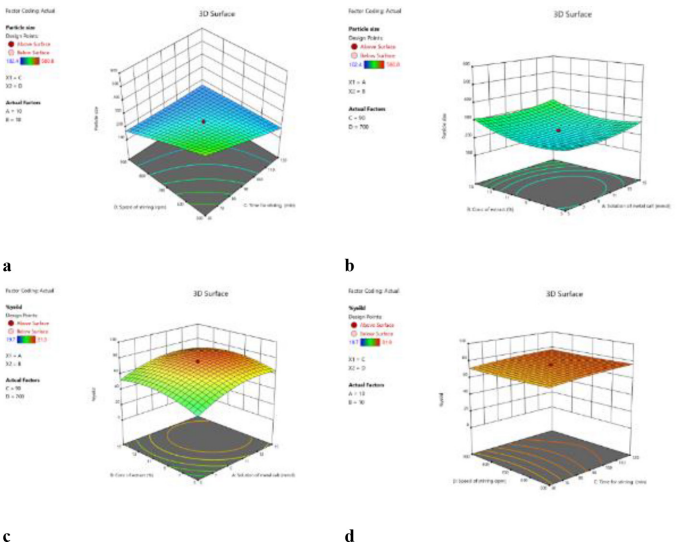

Figure 2: 3D responses.
a: Particle size: time for stirring, speed of stirring, b: Particle size: concentration of extract, solution of metal salt, c: % Yield: concentration of extract, solution of metal salt, d: % Yield: time for stirring, speed of stirring.

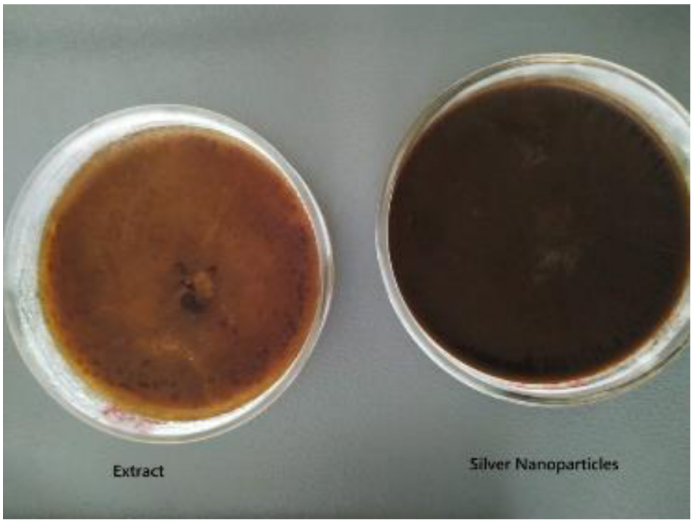

Figure 3: Color change.

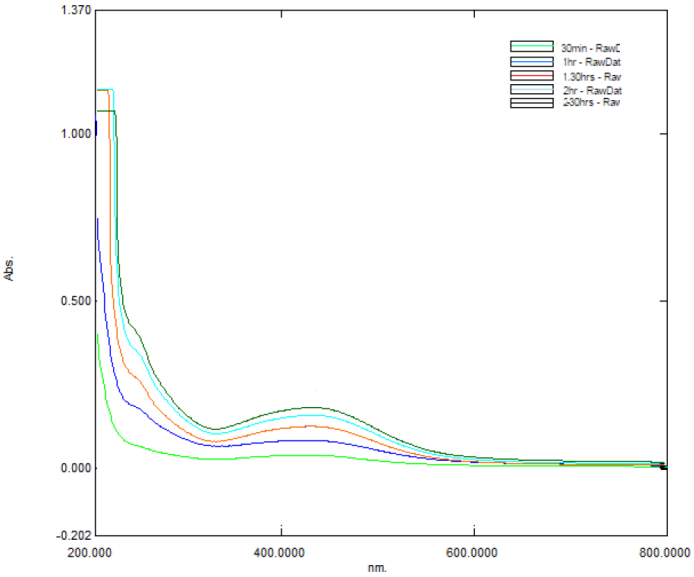

Figure 4: Effect of time on absorbance.

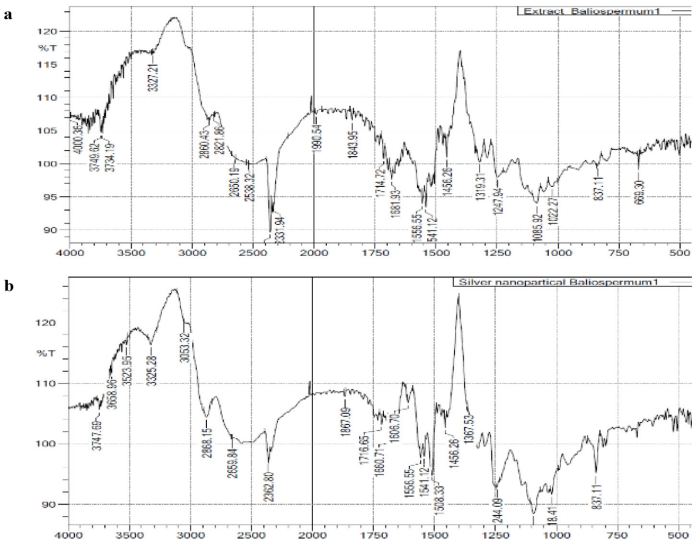

Figure 5: IR SPECTRA a: Extract, b: Silver nanoparticles.

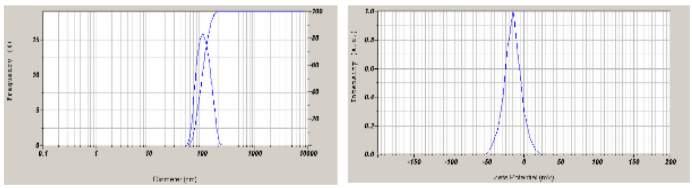

Figure 6 a: Particle size, b: Zeta potential.

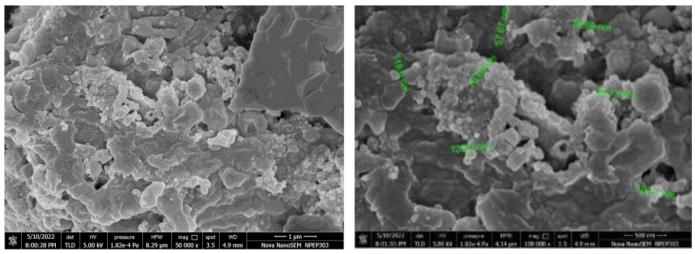

Figure 7: FESEM images of silver nanoparticles.

of the SPR peak grew as the reaction time increased, indicating that the silver ions continued to be reduced, and the increase in absorbance with reaction time suggests that the concentration of AgNPs is increasing, as seen in Figure 4. When the reaction time approached 2 hr, the peak broadened, indicating that the size of the nanoparticles had increased.[15]

FTIR spectra of Baliospermum extract and silver nanoparticles are shown in Figure 5A and 5B respectively. Addition and deletion of peaks change in intensity and shift in peak is observed by comparing both the graphs. Baliospermum extract and AgNPs have shown a shift in peak: 3647 due to O-H stretching in primary alcohol, 3300 due to N-H stretching, 2852 due to aliphatic C-H stretching, 1848-1867 due to C-H bending, 1714-1761 due to C=O stretching, 1319-1367 due to O-H bending, 1247-1244 due to C-O stretching, 1085-1093 due to O-H stretching. Addition of peaks observed at 3523 due to N-H stretching, 3053 due to O-H stretching, and 2659 due to C-H stretching. FTIR data strongly revealed the presence of phenolic compounds in the reduction, stabilization, and biosynthesis of AgNPs.[3]

The average particle size was found 105.6 nm (Figure 6 a) with a poly dispersibility index of 0.42. Zeta potentials (Mean) of AgNPs were −15 mV confirming the stability of silver nanoparticles (Figure 6 b).

In the FESEM images shown in Figure 7, the synthesized nanoparticles were visible. Although the majority of the nanoparticles were aggregated, a few single particles were also visible. The diameters of the synthesized AgNPs were listed from 70 to 140 nm.

The crystalline nature of nanoparticles was confirmed with the help of X-ray crystallography (Figure 8). AgNPs from Baliospermum extract is showing 14 intense peaks of 2θ values ranging from 20° to 80°. Nine strong Bragg reflections at 27.175°, 27.61°, 28.17°, 29.60°, 30.69°, 31.98°, 37.79°, 40.33°, 46.04°, 46.29°, 49.98°, 58.48°, 66.19° and 73.53° which can be indexed according to the facets of the face-centered cubic crystal structure of silver.[16]

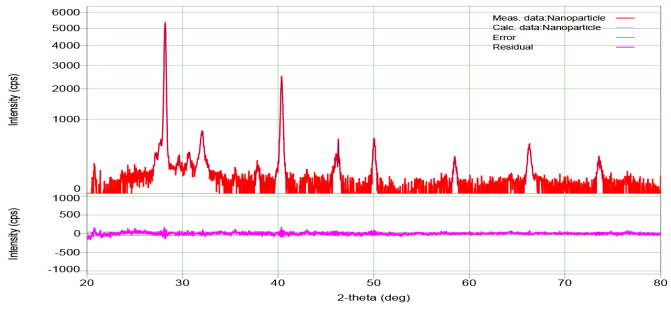

Figure 8: XRD graph of AgNPs.

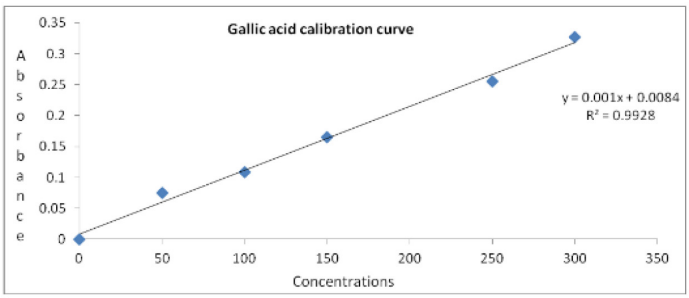

Figure 9: Calibration curve of gallic acid.

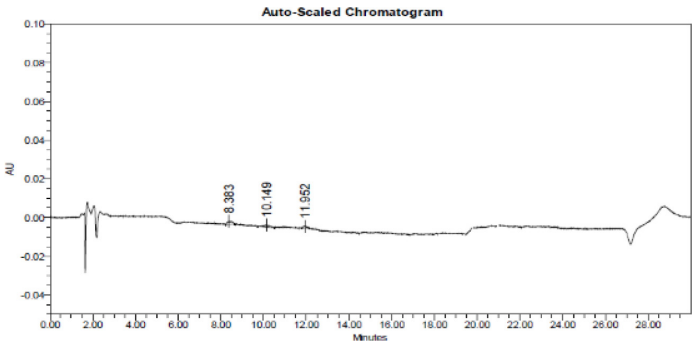

Figure 10: Phenol content profile by HPLC.

Phenolic Content in AgNPs

Total phenolic content was expressed as Gallic acid equivalent, mg/ml of solution, and was calculated with help of the Gallic acid calibration curve shown in Figure 9. Total phenolic content (Gallic acid equivalent) in Baliospermum extract was found 95.5 mg/ml of the extract.[14]

Phenolic compounds profile in Baliospermum AgNPs

As shown in Figure 10 phenolic compounds present in the extract as well as silver nanoparticles were catechins, ellagic acid, and quercetin with retention times 8.383, 10.149, and 11.952 respectively.[3]

DISCUSSION

AgNPs produced biologically have been reported as potential therapeutic molecules. The search for new nanoparticles with precise biological, physical, and chemical properties is still at the forefront of nano science research, despite the fact that many nanoparticles have been successfully synthesised using microorganisms and plants. This study described the green synthesis of AgNPs from Blaiospermum polyphenolic extract.[17] Less concentration of extract was reported to have the smallest size of silver nanoparticles, while higher concentrations of extract may increase the likelihood of particle agglomeration and, as a result, increase in nanoparticle size. Additionally, the presence of a lower concentration of extract enables the provision of an adequate quantity of antioxidants to synthesize silver nanoparticles with an acceptable size, whereas a higher concentration of extract may cause the reaction to continue and increase the size.[9] The relationship between stirring speed and AgNps sizes can be explained by the fact that faster stirring causes more agitation in the system, which leads to the formation of smaller nanoparticles. More stirring simply causes more particle dispersion, which leads to smaller particle sizes.[18] 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 pale yellow 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 raising as a result of an increase in absorbance. The peak broadened as the reaction time approached 2 hr, showing that the size of the nanoparticles had increased.[15] FTIR was employed for the detection of possible functional groups in the reduction, stabilization, and biosynthesis of AgNPs. Strong stretching vibrations of C-H alkyl halides are responsible for the FTIR band of 1367. The spectra for the synthesised AgNPs showed distinct peaks in the range of 669, 1085, 1367, 1526, 1681, 1761, 1867, 2659, 3053, and 3523 cm^{1}. The strong stretching vibration O-H functional group was identified in the spectrum of the FTIR peak at 3523.[3,19] Strong peaks in the IR spectrum were visible at 1635 cm-1 and 3300 cm-1, which correspond to the presence of phenolic -OH and C = C, respectively. The results show that baliospermum phenolic compounds are involved in the stabilisation of the size and shape of the nanoparticles as well as the reduction of silver ions.[20] Zeta potential of AgNPs was observed -15 mV, with a single peak indicating the presence of repulsion between the synthesised nanoparticles.[21] The image of biosynthesized AgNPs revealed by FESEM analysis showed that their predominant shape was spherical with some irregular shapes.[22] A thorough XRD analysis demonstrated that the silver nanoparticles produced from Baliospermum extract reduction of silver ions were crystalline in nature. Sharp peaks were visible in the XRD graphs of the biosynthesized Ag nanoparticles at positions 27.175°, 27.61°, 28.17°, 29.60°, 30.69°, 31.98°, 37.79°, 40.33°, 46.04°, 46.29°, 49.98°, 58.48°, 66.19° and 73.53° which correspond to the diffraction from the planes of Ag with the FCC lattice.[23] Phenolic contents play a major role as a reducing agent in reduction of silver nitrate to AgNPs. Phenolic content reported as important phytochemicals responsible for the antioxidant capacity. Polyphenols exhibit distinctive oxidative response inhibition patterns. Because of this, the higher phenolic content of baliospermum extract is a clear indication of its greater antioxidant capacity. It can be inferred that the AgNPs biosynthesized from the baliospermum extract have high antioxidant activity, which further suggests their potential for therapeutic use or can be used as a natural, renewable, and affordable bioreducing agent.[24]

CONCLUSION

Silver nanoparticles from the phenolic extract of Baliospermum leaves were successfully synthesized. Ellagic acid, catechins, and quercetin was the major compound found in AgNPs. Total phenolic content,

phenolic compound profile by HPLC, and FTIR data strongly revealed the presence of phenolic compounds in the reduction, stabilization, and biosynthesis of AgNPs. The central composite design (CCD) practice of response surface methodology (RSM) appeared to be an important tool for elucidating the discrete and reciprocated effects of numerous investigational data points and optimizing them for better silver nanoparticle biosynthesis in a cost-effective and time-effective manner. Optimized factors: 10mM $AgNO_3$, 10 % Baliospermum extract, 150 min, and 700 rpm were provided by central composite design.

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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, **UV:** Ultra violet.

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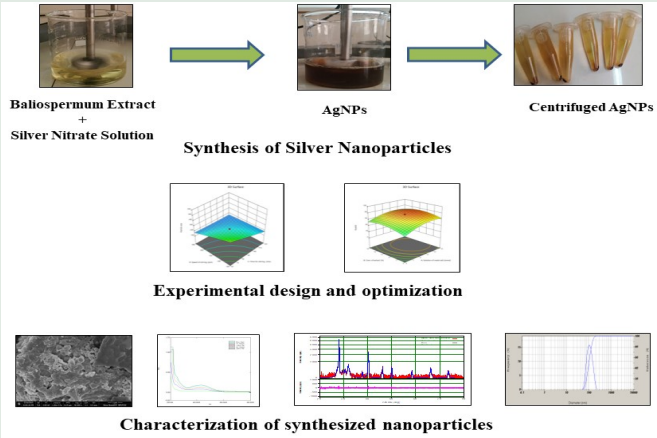
GRAPHICAL ABSTRACT



SUMMARY

This study revealed the successful synthesis of silver nanoparticles using the phenolic extract of Baliospermum leaves. Silver ion was successfully converted into silver particles by phenolic compounds found in the plant extract. The presence of phenolic compounds in the reduction, stabilization, and biosynthesis of AgNPs was strongly supported by the total phenolic content, phenolic compound profile by HPLC, and FTIR data. Ellagic acid, catechins, and quercetin was the major compound found in AgNPs. The central composite design (CCD) practice of response surface methodology (RSM) seemed to be a crucial tool for illuminating the discrete and reciprocated effects of numerous investigational data points and optimizing them for better silver nanoparticle biosynthesis in a time and cost-efficient manner.

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