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Design, Synthesis & Box-Behnken design based Optimization of Silver Nanoparticles of *Liquorice* root extract loaded Nano gel and evaluation of its Antimicrobial activity

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ABSTRACT

The current work presents a novel, fast, eco-friendly, and straightforward method for biologically synthesizing silver nanoparticles loaded nanogel using Liquorice (*Glycyrrhiza glabra* Lin.) root extract. In order to create stable silver nanoparticles through a biological reduction method, an aqueous stock solution of Liquorice extract was created and employed as a capping and reducing agent. Response surface analysis (RSA) based Box-Behnken design (BBD) was used to systematically optimize the method, accounting for the effects of temperature, reaction time, and concentration of silver nitrate ($AgNO_3$) on response. RSA was used in conjunction with a quadratic polynomial model for mathematical modeling to ascertain the relationship between the factors and the responses. 55°C, 60 mM $AgNO_3$, and 10 hours of incubation were ideal. In five hours at 55°C, silver ions (Ag+) can be transformed into silver nanoparticle using a methanolic stock solution containing glycyrrhizinic acid. The UV spectra of biosynthesised and optimized AgNPs show an SPR absorption peak at 419 nm. FTIR spectroscopy revealed that liquorice capped silver ions. Silver nanoparticle crystallinity was revealed by XRD. A 100 nm-sized spherical elemental silver particles were discovered using TEM. The average particle size, PDI and zeta potential were in desirable range. Silver nanogel of liquorice containing glycyrrhizinic acid silver nanoparticles demonstrated an upper limit of inhibition of 15 mm against *T. corporis* and 14 mm against *S. pyogenes* and 13 mm against *S. aureus*, respectively. Ultimately, there is strong antibacterial activity in the synthesized silver nanoparticles and their quality components, suggesting that this research can be applied to the development of practical biomedical products.

Keywords: Silver nanoparticle, Box-Behnken Design, Antimicrobial, Nanotchnology, Liquorice, Glycyrrhizinic acid.

INTRODUCTION

In terms of technological advancement, nanotechnology is a revolutionary approach that deals with the handling of materials at the nanoscale, or one billion times smaller than a meter. In real terms, nanotechnology refers to any technology that operates at the nanoscale and has a wide range of practical uses [1]. Metal and metal oxide nanostructures can be produced via a variety of physicochemical methods. These methods are usually applied to toxic, reactive reducing chemicals that affect plant and animal life as well as the environment. The nanomaterials were also produced by a variety of species, such as yeast, fungi, bacteria, and others, which resulted in a clean and environmentally friendly synthesis process [2, 3].

Plant derivatives with strong antitumor, anti-inflammatory, antifungal and bactericidal properties are of great interest to the health of humans and animals. These plant products can still be utilized in the synthesis of nanomaterials to link the properties of nanomaterials with those found in extracts and essential oils, and to lessen the negative effects of traditional synthesis routes based on toxic/hazardous chemical reagents [4, 5]. Plant-derived metal nanoparticles have a remarkable antibacterial action because of their high surface/volume ratio and nano range particle sizes. Because they are safe and effective antibacterial agents, environmentally friendly methods of synthesizing silver, copper, and zinc nanoparticles are widely employed in medicine [6, 7].

Special attention has been paid to silver nanoparticles (AgNPs), particularly in the field of biomedicine. AgNPs are well-known for their potent and broad-spectrum antimicrobial and anticancer properties. AgNPs have also been shown to have other biological activities, such as enhancing the immunogenicity of vaccines, causing bone healing and wound repair, and having anti-diabetic effects [8, 9]. AgNPs are a type of nanostructure that have long been used as antibacterial agents in the cosmetics, food storage, textile coatings, health and environmental industries [10]. AgNPs are utilized in a range of pharmaceutical formulations, including antibacterial clothes, burn creams, and coatings for medical equipment [11]. Significant inhibitory effects against both Gram-positive and Gram-negative bacterial biofilm development were exhibited by Central Venous Catheters (CVCs) coated with AgNPs [12]. AgNPs were also used in various fields of dentistry, such as dental prostheses, restorative and endodontic dentistry, and implantology [13]. AgNPs are essential to the creation and application of cutting-edge biomedical techniques because of their distinct physiochemical characteristics and biofunctional qualities, which include anti-inflammatory, antiplatelet, anti-

angiogenesis, antiviral, antibacterial and antifungal actions [14]. AgNPs are useful for fabrication of high-conductivity elements for printed electronics [15]. Applications involving biological sensing and imaging are well suited for silver nanoparticles [16]. For producing silver nanoparticles, the biological reduction process is recommended because of its one-step process and minimal environmental impact.

For many centuries, the dried root and rhizomes of the Glycyrrhiza genus have been used to make the amazing traditional Chinese medicine known as liquorice. It is composed of over 300 compounds, primarily classified as flavonoids, phenolic components, polysaccharides, volatile component, essential oils and triterpene saponins. Most active compounds of Liquorice (the roots of the leguminous plants *Glycyrrhiza glabra* L., *G. uralensis Fisch*. and *G. inflate Batalin*) is the triterpenoids glycyrrhizic acid (glycyrrhizin). Among these, a triterpenoid saponin known as glycyrrhizinic acid (GA) is regarded as the main chemical compound of Liquorice. Numerous biological activities, such as antitumor, antiviral, anti-inflammatory, antimicrobial, immunoregulatory, neuroprotective and cardioprotective properties, have been linked to the active ingredients in Liquorice. It is also used to treat several bodily dysfunctions such as fever, asthma, cough, sore throat, spasms, tonsilitis, gastric ulcer, dyspnea, arthritis, bronchitis, gastritis and skin diseases [17-20].

In this work, we synthesized stable silver nanoparticles (AgNPs) from glycyrrhizinic acid by using a biological reduction method. Application of Box-Behnken Design (BBD) of RSM (Response Surface Methodology), the goal of this work was to optimize several experimental parameters that are essential for the environmentally friendly synthesis of glycyrrhizinic acid AgNPs. Many analytical methods were used to determine the characteristics of the environmentally friendly and optimized AgNPs. Using further optimized AgNPs, the antimicrobial effectiveness against some human pathogens was evaluated.

MATERIAL AND METHODS

Materials

Glycyrrhizinic acid and silver nitrate were purchased from the Sigma Aldrich. All ingredients used in this study, including the double-distilled water, were of analytical quality.

Methodology

Synthesis and Characterization of AgNPs of Liquorice Root Extract

Liquorice root extract solution was prepared by adding 1.5 gm aqueous extract of Liquorice root in 50 ml of demineralized (D.M.) water (Solution 01). The AgNO3 solution was prepared in different concentrations (25-75 mM) by dissolving pre-weighed quantity of AgNO3 in D.M. Water (Solution 02). (Mol. Wt. of AgNO3 is 169.9 gm dissolve in 1000 ml of D.M. Water to prepare 1 M solution). The biosynthesis of AgNPs was carried out by using 25 ml of the Liquorice root extract solution (Solution 01) into 450 ml of 50 mM aqueous solution of AgNO3 (Solution 02) and stirring continued at room temperature for 10 min. Reduction takes place slowly which gives colloidal solution of AgNPs capped with Biomolecules of Liquorice root extract. After 24 hours, stabilized biomolecules capped AgNPs as shown by stable reddish brown colour of the solution. The above solution was added with Tween 20, a non-ionic surfactant then subjected to centrifugation at 12000 rpm for 30 minutes. The pellets obtained were washed with D.M. water and lyophilized for characterization and formulation development, shown in "Figure 1" [21].

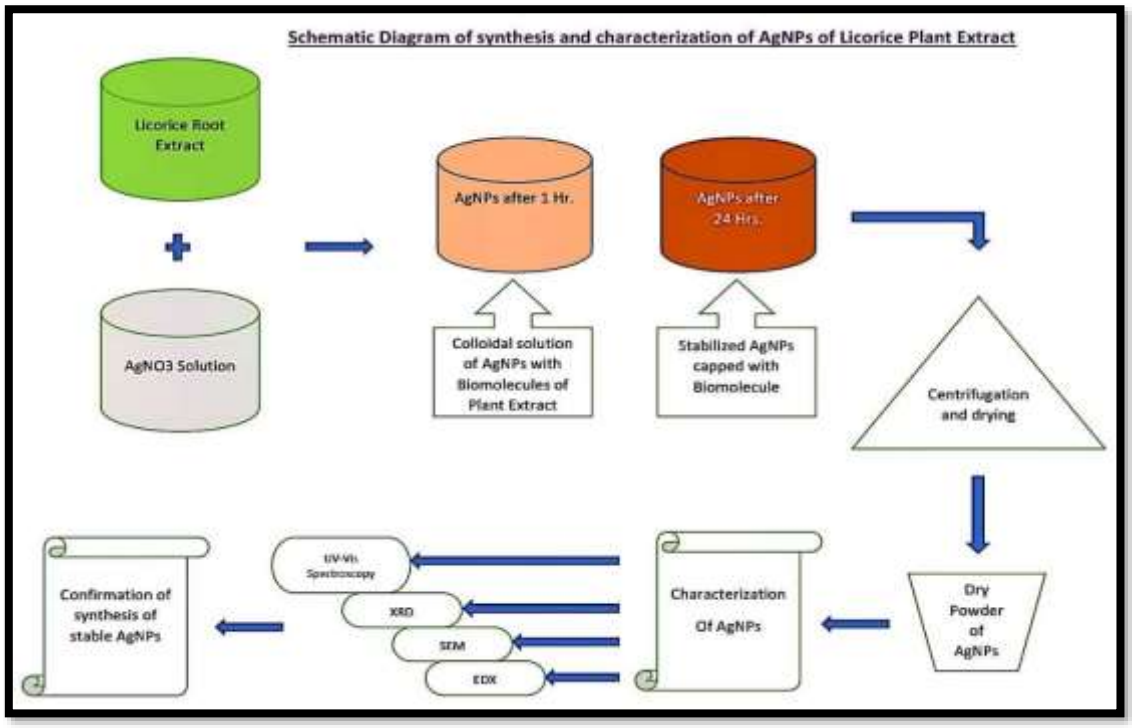

Figure 1. Synthesis and characterization of AgNPs of Liquorice root extract

Optimization of Silver Nanoparticles

The systematic optimization of silver nanoparticles was carried out using Box-Behnken design (BBD) with the help of Design Expert® ver.13 software (Stat-Ease Inc., Minneapolis, USA). Three most influential factors including concentration of AgNO3, temperature and reaction time were selected as independent variables (factors) for optimization at three different levels,

viz., low (−1), medium (0) and high (+1). A total of 17 experimental trials were suggested by the selected design as shown in Table 1. Particle size (nm) and polydispersity index of synthesized silver nanoparticles were analyzed as responses. After putting the data in BBD, mathematical modelling was performed to analyze the results. Quadratic second-order model was selected and the data-fitting with the model was analyzed by ANOVA along with other parameters like coefficient of correlation (R^2), adjusted R^2, predicted R^2 and predicted residual sum of squares. Optimized conditions required for silver nanoparticle synthesis were identified by the numerical desirability function and graphical optimization techniques [21]. "Table 1" summarizes the $AgNO_3$ nanoparticles of liquorice extract by factorial design.

Table 1. Synthesis of $AgNO_3$ nanoparticles of liquorice extract by factorial design

Factor 1	Factor 2	Factor 3
A: $AgNO_3$ (mM)	**B: Temperature (^{0}C)**	**C: Time (hr.)**
75	40	10
25	40	10
50	50	10
75	50	15
50	60	5
50	50	10
25	60	10
25	50	5
50	40	15
50	50	10
75	60	10
50	40	5
50	50	10
75	50	5
25	50	15
50	50	10
50	60	15

Characterization of Optimized Silver Nanoparticles

The characterization study of silver nanoparticles was done by the examining size, shape and quantity of particles. Various analytical techniques including UV-visible spectroscopy, Fourier Transmission

Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), Hydrodynamic Size and Zeta Potential Measurement and X-Ray Diffraction (XRD).

UV-Vis Spectroscopy

Absorbance spectroscopy is used to determine the optical properties of a solution. A Light is send through the sample solution and the amount of absorbed light is measured. When the wavelength is varied and the absorbance is measured at each wavelength. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law. The examination of nanoparticles, the optical properties are much more complicated. For instance, the measured absorbance spectrum does not necessarily show the actual absorbance but the extinction of the light is both the absorbed and the scattered light from the particles. These wavelengths arise due to the surface Plasmon resonance of the particle [21].

Fourier Transmission Infrared Spectroscopy

FTIR is a chemical analytical method which measures infrared intensity v/s wavelength or wave number of light. It used to analysis of possible bio molecule and also bonding interaction between themselves. IR spectroscopy detects the vibration characteristics of chemical functional groups of the sample. When an infrared light interacts with matter, chemical bonds will shows stretch, contract and bend form. This chemical functional group tends to adsorb infrared radiation in a specific wave number range of the structure of the rest of the molecule. The AgNPs synthesis, FTIR data measures interaction between Ag salts and proteins molecules, which accurate for the reduction of silver ions and stabilization of Ag NPS formed [21].

Transmission Electron Microscope

In order to better clarify the morphology and size of the particles, TEM analysis was applied. For the purpose of characterizing nanomaterials and obtaining quantitative measurements of particle and/or grain size, size distribution, and morphology, transmission electron microscopy (TEM) is a useful, widely used, and significant technique. The TEM image makes the morphology of the synthesized NPs visible. Since the hydrogel sample must be semi-transparent in order to transmit electron beams, it is imperative that this step be completed correctly for the TEM to work. It takes time to prepare the sample. The distance between the objective lens and its image plane as well as the distance between the objective lens and the specimen are the primary factors that determine the magnification. A thin specimen is exposed to an electron beam with a constant current density in a traditional TEM. A fluorescent screen is used to display the electron intensity distribution behind the specimen after it has been magnified using a three or four-stage lens system. A CCD camera can capture the image digitally, or directly through exposure of an image plate or photographic emulsion [22, 23].

Hydrodynamic Size and Zeta Potential Measurement of AgNPs

The average particle size along with its polydispersity index (PdI) and the zeta potential (ZP) of the nanoparticles was analysed by photon correlation spectroscopy and laser Doppler anemometry, respectively, using a Zetasizer Nano ZS (Malvern Instruments, UK). Prepared nanoparticles were separated and subjected to measurement following dilution with 0.45 μm filtered distilled water. Particle size and PDI measurements were performed at a scattering angle of 90∘ and at a temperature of 25∘C. The hydrodynamic diameter was calculated from the autocorrelation function of the intensity of light scattered from particles with the assumption that the particles had a spherical form. The samples for ZP were placed in a disposable zeta cell at a temperature of 25^0C and were measured using PALS technology. The measurement was repeated three times for each sample [24].

Energy Dispersive X-Ray Analysis (EDX)

The elemental analysis was performed using EDX, which is an attachment to the scanning electron microscopy. The sample powder of AgNPs was compressed to form tablets before analysis with EDX spectrum [24].

X-Ray Diffraction

XRD is a technique to used go study phase composition of a sample, crystal structure, texture or orientation. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffracted light at different angle. When X-ray passing through a crystal it produces a diffraction pattern, that diffraction gives the information about the atomic arrangement within the crystals. In AgNPs XRD gives phase structure and purity of the particle [21].

Formulation Development of Nano Gel Containing AgNPs of Liquorice Root Extract

The measured quantity of AgNPs of Liquorice root extract was dissolved in 50 ml. of D.M. water followed by addition of Polysobate-80 with continuous stirring by using magnetic stirrer. The measured amount of Glycerol, Methyl Paraben and Propyl Paraben were added to the solution. Carbopol 940/Guar gum according to the formulations (F1-F4) and Polyacrylamide was added gradually with continuous stirring for 30 minutes. Then neutralize the preparation by adding slow and constant stirring Triethanolamine solution until the Nano Gel preparation formed [25]. The composition of nano gel was given in “table 2”.

Table 2: Composition of Liquorice root extract AgNPs nano gel

Sr. No.	Ingredients	Unit Formula (mg) (For 50 gm Nano-gel)			
		F-1	F-2	F-3	F-4
1	AgNPs of Liquorice root extract	100	100	100	100

2	Guar gum	100	-	200	-
3	Carbopol 940	-	100	-	200
4	Polyacrylamide	750	750	750	750
5	Glycerol	0.5	0.5	0.5	0.5
6	PEG 600	0.2	0.2	0.2	0.2
8	Methyl Paraben	80	80	80	80
9	Propyl Paraben	10	10	10	10
10	Triethanolamine	300	300	300	300
11	Polysorbate 80	250	250	250	250
12	D.M. water (ml) Q.S.	50	50	50	50

Evaluation of Silver NPs loaded Nano Gel Formulation

Appearance and Consistency

The physical appearance was virtually checked for the texture of silver nano gel and observation reported.

Washability

Prepared formulation was added to the skin and then manually tested for ease and degree of washing with water and findings were recorded.

Extrudability Determination of Formulation

The mixtures were put into flexible aluminum tubes. The tubes were compressed in order to extrude a gel ribbon measuring 0.5 cm within a 10-second timeframe, and the extrudability of the formulations was subsequently assessed.

Determination of pH

pH of silver nano gel was checked by using numerical pH metre. 1gm of gel was dissolved in 25 ml of purified water and the electrode was then dipped into gel solution until steady reading was achieved. Measurements of pH were noted too time for individual formulation.

Viscosity

The viscosity of the formulation batches was determined using a (Visco QC100) viscometer with spindle L4 at 50 rpm. The spindle was allowed to move freshly into the silver nanogel, and the reading was noted.

Determination of Spreadability

For determined the spread ability of the gel, 0.5 g gel was placed within a circle of 1 cm diameter pre marked on a glass plate over which a second glass plate was placed. A weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in the diameter due to spreading of the gels was noted.

$$\textbf{\textit{Spreadability (S) = m x lt}}$$

Where,

S = Spreadability (gcm/sec)

m = weight tied to the higher slide i.e. 20 gm

l = length of glass slides i.e. 6 cm

t = time taken in seconds

Drug Content

The composition of the silver nanogel was measured by taking 1 gm. of gel mixed with methanol in 10 ml volumetric flask. The mixture was vortexed for 15 seconds and after that take the absorbance in UV at pre-determined λmax.

In-vitro Drug release

Franz diffusion cell was used to determine the release profile of silver nanogel. A thin membrane (dialysis membrane) was placed over the donor compartment. Capacity of receptor chamber was 18 ml. A magnetic bead was placed in the receptor chamber. The diffusion medium consisted of phosphate buffer pH 7.4. Whole assembly was put on magnetic stirrer at 50 rpm stirring speed and temperature was maintained at 37.0 ± 0.5^0 C. The 1g samples were kept over the membrane in donor compartment and stirred. 2ml samples were withdrawn from the receptor compartment at predetermined time intervals (0.5h, 1h, 2h, 3h, 4h, 5h, 6h, 8h) and the volume was replenished with same volume of buffer medium. Addition of buffer medium to the receptor compartment was performed with great care to avoid trapping air beneath the diffusion membrane. The samples were analyzed by UV at pre-determined λmax. The % drug release was calculated, and graph of % drug release v/s time was plotted.

Antibacterial Activity of AgNPs of Liquorice Root Extract Nanogel

Agar well diffusion assays were performed to check for antibacterial activity of green synthesised glycyrrhizinic 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, Streptococcus pyogenes*, *Tineacorporis*, *T. cruris*, *T. pedia* 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 was 6 mm in diameter. Then, the well was loaded with 10 μg of ampicillin, while the other

wells contained positive control silver nanoparticles ($AgNO_3$), 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 [20].

RESULTS AND DISCUSSION

Synthesis and characterization of Silver nanoparticles of Liquorice extract

During past few years, numerous plant extracts are being employed for the synthesis of silver nanoparticles by biological reduction method [1]. Glycyrrhizinic acid is the most active compound of Liquorice and comprised of various biological activities viz., antimicrobial, antiulcer, immunomodulatory, antiviral, anti-inflammatory and anti-oxidant activity [2]. In the current research work, silver nanoparticles were synthesized using aqueous extract of liquorice root via biological reduction method. Silver nitrate (AgNO3) was used as a precursor, and glycyrrhizinic acid (GL) was used as a reducing agent to synthesize AgNPs of Liquorice extract. Visually, the color change from colorless to yellow confirmed the formation of AgNPs, which was further supported by the SPR band. The colloidal silver prepared under several different reaction conditions appeared as different shades of yellow under natural light. However, they were all clear and transparent without precipitation.

Optimization of Silver Nanoparticles of Liquorice extract

The systematic optimization of silver nanoparticles was carried out using Box-Behnken design (BBD) with the help of Design Expert® ver.13.0 software (Stat-Ease Inc., Minneapolis, USA). Particle size (nm) and polydispersity index of these silver nanoparticles were optimized as responses. $AgNO_3$ concentration, temperature and reaction time were selected as independent variables (factors) at three different levels, viz., low (−1), medium (0) and high (+1). A total of 17 experimental trials were suggested by BBD by Design Expert® ver. 13 software. Eqs. (1) and (2) were obtained as the polynomial equations generated after the data modelling, which indicated presence of both interaction and curvature effect for both the response variables analyzed (particle size and polydispersity index). The parameters like coefficient of correlation were found excellent in the range between 0.9719 (for particle size) and 0.9927 (for polydispersity index), along with good values of adjusted and predicted R^2. Eqs. (1) and (2) were obtained as the polynomial equations generated after the data modelling, which indicated presence of both interaction and curvature effect for both the response variables analyzed (particle size and polydispersity index).

Particle size=

126.79-130.47*A-20.58*B-13.38*C+4.08*AB+35.60*AC+7.90*BC+98.38*A2+51.49*B2+48.73*C2…. Eq.1

Polydispersity index=

0.753+0.158*A+0.031*B-0.011*C+0.025*AB+0.030*AC-0.026*BC-0.191*A2-0.155*B2-0.140*C2……Eq.2

Box-Behnken Design was used to determine the optimal AgNPs synthesis conditions. Table 3 & 4 displayed the ANOVA results for Particle size and PDI indicating that the quadratic regression model was statistically significant for predicting the relationship between particle size and PDI.

Table 3. ANOVA analysis of Response 1 (Particle size)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2.145E+05	9	23830.61	26.90	0.0001	significant
A-AgNO3	1.362E+05	1	1.362E+05	153.69	< 0.0001	
B-Temperature	3387.06	1	3387.06	3.82	0.0915	
C-Time	1431.93	1	1431.93	1.62	0.2442	
AB	66.50	1	66.50	0.0751	0.7920	
AC	5070.15	1	5070.15	5.72	0.0480	
BC	249.64	1	249.64	0.2817	0.6120	
A^2	40750.03	1	40750.03	45.99	0.0003	
B^2	11163.03	1	11163.03	12.60	0.0094	
C^2	9998.37	1	9998.37	11.28	0.0121	
Residual	6202.25	7	886.04			
Lack of Fit	6193.63	3	2064.54	957.89	< 0.0001	significant
Pure Error	8.62	4	2.16			
Cor Total	2.207E+05	16				

The Model F-value of 26.90 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 957.89 implies the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value this large could occur due to noise. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 14.549 indicates an adequate signal. This model can be used to navigate the design space.

Table 4. ANOVA analysis of Response 2 (PDI)

Source	Sum of Squares	df	Mean Square	F-value	p-value	

Model	0.5970	9	0.0663	105.51	< 0.0001	significant
A-AgNO3	0.2010	1	0.2010	319.66	< 0.0001	
B-Temperature	0.0078	1	0.0078	12.33	0.0098	
C-Time	0.0011	1	0.0011	1.79	0.2223	
AB	0.0026	1	0.0026	4.14	0.0814	
AC	0.0036	1	0.0036	5.73	0.0480	
BC	0.0029	1	0.0029	4.55	0.0703	
A^2	0.1549	1	0.1549	246.30	< 0.0001	
B^2	0.1012	1	0.1012	160.95	< 0.0001	
C^2	0.0831	1	0.0831	132.25	< 0.0001	
Residual	0.0044	7	0.0006			
Lack of Fit	0.0042	3	0.0014	25.49	0.0046	significant
Pure Error	0.0002	4	0.0001			
Cor Total	0.6014	16				

The Model F-value of 105.51 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The Lack of Fit F-value of 25.49 implies the Lack of Fit is significant. There is only a 0.46% chance that a Lack of Fit F-value this large could occur due to noise.

Figure 2 represented the 2D contour plot showing the influence of concentration of AgNO3 and temperature on Particle size (a) and 3D response surface plot showing the influence of concentration of AgNO3 and temperature on Particle size. “Figure 3” represented the 2D contour plot showing the influence of AgNO3 concentration & temperature on PDI, and 3D response surface plot of PDI.

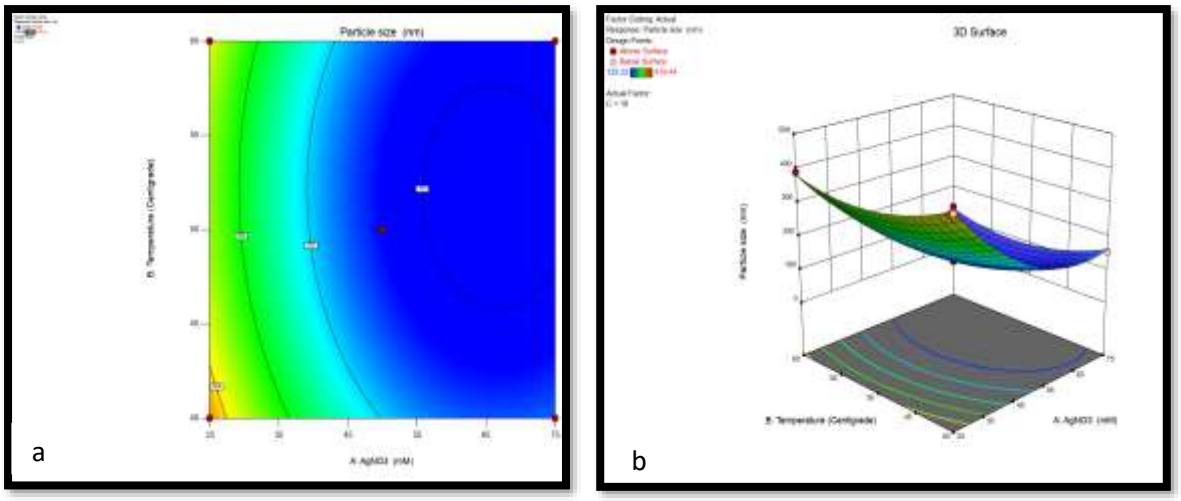

Figure 2: (a)2D contour plot showing the effect of AgNO3 concentration and Temperature on Particle size, (b) 3D response surface plot of particle size

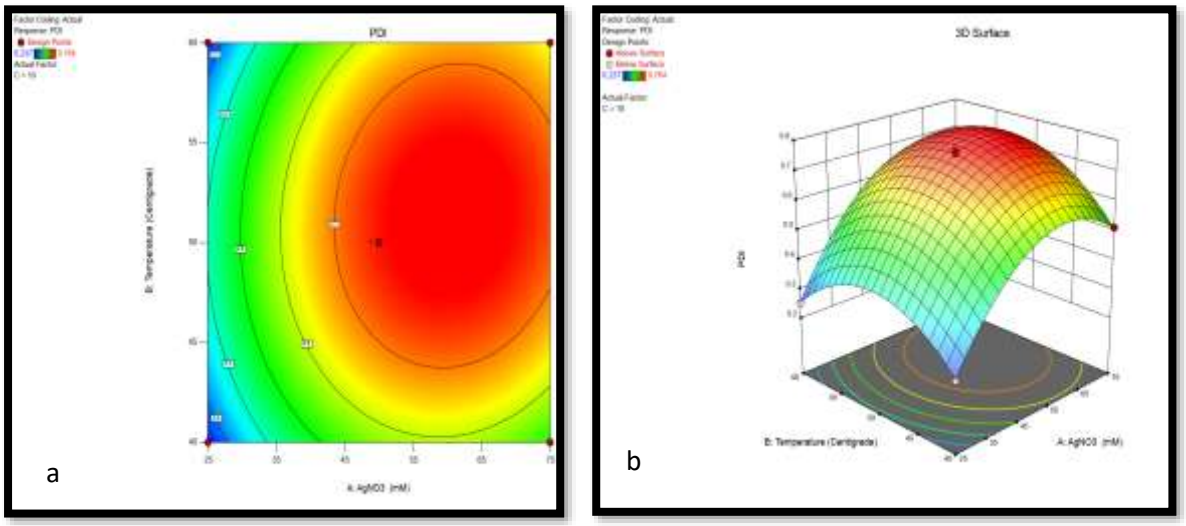

Figure 3.(a) 2D contour plot showing the effect of AgNO3 concentration and Temperature on PDI, (b) 3D response surface plot of PDI

Based on the data, we can conclude that AgNO3 concentration (mM), temperature (°C) and reaction time (hr.) are all significantly related to one another. By using BBD, the best values of the variables were found. After optimising the amount of AgNO3 (60.417 mM), the time of reaction (10 hrs), and the temperature (50.55°C), the model indicated that particles with the desirable particle size and PDI 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. Graphs showed how the different factors affected the process of making the best silver nanoparticles. Based on the above results, an optimized batch was selected which was further evaluated for various parameters.

Characterization of AgNPs of Liquorice extract

Prepared silver nanoparticles of Liquorice extract were evaluated for various parameters.

UV-Vis Spectroscopy

Absorption spectra of optimised silver nanoparticles were measured using UV-visible spectrophotometry, as displayed in Figure 4. Optimised silver nanoparticles were synthesised and stored at room temperature for 24 hrs at optimum concentration of silver nitrate, temperature and reaction time. 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 reaction 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) while the sharp absorption peaks indicates formation of homogeneous silver nanoparticles.

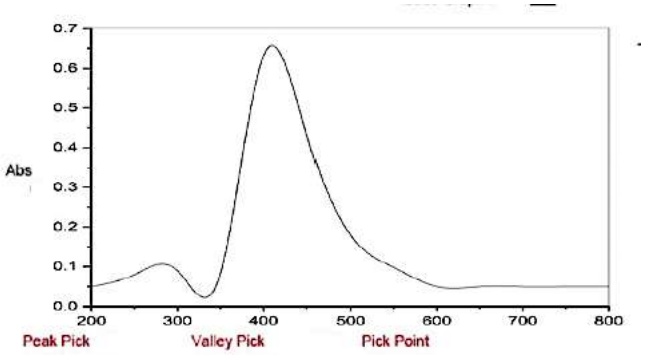

Figure 4: UV-visible absorption spectra of AgNPs of Liquorice extract (optimized batch)

FTIR study

FTIR spectrum of synthesised Liquorice silver nanoparticles was presented in figure 5. FTIR investigations examined functional groups that reduce, cap and stabilize Glycyrrhizinic acid synthesized nanoparticles. The peak bands for Glycyrrhizinic acid are OH stretching modes at 3423 cm^{-1}, aliphatic C-H at 2920 cm^{-1} and amide I at 1630 cm^{-1}. The strong peak at 1042 cm^{-1} showed methyl C-H stretching vibration, while the peak at 858 cm^{-1} represents beta-glucosidic linkage. The FTIR spectra of synthesized nanoparticles of Glycyrrhizinic acid followed the spectra of pure drug.

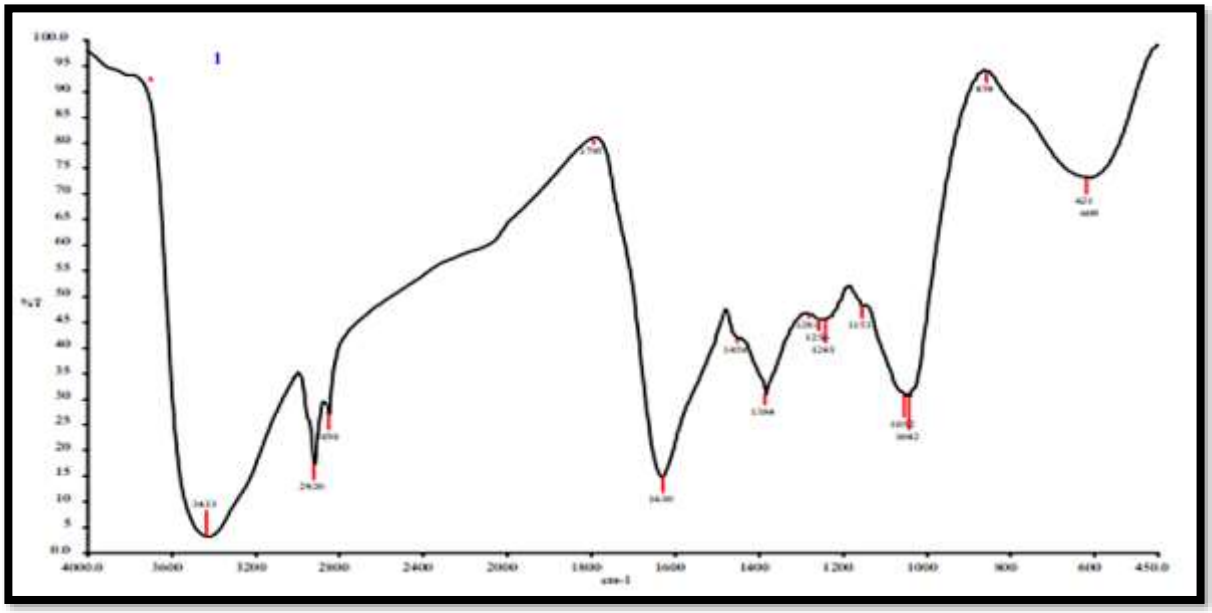

Figure 5: FTIR spectra of AgNPs of Liquorice extract (optimized batch)

Transmission Electron Microscopy (TEM)

The TEM figures out the spherical morphology of the formed AgNPs of Liquorice extract as displayed in Figures 6A. It was observed that the size of the colloidal solution of AgNPs coated with liquorice have small size with marginal increase by increasing the concentration of Ag ions in the solution upto 50 mM. Further increase in the Ag ions up to 75 mM led to highly aggregated products without complete reduction of Ag ions.

Hydrodynamic Size and Zeta Potential Measurement of AgNPs

Mean PS diameter and PDI were all measured in solutions using dynamic light scattering (DLS). The size of the colloidal AgNPs and their granulometric are disclosed (Figure 6(B)). As is evident, the size of the particles is between 10 and 100 nm. PS analyzer displays the presence of AgNPs with desirable PDI. Figure 6(C) showed the ZP measurement of AgNPs at concentration equal to 50mM in a solution form. For the obtained AgNPs, ZP value was measured and found to be −28.61mV. Current data are in conformation with the literature; solutions with ZP above +20mV or below −20mV are considered stable.The average size and PDI determined by DLS are in concurrence with the the results of TEM image and the particle size distribution evaluated using Image J 1.45 s software. By studying the dynamic light scattering, it is clear that the AgNPs exhibit a narrow size distribution for the nanoparticles.

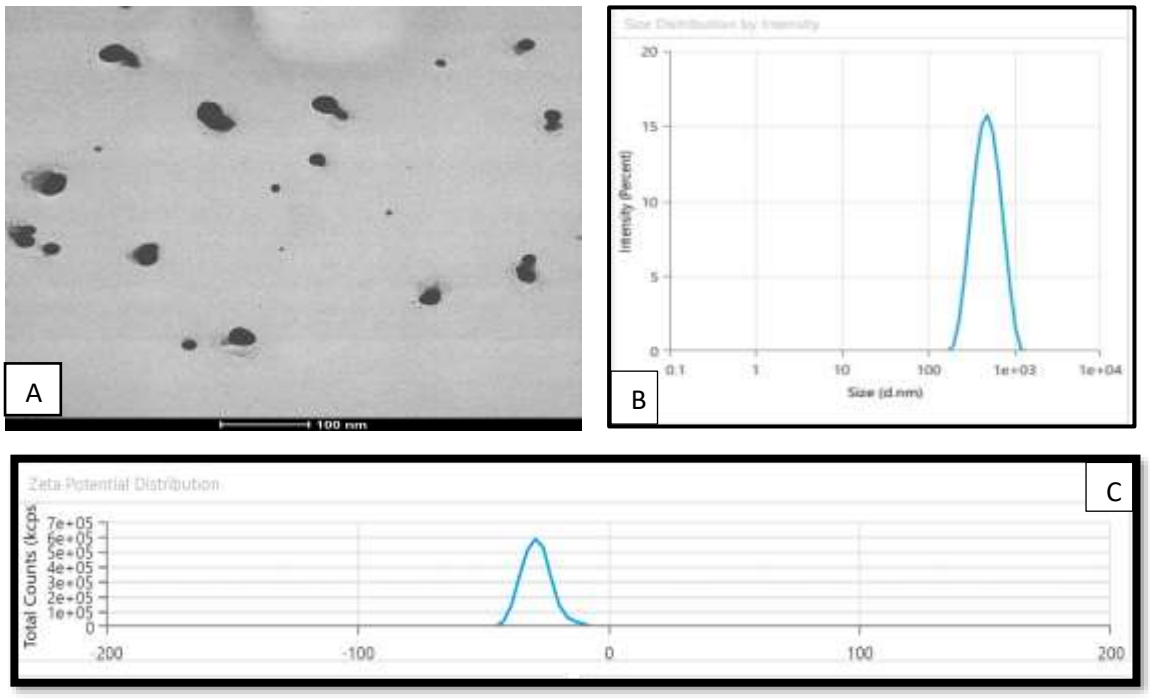

Figure 6: TEM image of synthesized AgNPs of Liquorice extract (optimized batch) (6A), Particle size of synthesized AgNPs (6B) and zeta potential of synthesized AgNPs (6C)

Energy Dispersive X-Ray Analysis (EDX)

Chemical analysis of the produced AgNPs was accomplished by means of EDX, which confirmed both the existence of Ag and the Glycyrhizinic acid present in Liquorice extract that covers the AgNPs; the latter is implied by the presence of the C, O, and Na peaks in the EDX spectra (Figure 7(a)). Metallic silver nanocrystals generally show typical optical observation peak approximately at 3 keV due to surface plasmon resonance. The EDX spectra also proved that the Ag nanoparticles are in metallic form, with no formation of Ag2O in them and free from any other impurities. Figure 7(a) shows the elemental map of AgNPs. The results indicated that AgNPs are homogeneous and stabilized by Glycyrrhizin present in liquorice extract.

XRD Studies of AgNPs of Liquorice extract

X-ray diffraction (XRD) was performed to ascertain the identity of the final products. The XRD results of AgNPs of Liquorice extract were shown in Figure 7 (b). The Ag- NPs coated with Liquorice extract, the four diffraction peaks obtained at angles 2θ of 28.1952∘, 34.2987∘, 39.4087∘, and 47.6079∘, respectively, are attributed solely to the face-centred cubic (fcc) crystalline silver content of the sample. Furthermore, no additional peaks in the XRD were observed for Ag2O revealing the high purity of the as synthesized silver crystal.

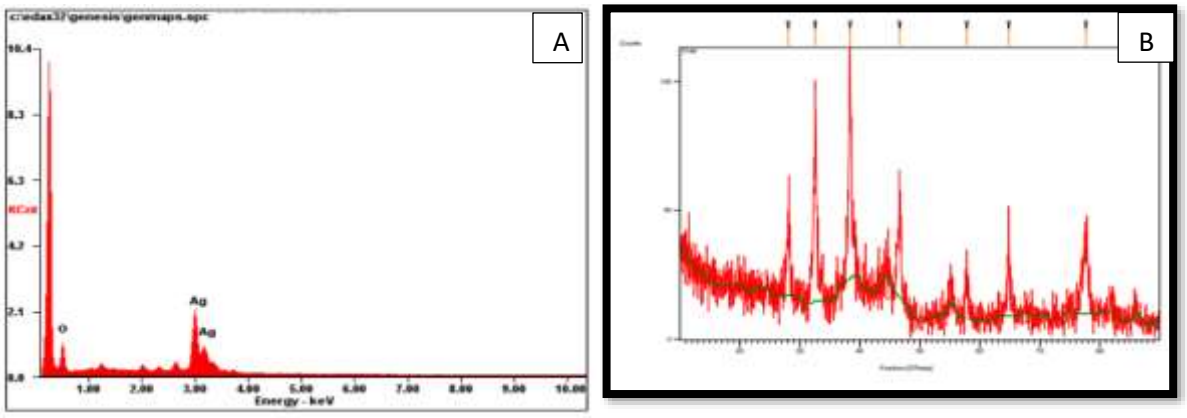

Figure 7: EDX spectra of synthesized NPs of Liquorice extract (Optimized batch) (7A), X-ray Diffraction pattern of synthesized NPs of Liquorice extract (7B)

Formulation and Evaluation of Silver Nano Gel Formulation

The optimized batch of thus formulated and evaluated, was further converted into nanogel utilizing guar gum and Carbopol 940 and results were depicted in following sections.

Evaluation of physical characteristics of Nano gel

During checking of physical characters of prepared silver nanoparticles laden nano gel, it was found that prepared gel was light brown in colour and homogeneous with smooth texture. No clogging was seen in the nano gel and it was easily washable (Table 5).

Table 5: Physical characteristics of Nano gel formulation

FORMULATION CODE	COLOUR	CLOGGING	HOMOGENEITY	TEXTURE	WASHABILITY	EXTRUDABILITY
F1	BROWN	ABSENT	GOOD	SMOOTH	GOOD	GOOD
F2	BROWN	ABSENT	GOOD	SMOOTH	GOOD	GOOD
F3	BROWN	ABSENT	GOOD	SMOOTH	GOOD	GOOD
F4	BROWN	ABSENT	GOOD	SMOOTH	GOOD	GOOD

Evaluation of nano gel formulation

The nano gels prepared with silver nanoparticles found with good spreadability, viscous with pH ranged in between 6.50 to 6.87 (Table 6).

Table 6: Results of Spreadability, Viscosity, drug Content and pH of nano gel

FORMULATION CODE	Spreadability* (gcm/sec)	Viscosity* (cp)	Drug (Glycerrhizin) Content (%)	pH
F1	27.193	2916.35	96.84	6.50
F2	27.329	3259.26	97.12	6.65
F3	20.783	8128.11	97.86	6.79
F4	18.124	6320.05	98.98	6.87

The results of evaluation of Liquorice extract AGNPs showed that promising results were represented by formulation F4 among other three formulations, thus it was selected as best batch and further evaluated for in-vitro drug release study and anti-microbial activity.

The in-vitro drug release profile of best batch was depicted in figure 8.

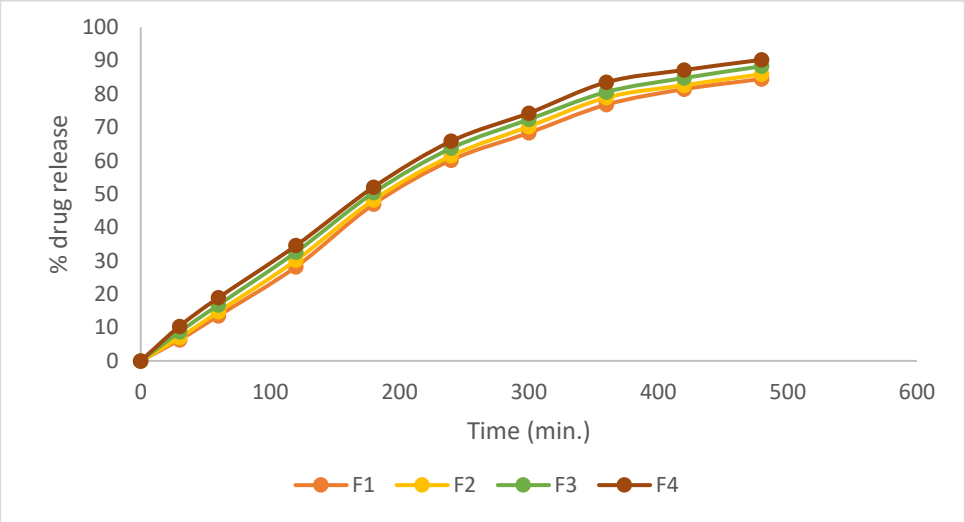

Figure 8: *In-vitro* release profile of nano gel formulation of optimized batch of AgNPs of Liquorice extract

Antibacterial Activity of AgNPs of Liquorice Root Extract Nanogel

Antibacterial activity of prepared nano gel was evaluated against of some human pathogens. Pure drug (glycyrrhizin) showed less activity than nano gel prepared with AGNPs of liquorice extract. The results revealed that Liquorice containing Glycyrrhizinic acid AgNPs nano gel have effective antimicrobial activity against *E. coli*, *S. pyogenes*, *S. aureus*, *T. corporis*, *T. Cruris* and *T. pedia*. Zone of inhibition was seen against *T. corporis* (15 mm), *S. pyogenes* (14 mm), *S. aureus* (13 mm). *T. Cruris* (12 nm) and *T. pedia* (10 mm) and *E.coli* (10 mm).

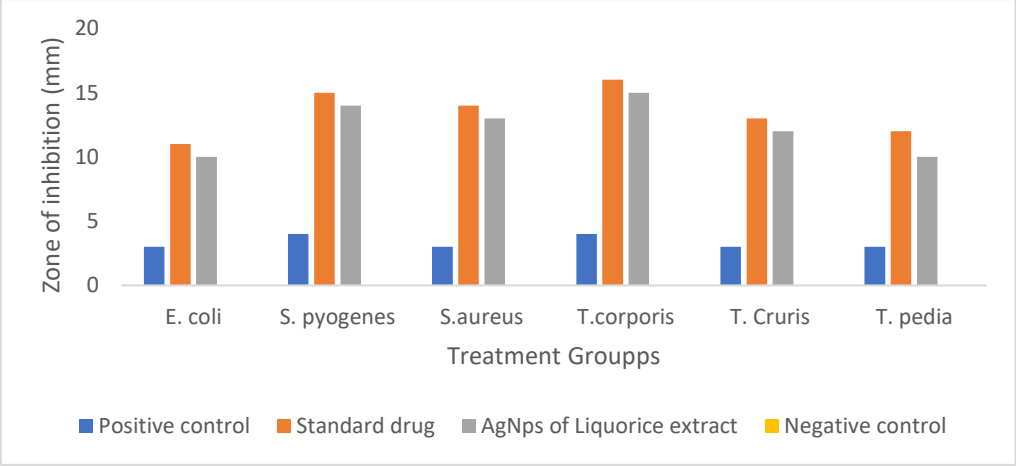

Figure 9: Antimicrobial activity of Glycyrrhizinic acid (liquorice extract) silver nanoparticles

Figure 9 depicted the results of zone of inhibition measurements. Because of its versatile anti-bacterial activity again some pathogenic organisms, it can be beneficial in treating such diseases.

CONCLUSION

Here, we showed that the plant- active compound i.e., Glycyrrhizinic acid in Liquorice extract, may be used in combination with a simple and in-expensive synthesis methodology to create stable silver nanoparticles through a biological reduction method. Synthesised silver nanoparticles based on glycyrrhizinic acid underwent rigorous optimisation of particle size and PDI using a Box-Behnken design. This was accomplished by factoring in the influence of several independent variables (factors), such as AgNO3 concentration, reaction time, and temperature. TEM, EDX, UV-visible, FT-IR, and X-ray diffraction were all working to learn more about AgNPs' composition and structure. Under a TEM, silver nanoparticles that were made in the best way were found to be round and about 100 nm in size. Antibacterial assessment of these optimized silver nanoparticles revealed that the plant active in Liquorice extract contributed most towards silver ions reduction and stability. In addition, the silver nanoparticles synthesised in this study displayed strong antimicrobial activity against some pathogens. It is ecofriendly and may contribute to its role as potential powerful weapons as an antimicrobial agent. It can be deduced from the present study that Glycyrrhizinic acid-based synthesized silver nanoparticles is a better alternative to synthetic counterparts.

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