RESEARCH ARTICLE

Box–Behnken Design Based Optimization of Process Variables for the Green Synthesis of 18-Beta–Glycyrrhetinic Acid Silver Nanoparticles and Evaluation of its Antioxidant, Antimicrobial Activity

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ABSTRACT

Using 18-beta-glycyrrhetinic acid, a well-known component of licorice (*Glycyrrhiza glabra* Lin.), the current study offers a unique rapid ecological & simple approach of biologically synthesizing of silver nanoparticles (AgNPs). To synthesise stable silver nanoparticles via a biological reduction technique, a methanolic stock solution of 18-β-glycyrrhetinic acid was produced and used as capping and reducing agent. The approach was methodically optimized using response surface analysis (RSA) based Box-Behnken design (BBD), taking into account influence of parameters like as silver nitrate ($AgNO_3$) concentration, incubation time and temperature on response. RSA was utilized to determine the association among factors and the responses by mathematical modelling with a quadratic polynomial model. $AgNO_3$ (1mM), 55°C, and 5 hours incubation were optimal. 18-β-glycyrrhetinic acid methanolic stock solution can convert silver ions (Ag+) into silver nanoparticles (AgNPs) in 5 hours at 55°C. Biosynthesized and optimized AgNPs have an SPR absorption peak at 419 nm in their UV spectra. 18-β-glycyrrhetinic acid reduced and capped silver ions according to FTIR spectroscopy. XRD showed AgNPs' crystallinity. SEM revealed spherical elemental silver with particle size of 100 nm. Average particle size, PDI & Zeta were 83.36 nm, 0.462 and -35.4mV, respectively, at 100% intensity. Silver nanoparticles (GAAgNPs) are stable. DPPH experiment showed substantial antioxidant activity in GAAgNPs compared to ascorbic acid. At 10 µg/mL, AgNPs showed utmost region of inhibition of 15 and 14 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively. Finally, the synthesized AgNPs and their quality components have strong antioxidant and antibacterial activity, indicating that this research can be used to formulate useful biomedical goods.

Keywords: Silver Nanoparticles, Green synthesis, Response surface methodology, Box-Behnken Design, Antimicrobial, and Antioxidant.

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INTRODUCTION

Nanotechnology has been more important in the field of modern biology.[1-3] The last few decades have seen a rise in interest in the pharmaceutical and environmental cleaning applications of green-mediated production of metal nanoparticles since it is most economical, non-toxic, and ecologically acceptable technology.[4,5] Synthesised nanoparticles derived from diverse plant parts, including seeds, stems, flowers, leaves, and fruit skins. Green nanoparticles synthesized from plant extract have been discovered to be applicable in medication delivery and cosmetics.[6] Due to their nano range particle sizes and high surface/volume ratio, metal nanoparticles produced from plant sources exhibit remarkable antibacterial action.[7,8] Silver, copper, and zinc nanoparticles synthesized using environmentally friendly methods are frequently used as antibacterial agents in medicine because of their effectiveness and safety.[9,10]

Cosmetics, purified water, medical diagnostics, textiles, electronics, and even domestic appliances can all benefit from silver nanoparticles' antibacterial properties.[11] Silver nanoparticles are well suited for biological sensing and imaging applications[12] due to their antibacterial characteristics and powerful optical features. Nanoparticles of silver are employed in a wide variety of electrical devices due to the metal's high conductivity.[13] Styrene oxidation is facilitated by its employment as a catalyst in a number of chemical processes.[14,15] Silver nanoparticles are currently having several uses, including management of burn wounds, dentistry,

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and catheters.[16-20] Due to its one-step procedure and low environmental impact, the biological reduction process is preferred for making silver nanoparticles.

The biological effects of licorice, which include antimicrobial, antiulcer, immunomodulatory, antiviral, inflammatory, and antioxidant properties, have led to its widespread use in the cosmetic and pharmaceutical industries.[21–27] Most active compounds of licorice (the roots of the leguminous plants *Glycyrrhiza glabra L.*, *G. uralensis Fisch.* and *G. inflate Batalin*) is the triterpenoids glycyrrhizic acid (glycyrrhizin) and the main product of its metabolism is a pentacyclic triterpene compound, the aglycone 18-*β*-glycyrrhetinic acid,[22,23] which is a key bioactive constituent of licorice.[28-31]

In this study, we attempted to use a biological reduction approach to create stable silver nanoparticles (AgNPs) from 18-*β*-glycyrrhetinic acid. Using Box-Behnken Design (BBD) of RSM, this study sought to optimize a number of experimental parameters critical to the eco-friendly synthesis of AgNPs of 18-*β*-glycyrrhetinic acid. In order to learn more about the properties of the optimized and environmentally friendly AgNPs, a number of analytical techniques were applied. Antimicrobial efficacy against four human pathogens was tested using further optimized AgNPs.

MATERIAL AND METHODS

Consumables

18-*β*-glycyrrhetinic acid and silver nitrate, both used in plants, were purchased from the American company Sigma Aldrich. All ingredients used in this study, including the double-distilled water, were of analytical quality.

Green Synthesis of18-β-Glycyrrhetinic Acid silver Nanoparticles (GAAgNPs)

Our GAAgNPs were biologically synthesized using a customized description of the procedures.[32–35] However, by adjusting a number of synthesis-related parameters, the synthesis conditions for the present reaction were optimized.

Stock Solution of 18-β Glycyrrhetinic Acid (GA)

The medication was diluted to 20 mL from 2 mg by using methanol. It was put away at 4°C until needed.

Silver Nitrate Aqueous Solution (1mM)

A total $AgNO_3$ (0.017 g) was mixed with water (100 mL) that had been distilled twice and put in an amber jar.

Silver Nanoparticles (GAAgNPs)

A total of 5 mL of methanolic stock solution of 18-β-Glycyrrhetinic acid was taken in conical flask separately and subjected to hot plate attached magnetic stirrer. Over 30 seconds, 50 mL of 1 mM $AgNO_3$ was mixed with mixture using a peristaltic pump (dropper/syringe) with constant stirring at 120 rpm. Between 50 and 60°C, the solution was warmed. Phytoconstituent will act as a reducing and stabilizing agent for the silver nitrate solution in water. A pH of 7.5 was achieved in the solution slurry. Nanoparticles of silver (Ag°) are produced when silver ions (Ag^+) are reduced to silver. After 5 hours, the medium had changed color from clear to dark brown. Highly stable *GAAgNPs* were synthesized through reducing aqueous silver ions using a methanolic stock solution of 18-β-Glycyrrhetinic acid.

Separation of Silver Nanoparticles

REMI centrifuge separation was used on the freshly synthesized silver nanoparticles at 10,000 rpm for 15 minutes. After being separated, the supernatant liquid was resuspended in sterile double-distilled water. The technique was carried out several times to guarantee that no uncoordinated biomolecules would survive. The pellets were collected after the reaction period and stored at 4°C while the supernatant liquid was discarded.

Lyophilization

To improve stability of silver nanoparticles, the pellet was lyophilized using a freeze dryer (TIC 4000, Thermotech, Japan make). A cryoprotective agent (mannitol) is used to lyophilize freshly synthesized GAAgNPs (-50°C/2 h) at 1.03 m bar (primary drying) and 0.001 m bar (secondary drying), respectively. The produced AgNPs were lyophilized & set aside at 4°C for further usage.

Optimization of AgNPs Synthesis by Box-Behnken Design based

Box-Behnken design of RSM optimized green-mediated AgNP synthesis process parameters. BBD optimized green AgNPs synthesis by considering three experimental factors: $AgNO_3$ (A) concentration, (B) incubation time and (C) temperature. Box-Behnken Design analysed these three factors at three levels (-1, 0 and +1). The design recommended 17 runs. Responses included synthesized AgNPs' particle size (nm) and zeta potential (mV). Mathematical modeling analyzed the data in BBD. ANOVA was used to analyze data-fitting with the second-order quadratic model, including r^2, adjusted r^2, anticipated r^2 and predicted residual sum of squares. Numerical desirability and graphical optimization determined AgNPs manufacturing parameters. Each variable is varied at these levels, and BBD looks at linearity.

Silver Nanoparticles Analysis

Particle size and zeta potential study

Dynamic light scattering was used to assess GAAgNPs' mean polydispersity index (PI), particle size (z-average), and zeta potential. Before measurement, the freeze-dried powder was dissolved in water.

UV-visible spectroscopy

The optimized GAAgNPs synthesised with the Box-Behnken design were verified using a Systronics UV-2201 PC. Transmission UV-visible spectra were taken from distilled water to serve as a blank. Absorption climbed as the manufacture of green AgNP progressed.

FTIR

FTIR study of biosynthesized AgNPs determined biomolecule functional groups. KBr pellet technique was used to analyze

400 to 4000 cm^{-1} at room temperature. Bruker Corporation (USA) type FTIR instrument analyzed pellet.

XRD measurement

Powder samples of synthesized silver nanoparticles were sited on a glass substrate for XRD analysis. Using an XRD apparatus manufactured by Bruker Corporation (USA), we captured diffraction intensity against 2θ XRD patterns at 'k' of 1.54 A°, operating at 40 kV and 30 mA in the tube.

SEM analysis

SEM analysed synthesised AgNPs with an EM-30 (COXEM SEM-3000, South Korea manufacture). Sample powder was prepared for SEM analysis by dropping a small amount onto a carbon-coated grid, wiping off the surplus using blotting paper & allowing the film to dry.

Antioxidant action of silver nanoparticles

18-β-Glycyrrhetinic acid silver nanoparticles (GAAgNPs) were tested for the capability to quench free radicals using the DPPH spectrophotometric assay.[36] Two mL of silver nanoparticle solution (10–800 µg/mL) and one mL of DPPH (0.5 mM in methanol) were combined in a cuvette; analyzed at 517 nm (30 min) at room temperature. There are three tests performed on this analysis. In order to calculate antioxidant activity, the following equation was used:

% Antioxidant Activity [AA] = 100 – [{(Abs sample – Abs blank) X 100}/Abs control]

The blank was methanol (1.0 mL) + 2.0 mL of 18-β-Glycyrrhetinic acid silver nanoparticles (GAAgNPs) solution, and the negative control was DPPH (0.5 mM) with methanol (2 mL; ascorbic acid (standard).

Antimicrobial prospective of AgNPs

Agar well diffusion assays were performed to check for antibacterial activity of green synthesised 18-β-glycyrrhetinic acid AgNPs. Green synthesized silver nanoparticles' high surface-to-volume ratio, precision tailoring nanoparticle size with plant bioactive compounds, and close interaction with microbial membranes make them antibacterial. *S. aureus, B. subtilis*, *E. coli* and *P. aeruginosa* bacterial cultures were inoculated in sterile petri plates using the spread plate technique and allowed to grow for 24 hours before being used in the experiment. The circular wells used in agar well diffusion were 6 mm in diameter. Then, the well was loaded with 10 μg of ampicillin, while the other wells contained positive control silver nanoparticles (AgNO3), pure 18-β-glycyrrhetinic acid (10 μg/mL), loaded silver nanoparticles, and distilled water (negative control). Triplicate plates of each culture were incubated at 37°C for a full 24 hours.

Data, Values and Validation

Box-Behnken design

Box-Behnken Design determined optimal AgNPs synthesis conditions. Table 1 illustrates optimization. Table 2 displays the results of a Fisher's F-test results indicating that the quadratic regression model is statistically significant for predicting the relationship between particle size and zeta potential. Model term was significant when 'Prob> F' was 0.0363 for particle size and <0.0001 for zeta potential. Model F-values of 4.18 and 60.08 indicate significance. Noise only caused model F-values 3.63% (particle size) and 0.01% (zeta potential). The predicted R^2 (-1.5102 and 0.8636) and adjusted R^2 (0.6414 and 0.9708) values for AgNPs synthesis were close to 1.0 (Best fitted).

All of the contour diagrams, graphic pictures, and 3D plots can be seen in Figures 1 and 2. Based on the data, we can conclude that $AgNO_3$ concentration (mM), incubation period (hrs), and temperature (°C) are all significantly related to one another. Using plant-active 18-β-glycyrrhetinic acid was the best way to make AgNPs. By using BBD, the best values of the variables were found. After optimising the amount of $AgNO_3$ (1 mM), the time of incubation (5 hrs), and the temperature (55°C), the model indicated that particles with the right size and zeta potential would form. The analysis was conducted under the assumption that the model is correct. The values predicted by the model came extremely close to those observed in the experiments. This shows that the response model isn't as accurate as it was thought to be and that optimal conditions aren't necessary. Graphs showed how the different factors affected the process of making the best silver nanoparticles. The following regression equation was produced after calculating coefficients of response 1 and 2.

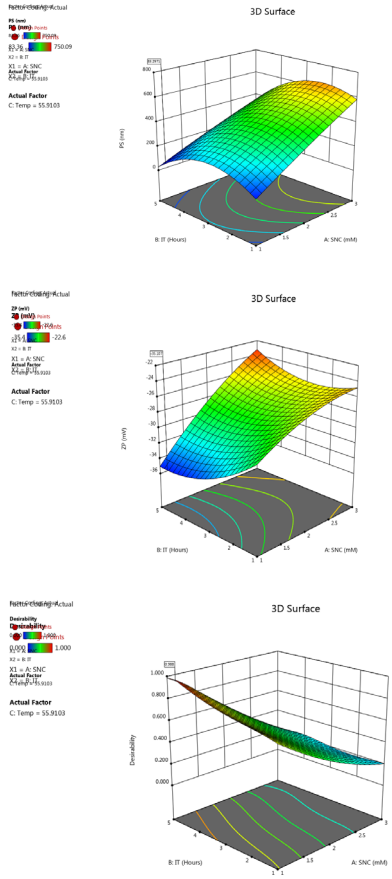

Figure 1: 3D plots with combine effect of 2 factors on biosynthesis ovfAgNPs from 18-β-Glycyrrhetinic acid

Table 1: Box-behnken design for AgNPs

	Runs	*Factor 1 $AgNO_3$ Conc. (mM)*	*Factor 2 Incubation Period (h)*	*Factor 3 Tem.(°C)*	*Response 1 Particle Size (nm)*	*Response 2 Zeta Potential (mV)*
12	1	2	5	60	242.84	-27.3
7	2	1	3	60	94.42	-32.1
9	3	2	1	50	410.09	-24.8
4	4	3	5	55	205.67	-22.6
3	5	1	5	55	83.36	-35.4
8	6	3	3	60	692.24	-25.2
17	7	2	3	55	443.77	-28.1
11	8	2	1	60	205.1	-24.3
14	9	2	3	55	445.44	-29.2
13	10	2	3	55	444.36	-28.5
2	11	3	1	55	540.76	-24.4
16	12	2	3	55	442.81	-29.2
5	13	1	3	50	99.88	-31.8
15	14	2	3	55	444.28	-28.9
6	15	3	3	50	750.09	-23.7
1	16	1	1	55	255.23	-30.1
10	17	2	5	50	185.52	-25.4

Table 2: ANOVA table of for model

	Source	Sum of Squares	dF	Mean Square	F value	P value	
PS	Model	5.478E+05	9	60868.06	4.18	0.0363	Significant
	Std. Dev.	120.68	R^2	0.8431	C.V. %	34.27	
	Mean	352.11					
ZP	Source	Sum of Squares	dF	Mean Square	F value	P value	
	Model	190.07	9	21.12	60.08	< 0.0001	Significant
	Std. Dev.	0.5929	R^2	0.9872	C.V. %	2.14	
	Mean	-27.71					

Y (Particle size = 83.36 nm)
=+444.13+206.98A-86.72B-26.37C-40.80AB-13.10AC+65.58BC-12.30A^2-160.57B^2-22.67C^2
Y (Zeta potential = -35.4mV)
=-28.78+4.19A-0.8875B-0.4000C+1.77AB-0.3000AC-0.6000BC-1.05A^2+1.70B^2+1.63C^2
Where A is the conc. of $AgNO_3$, B is the incubation duration, and C is the temperature, and Y is the synthesis of silver nanoparticles (AgNPs) (for particle size 83.36 nm and zeta potential -35.4mV). RSM has a good chance of being accurate and useful in optimising the silver nanoparticle manufacturing process due to the high degree of similarity between the observed values.[37]

Characterization of Green Synthesized AgNPs

Particle size and zeta potential study

Stability, dissolution and solubility of synthesised silver nanoparticles are all governed by particle size, size distribution and zeta potential.[38] The zeta potential and polydispersity index (PDI) of the synthesised silver nanoparticles are calculated using the Zeta Size Analyzer (ZSU 3200).

Particle size examination

Particle diameter (Average) and PDI in solutions measured using PCS. Number of particles per unit volume and size distribution of colloidal nanoparticles were analysed and expressed.[39]

Particles size, on average (as measured by z), was 83.36 nm. Nanoparticles with a PDI of 0.462 and an intercept of 0.663 are produced through particle size analysis. Table 3 and Figure 3 show the results.

Zeta potential properties

It is for calculation of surface potential of the synthesised AgNPs. The stability of aqueous AgNPs can be measured, in part, by their zeta potential. Silver nanoparticles that is stable in the environment need to have a zeta potential of at least + 30mV

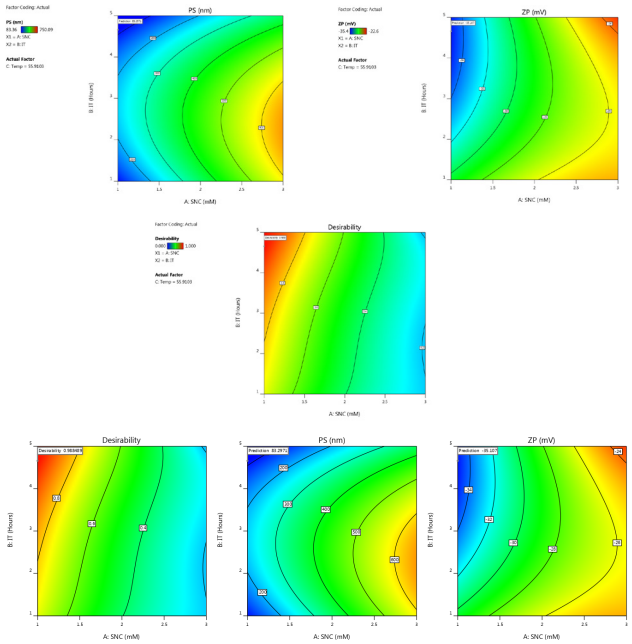

Figure 2: 2D Contour plot of synthesized AgNPs

Table 3: Mean Particle Size & PDI of GAAgNPs

Parameter	*Value*	*Peak no*	*Peak size (d.nm)*	*Peak intensity (%)*	*Peak width (d.nm)*
Z-Average (d.nm)	83.36	1	147.0	98.2	50.8
PDI	0.462	2	6.08	1.8	10.12
Intercept	0.663	3	0.000	0.0	0.000

in order to be detected. Silver nanoparticles synthesised in this way have been measured to have a zeta potential of -35.4mV, with a peak area of 100% intensity. Complete nanoparticle stability is achieved at these levels, which may explain why a restricted size distribution index (Figure 4) is produced during synthesis.

UV spectroscopy

Absorption spectra of optimised silver nanoparticles were measured using UV-visible spectrophotometry, as displayed in Figure 5. Optimised silver nanoparticles were synthesised and stored at room temperature for 24 hours at optimum concentration of silver nitrate, incubation period, and temperature. Surface Plasmon resonance of artificial silver nanoparticles was shown responsible for peak of the spectrum at 419 nm and the dark brown hue of the dispersion. The incubation times were also changed during the course of the investigation. Raising the incubation time or temperature resulted in peaks, showing that AgNPs were shrinking in size. In contrast, increasing the silver concentration in the solution causes the absorption peaks to shift to longer wavelengths (red shift occurs), indicating the creation of silver nanoparticles with bigger diameters (nucleation effects).[40,41]

FTIR study

Figure 6 showed spectrum of 18-β-glycyrrhetinic acid and its synthesised silver nanoparticles. FTIR investigations

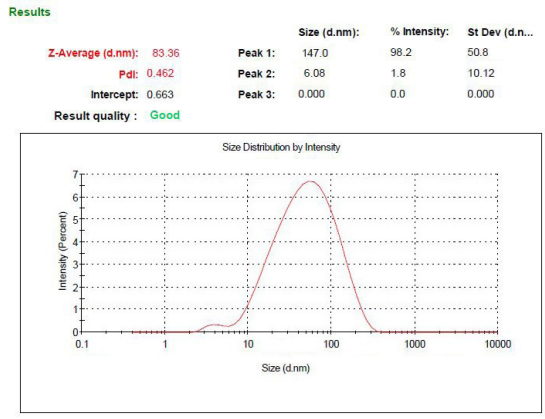

Figure 3: Particle size distribution intensity of synthesized silver nanoparticles

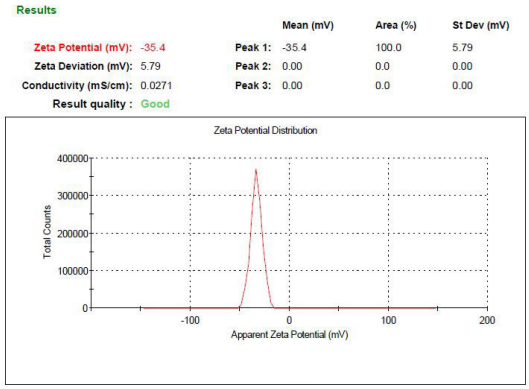

Figure 4: Zeta potential distribution of synthesized silver nanoparticles

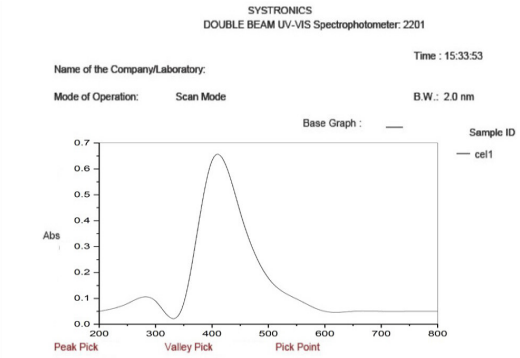

Figure 5: UV- Visible absorption spectra for Green synthesized AgNPs from 18-β-Glycyrrhetinic acid.

examined functional groups that reduce, cap, and stabilise 18-β-glycyrrhetinic acid-synthesized metallic nanoparticles. The peak bands for 18-β-glycyrrhetinic acidin (A) are OH stretching modes at 3430 cm^{-1}, aliphatic C-H at 2923 cm^{-1}, and amide I at 1662 cm^{-1}. The strong peak at 1024 cm^{-1} shows methyl C-H stretching vibration, while the peak at 817 cm^{-1}represents β-glucosidic linkage. FTIR spectra of synthesised AgNPs (B) showed aliphatic C-H stretch at 2623 cm^{-1} and C=O stretch at 1446cm^{-1}.[42]

Powder XRD measurement

The XRD patterns of silver nanoparticles synthesised from 18-β-glycyrrhetinic acid after they have been vacuum-dried are

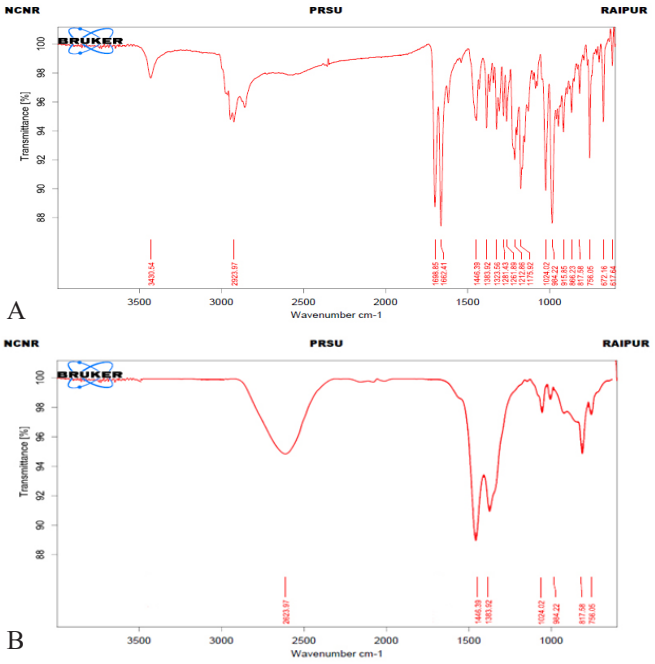

Figure 6: (A) FTIR spectrum of 18-β-Glycyrrhetinic acid; (B) GAAgNPs

displayed in Figure 7. AgNPs (cubic structure) showed by their XRD patterns when exposed to Ag/18-β-glycyrrhetinic acid. The XRD peaks at 2θ values of 38.2°, 44.3°, 64.8°, and 77.8° represent planes at 111, 200, 220, and 311. Thus, the findings crystallised and demonstrated the biosynthesis of AgNPs. Results were consistent with metallic silver (JCPDS No. 89-3722), it is concluded. The plant actives organic compounds are to thank for these peaks[43], as they are what reduce silver ions and keep the resulting nanoparticles stable.

SEM study

The silver nanoparticles generated through the mediation of 18-β-Glycyrrhetinic acid were studied by SEM to determine the precise morphology and size. Microscopy image that resulted (Figure 8) showed spherical shaped nanoparticles; averaged around 100 nm in size, and that they agglomerated into a cluster-like structure and nano crystal. AgNPS were found on plane of the cells in a variety of amounts, as seen in scanning electron microscopy photos. AgNPs were successfully biosynthesized by BBD, as shown by the strong signals of silver nanoparticles such as nano crystals and clusters. Silver nanoparticles were also found to correlate with the XRD patterns in biosynthesized material.[44]

Antioxidant potential of AgNPs

Free radicals play substantial roles in many different diseases and disorders. To nullify the free radicals and prohibit them from spreading illnesses antioxidants were used. This is done by ROS scavenger mechanism. Natural goods' electron-donating capacity can be measured with the help of the purple DPPH solution bleaching method. Scavenging DPPH via addition of a radical species or antioxidant renders the DPPH solution colourless. The strength of the colour change

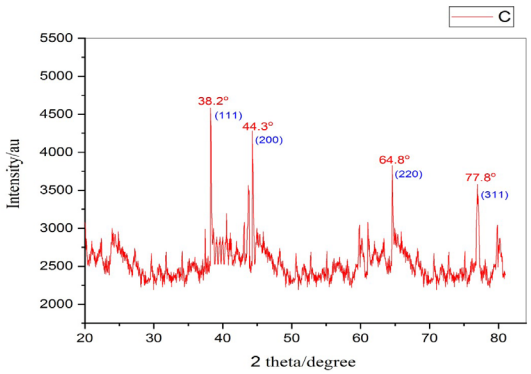

Figure 7: XRDimageof Synthesized Silver Nanoparticles.

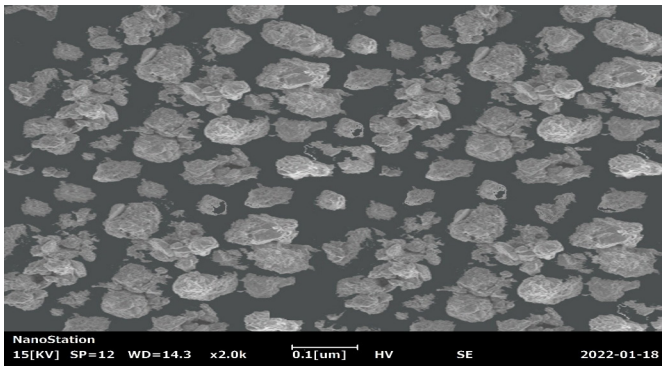

Figure 8: SEM image of green synthesized silver nanoparticles from18-β-Glycyrrhetinic Acid (GAAgNPs).

Table 4: DPPH assay for Antioxidant activity of synthesized AgNPs

S. no	*GAAgNPs Test sample*		*Standard Absorbace Ascorbic Acid (µg/mL)*	
	Conc. (ug/mL)	*% Scavenging*	*Conc. (ug/mL)*	*% Scavenging*
1.	10	35.38 ± 0.66	10	75.15 ± 0.81
2.	50	50.06 ± 0.82	50	75.76 ± 0.71
3.	100	61.67 ± 0.78	100	76.31 ± 0.88
4.	200	69.45 ± 0.67	200	79.27 ± 0.70
5.	400	76.05 ± 1.37	400	83.41 ± 0.72
6.	800	82.49 ± 1.06	800	87.03 ± 0.91

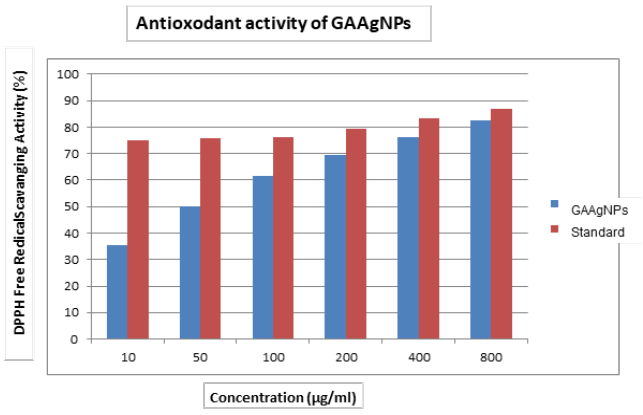

Figure 9: Antioxidant activities of GAAgNPs

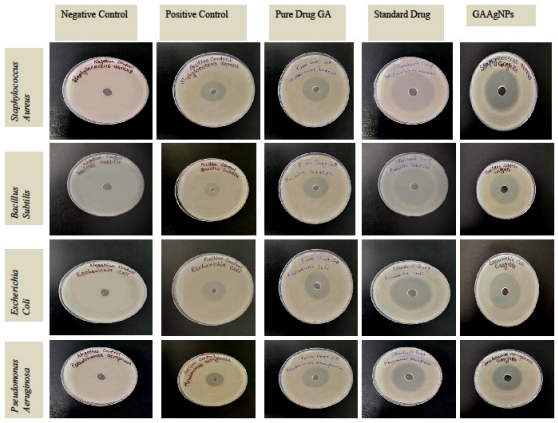

Figure 10: Antimicrobial activity of synthesized 18-*β*-Glycyrrhetinic acid silver nanoparticles (GAAgNPs) against 4 various human bacteria pathogens

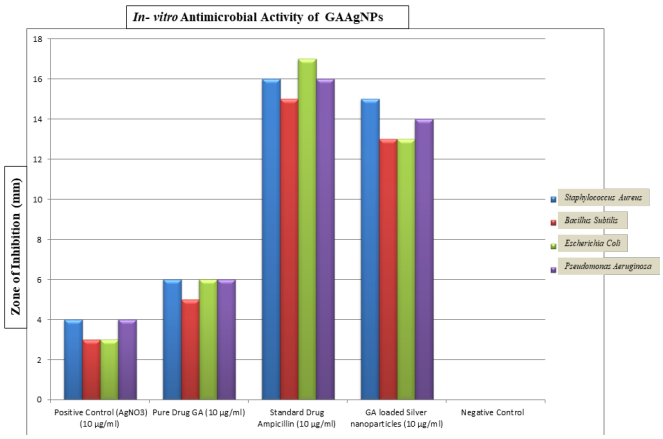

Figure 11: Antimicrobial action of 18-*β*-Glycyrrhetinic acid and its synthesized silver nanoparticles

Table 5: *In-vitro* antimicrobial activity of synthesized 18-β-Glycyrrhetinic acid silver nanoparticles (GAAgNPs)

Sample	*Experiential zone of inhibition in various pathogens (mm)*			
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
negative Control (Distilled Water)	0	0	0	0
Dummy Silver Nanoparticles as Positive Control ($AgNO_3$) (10 µg/mL)	4	3	3	4
Pure Drug 18-β-Glycyrrhetinic acid (10 µg/mL)	6	5	6	6
Standard Drug Ampicillin (10 µg/mL)	16	15	17	16
18-β-Glycyrrhetinic acid loaded Silver nanoparticles (GAAgNPs) (10 µg/mL)	15	13	13	14

is controlled by the antioxidant content. A drastic drop is indicative of the molecule's potent free radical scavenging capability. The current study demonstrated that synthesised silver nanoparticles (GAAgNPs) have antioxidant activity that is orders of magnitude more than that of ascorbic acid at high doses. At 800 µg/mL, the molecule demonstrated 82.49 ± 1.06% activity, which is high compared to the 87.03 ± 0.91% activity of conventional ascorbic acid. (Table 4 and Figure 9).[45]

Antimicrobial Activity of Silver Nanoparticles

Figure 10 & 11 display results of the antibacterial activity of GAAgNPs against 4 human bacterial pathogenic strains. Pure plant active18-β-Glycyrrhetinic Acidshows less antimicrobial activity thanits green synthesized silver nanoparticles (GAAgNPs). The results revealed that GAAgNPs have effective antimicrobial activityagainst *Staphylococcus aureus* and *Pseudomonas aeruginosa*, when compare with 2 extra clinical-pathogens. Zone of inhibition was seen against *S. aureus* (15 mm) and *P. aeruginosa* (14 mm) when using optimised GAAgNPs derived from 18-β-Glycyrrhetinic acid at a dose of 10 µg/mL. Table 5 displays the results of the zone of inhibition measurements. Because of its exceptional antibacterial activity alongside human medical pathogens like *S. aureus* and *P. aeruginosa*, RSM optimised GAAgNPs of plant active 18-β-Glycyrrhetinic acid are beneficial for treating multi drug-resistant bacteria pathogens.[46,47]

CONCLUSION

Here, we show that the plant-active compound 18-β-Glycyrrhetinic acid may be used in combination with a simple and inexpensive green synthesis methodology to create stable silver nanoparticles through a biological reduction method. Synthesised silver nanoparticles based on 18-β-glycyrrhetinic acid underwent rigorous optimisation of particle size and zeta potential using a Box-Behnken design. This was accomplished by factoring in the influence of several independent variables (factors), such as $AgNO_3$ concentration, incubation period, and temperature. SEM, XRD, UV-visible, FT-IR, and X-ray diffraction were all working to learn more about AgNPs' composition and structure. Under a SEM, silver nanoparticles that were made in the best way were found to be round and about 100 nm in size. With a PDI of 0.462, we found that the average particle size was 83.36 nm. When tested with

the DPPH method, it was found that the synthesised AgNPs had much more antioxidant activity than the standard antioxidant, ascorbic acid. Additionally, antibacterial assessment of these optimized silver nanoparticles revealed that the plant active 18-β-Glycyrrhetinic Acid, contributed most towards silver ions reduction and stability. In addition, the silver nanoparticles synthesised in this study displayed strong antimicrobial activity against four human diseases. It is ecofriendly and may contribute to its role as potential powerful weapons as antioxidant and antimicrobial agent, it can be deduced from the present study that 18-β-Glycyrrhetinic Acid-based synthesized silver nanoparticles is a better alternative to chemical synthesis.

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