Endemic *Inula Viscosa (L.)* Extracts and Their Potential for Both Biosynthesizing Silver Nanoparticles and Anti-microbial Activity

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Endemic *Inula Viscosa (L.)* Extracts and Their Potential for Both Biosynthesizing Silver Nanoparticles and Anti-microbial Activity

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Abstract

Green synthesis has recently become one of the most popular methods, as it is both low-budget and environmentally friendly. One of the important considerations in green synthesis is to perform an optimization study because it is necessary to understand how different application conditions (pH, incubation time, metal concentration, etc.) can affect the formation of nanoparticles with different morphology and efficiency, underlining the need for optimization of the process. In this study, firstly the endemic *Inula Viscosa (L.)* plant, popularly known as cancer grass, was extracted using distillation method. Then, silver nanoparticle (AgNPs) biosynthesis was carried out using the extract of *Inula Viscosa (L.)* plant. Their physicochemical characterization was conducted using Fourier-transformed infrared spectroscopy (FTIR), UV-visible spectrophotometry (UV-Vis), Scanning Electron Microscopy (SEM), and Dynamic Light Scattering (DLS). The time, pH, and AgNO3 concentration, which affect the characteristic and morphological properties of AgNPs, were optimized with the Box Behnken Design (BBD) method, with statistical and experimental design determined by means of a Design Expert statistical software program. The disk diffusion method was also implemented and optimized to increase antimicrobial activity. The study determined the optimal levels of AgNPs, which were green synthesized by *Inula Viscosa (L.)*, provided proof of its antimicrobial properties, and demonstrated their potential to be used as a low-budget aid to new generation clinical treatment methods.

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Keywords

- Silver nanoparticles
- Biosynthesis
- *Inula Viscosa (L.)*
- Antimicrobial activity

1 Introduction

Nanotechnology focuses on structures with dimensions below 100nm, and can be defined as an interdisciplinary technology. Recently, in nanotechnology, there has been a trend towards green synthesis method, which is low cost, environmentally friendly and relatively easy to synthesize. In chemical synthesis, the product is synthesized in a shorter time and in desired sizes, but this is a toxic and expensive method. Also, the yield is higher in green synthesis, which uses various biological sources such as plant extracts, bacteria, microalgae and fungi as reducing agents [1].

Inula viscosa (L), which was used in the study, is an endemic species in countries such as Turkey, Algeria, and Tunisia, is also popularly called ‘cancer grass’ and is a plant widely used in traditional medicine. Among the phytochemicals in *Inula Viscosa (L.)*, tomentosin in particular, has anticancer, antimicrobial, and anti-inflammatory properties. However, there are few medical studies on this plant in the literature [2].

These few preliminary studies show that it is important to investigate the potential of the plant in nanoparticle synthesis. Thus, we focused on the effect of the extract of this plant on silver nanoparticle synthesis. One of the major reasons for the selection of silver nanoparticles is that these are known to play an effective role in destroying the permeability of the bacterial membrane [3]. Chemical methods used to synthesize silver nanoparticles increase the harmfulness of the product by using toxic solvents, and reduce its potential. Due to these limitations, it was considered more appropriate to select the biosynthesis method, which is environmentally friendly and has less toxic effects [4]. So far, there has been no attempt to achieve silver nanoparticle biosynthesis from the extracts of *Inula viscosa (L.)* or its species in the literature.

This study sets out to determine the nanoparticle synthesis potential and usage areas of *Inula viscosa (L.)*, which have not yet been studied, and also to optimize efficiency, which has never been previously attempted. At the same time, optimization of the process is important for biological synthesis, considering that different application conditions (pH, incubation time, metal concentration, etc.) may cause nanoparticle formation with different morphology and efficiency [5]. Box Behnken Design (BBD) method, a response surface methodology, was used for optimization steps with Design-Expert program and optimization was found for the effect of incubation time (30–240 min), pH (4–8) and AgNO3 (metal) concentration (1–8 mM) on the average size diameter in nanoparticle synthesis. After nanoparticle production, characterization was performed [6] and the characterization of nanoparticles was conducted using Fourier-Transformed Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), UV-Vis spectrophotometry and Dynamic Light Scattering (DLS), and disc diffusion method.

2 Material and Method

2.1 The Extraction of the Plant

Extract of *Inula viscosa (L.)* plant was obtained using the infusion technique of extraction process, which included distilled water as a solvent. In the first step, the raw plant was passed through the grinder to obtain its ground form, and in the second, the infusion system was established. In the infusion system, distilled water is transferred to a 500 mL beaker, and the magnetic stirrer is run at 350 rpm and set at 60 ℃. Then the ground herb is added to the beaker, and allowed to infuse for 20–30 min. The temperature is maintained at 60 ℃ or below in order to obtain the most optimal level of the plant extract without losing its phytochemical and antimicrobial properties. After this process, a vacuum filtration device and Whatman No:1 filter paper were used to eliminate unwanted particles. The plant extract obtained after filtration was stored at + 4 ℃ for use in nanoparticle synthesis.

2.2 The Experiment Management with the Box Behnken Design Method

A three-level fractional factorial design developed by Box Behnken Design (BBD) was applied to determine the nature of the surface response in an experimental region. This design has many advantages, such as having three levels that can be coded as 1 (low), 0 (medium), and + 1 (high), creating an independent quadratic design and facilitating the organization and interpretation of results. Each block consists of the maximum and minimum values, the factorial design values, and the factors’ central values. The BBD method has the advantage that it saves time as it has fewer factor levels and has no excessively high or low number/levels of experiments. The BBD method reduces the number of experimental sets and is widely used in biotechnological research [7].

The parameters to be used in nanoparticle optimization in this project and their minimum and maximum values are as follows.

- Incubation time (30–240 min)
- pH (4- 8).
- $AgNO_3$ concentration (1–8 mM) [8, 9].

2.3 The Synthesis Silver Nanoparticles (AgNPs) by *Using Inula Viscosa (L.)* Extract

The BBD method was used to both identify and optimize parameters that affect various unique properties of nanoparticles after plant extraction. For optimum nanoparticle synthesis using the extract of *Inula Viscosa (L.)* plant, 17 experimental design runs were determined by Design Expert program. This involved the use of metal concentrations of 1, 4.5 and 8 mM $AgNO_3$. The pH values of 3 different metal concentrations were adjusted as 4, 6 and 8 using a pH meter. Then, for the synthesis of silver nanoparticles, the metal solution and plant extract were prepared in a 1:1 ratio in a working volume of 40 mL. The stock solution containing the plant extract was added dropwise to the medium containing $AgNO_3$ at a rate of 1 drop/s, thus initiating the nanoparticle production process. Incubation was completed at 25 ℃ at a mixing speed of 200 rpm using a shaking incubator at intervals appropriate to the experimental design. The aim of stirring was to increase the interaction of the plant extract with the metal solution, to produce nanoparticles in smaller sizes, and to prevent aggregation and homogeneous distribution. After the incubation period for all experimental trials, procedures were applied to calculate the nanoparticle concentration and the samples were dried and stored using the lyophilization process before characterization.

2.4 The Characterization of Nanoparticles

In this study, UV-Vis spectrophotometry, FTIR, SEM and DLS characterization methods were used to determine the properties of the synthesized nanoparticles.

UV-Vis spectrophotometry is a technique used to measure the light absorbed and emitted by a sample. UV-Vis spectrophotometry is very important for the characterization and examination of nanoparticles due to its optical properties, which are sensitive to nanoparticles. UV visible region devices operate between 200 and 900 nm [10].

In order to identify structural, compositional and functional groups associated with AgNPs formations, Infra-red spectra analysis of AgNPs was performed using Per-kin Elmer Spectrum FTIR Spectrometer at room temperature, within the range of 400- 4000 cm^{-1} [9, 10].

Scanning Electron Microscopy (SEM) works by scanning the particle surface with high-energy electrons to characterize the morphology of nanoparticles [11, 12]. AgNPs coated with Au-Pd under 7.50 kV high vacuum were recorded by Scanning Electron Microscopy (Thermo Scientific Apreo S).

Malvern Zeta Sizer was used to find the harmonic mean diameters and polydispersity index (PI).

2.5 The Antimicrobial Activity Test

Agar disk diffusion method was used to examine the antimicrobial activity of plant-based nanoparticles [13]. The test was performed on gram-negative (*Escherichia coli* (EA)) and gram-positive (*Staphylococcus aureus* (SA)) bacteria, and the antimicrobial effect of nanoparticles was compared with the control group in terms of the diameter of white plaque formed on the agar surface after incubation under appropriate conditions. All steps were followed inside the Class II Laminar Flow Biosafety Cabinet to ensure aseptic conditions. The results were evaluated in the Design Expert program.

3 Result and Discussion

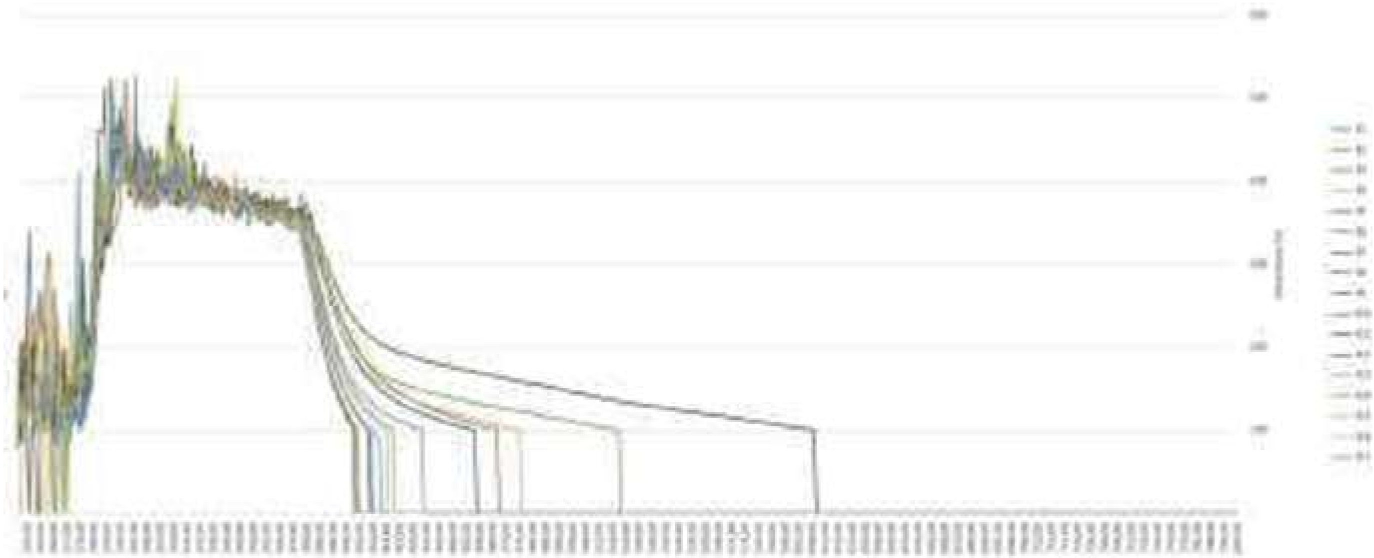
The nanoparticles synthesized by green synthesis were produced sustainably from the plant source, and during this process, cell metabolites were also used. In this study, optimized results were obtained after characterization. This study can also provide a model for further studies on biological synthesis for this selected *Inula Viscosa (L.)* plant species. In addition, there are very few studies on *Inula viscosa (L.)* plant used in the study at either national or international levels. The reasons for choosing to study a flowering plant species native to Turkey, and its- other bioactive compounds are the plant’s anti-cancer, anti-microbial, total flavonoid content, total phenolic values, and the resulting high antioxidant activity. At the same time, antimicrobial tests have proven the antimicrobial effects of nanoparticles biosynthesized with the extract of this plant. Addressing the lack of research on this herb is one of the main goals of the study. The anticancer and antimicrobial effects of the plant species *Inula Viscosa (L.)* have already been reported [14], but the current study is notable as the first to carry out the green synthesis of *Inula viscosa (L.)* plant based nanoparticles with silver.

3.1 The Results from Characterization Studies

The factors chosen for optimization are metal concentration, incubation time and pH values that affect the biosynthesis of nanoparticles.

UV-Vis spectrophotometry is a technique used to measure the light absorbed and emitted by a sample, using the measurement of the intensity of a beam after passing through it. The absorption here is mostly due to the excitation of bond electrons in the molecules. It is known that silver nanoparticles exhibit a yellowish or brownish color in aqueous solutions due to their dispersion and surface plasmon resonance absorption, which has characteristic optical absorption in the visible region. The absorbance value giving the highest peak is Run 11 at approximately 270 nm. For test run 1, the wave-length value is 258.97 nm, the absorbance value is 9.7217, and the full width at half maximum (FWHM) value is 1.149 nm. For test trial 4, the wavelength value is 293.78 nm, the absorbance value is 10, and the FWHM value is 6.7897 nm. For test trial 11, the wavelength value is 295.26 nm, the absorbance value is 10, and the FWHM value is 4.7154 nm. In general, absorbance and wavelength values were very similar.. In addition, the peak points are also within the range given for silver nanoparticles in the literature, i.e., in the region of 300–450 nm [15]. The surface plasmon bands of all NPs were observed in the range of 290–400 nm, which is as expected for this metal (Fig. 1).

Fig. 1.

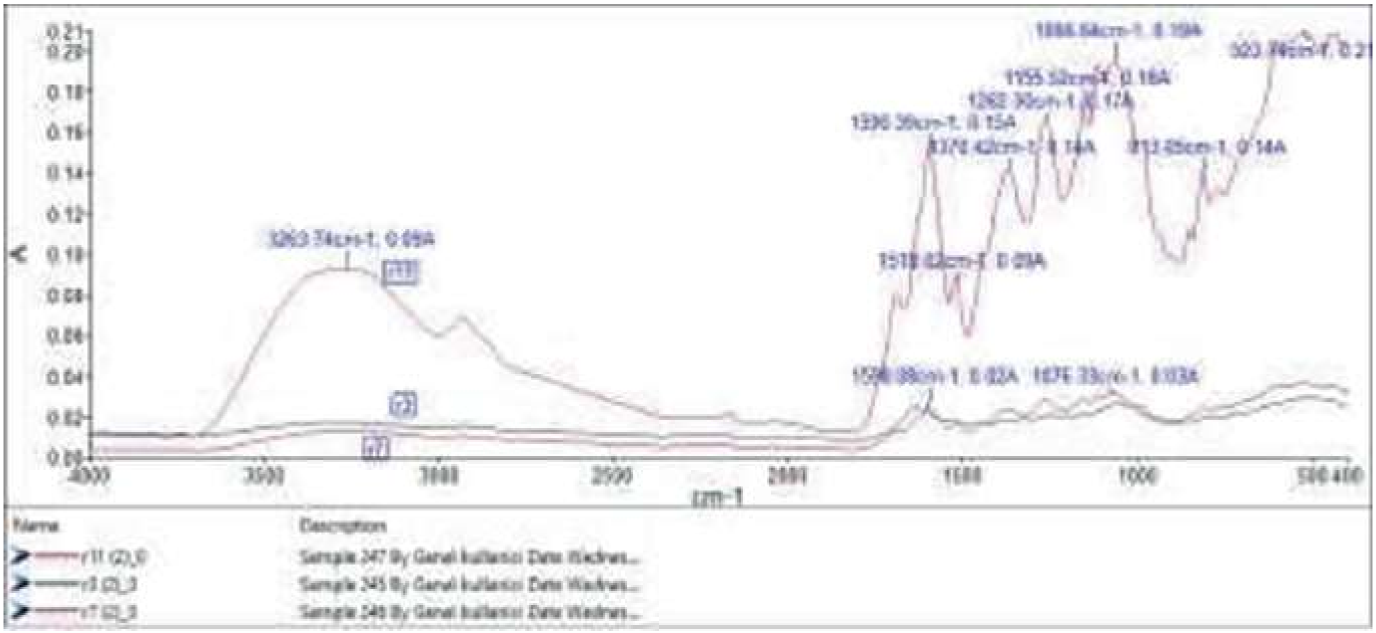


Surface plasmon resonance values of nanoparticles

Full size image

The FTIR spectra of silver nanoparticles synthesized by using *Inula viscosa (L.)* plant extract is shown in Fig. 2. FTIR results for AgNPs with average 56 nm, 200 nm size depicted in Fig. 2 coded as r11 and r3, respectively. Distinct peaks were observed at 3263.74, 1519.02, 1596.39, 1370.42, 1262.30, 1155.52, 1066.64, 813.05 and 523.74 cm^{-1} for AgNPs whereas for r3 coded AgNPs, the peaks were observed at 1598.08, 1076.3 cm^{-1} only. The peaks at around 3400 cm^{-1} are the bond vibration of water molecules [5]. Vibrations of C = O and CC = groups ranged from 1650 and 1500 cm^{-1} [5].

Fig. 2.



FTIR-ATR analysis results

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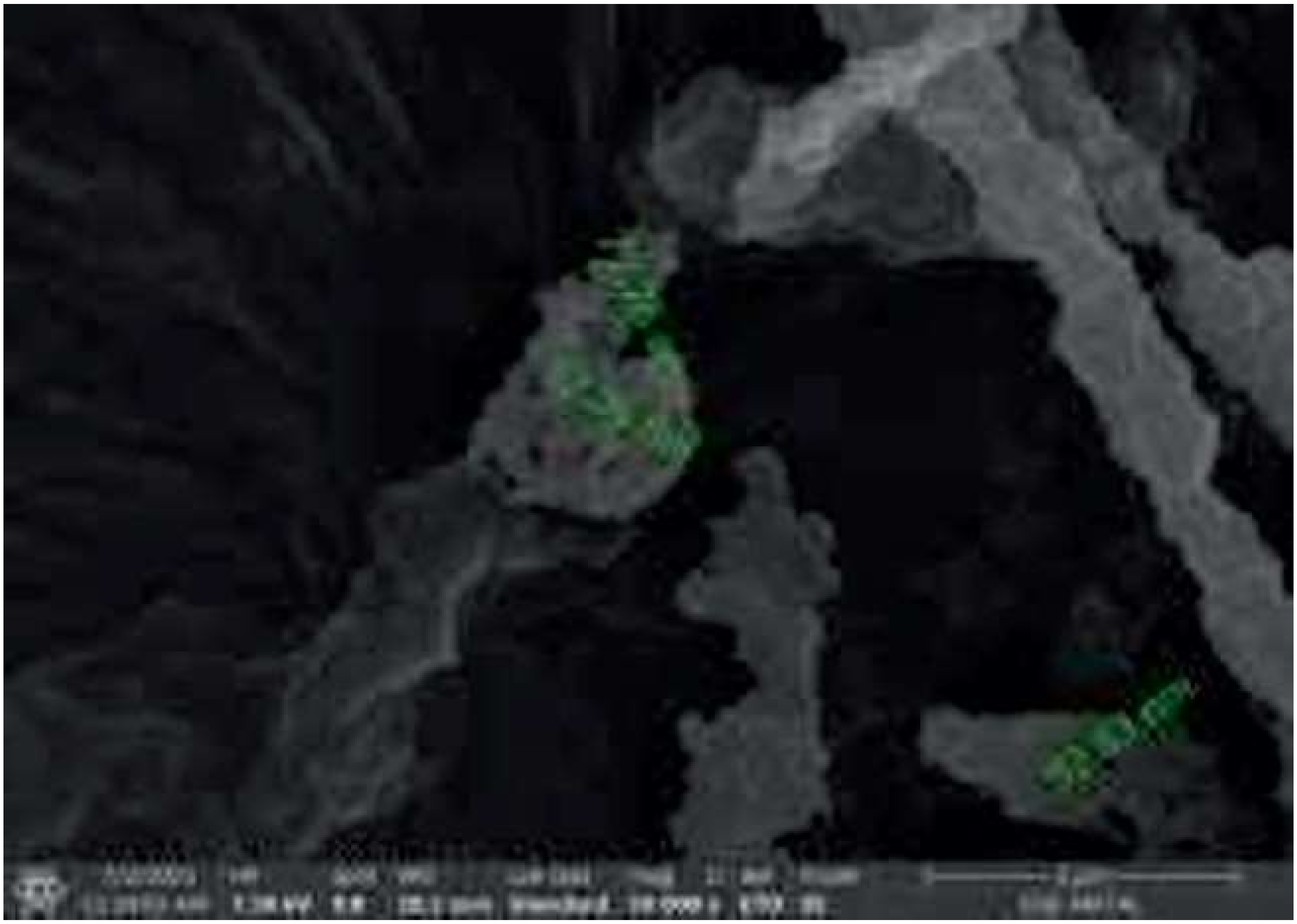
The surface morphology and size distribution of the AgNPs was examined by scanning electron microscopy (SEM). SEM images show that spherical shaped particles in range from 40 to 250 nm are prevalent (Fig. 3).

Fig. 3.



SEM images of AgNPs

Full size image

The minimum nanoparticle sizes are as follows, respectively; Trial 1 is 59.42-59.23–62.98 nm, Trial 4 is 48.26–45.94–52.11 nm, and Trial 11 is 42.66–62.58–62.98 nm. The nanoparticle sizes synthesized by green synthesis under normal conditions are expected to be larger than 100–1000, and sometimes as much as 200 nm. Nanoparticles synthesized with chemical substances are generally very likely to be smaller than 50 nm because their skeletons have been formed beforehand [16]. On the other hand, according to the biosynthesis and extraction process using water only in this project, the nanoparticle sizes are generally below 100. This provides the desired size range, according to the literature.

The produced nanoparticle had “Good” quality, shown by the zeta-potential value, low aggregation formation, harmonic mean diameter of nanoparticle size obtained as a result of DLS characterization. AgNPs showed a homogeneous distribution with a polydispersity index (PI) value as a 0.1938.

This value represents the distribution of size within a sample. PDI values of 0.2 and below are acceptable for metal nanoparticles [17]. The DLS results in our study appear to have been successful in terms of monodispersal and stability. In addition, the nanoparticle concentration after lyophilization was calculated to be approximately 1.5 mgNP/mL.

According to the BBD, the F value of the Model is 33.23%. A value of $p < 0.0001$ indicates that the model is significant (Table 1). The probability of such a large “Model F-Value” due to noise is only 0.01%. Overall values of Probe > F less than 0.05 indicate that the model terms are significant. In this case B (incubation time), C (metal concentration), AB (pH-incubation time), AC (pH-metal concentration), BC (Incubation time-metal concentration), B^2 (incubation time-incubation time), C^2(metal concentration-metal concentration) can be considered important model terms, as long as there is another parameter with values greater than 0.1, indicating that the model terms are not significant, i.e. the model reduction option model. However, there is no trivial model term in this study. A “Lack of Fit F-value” of 0.13 means no Missing Fit significant to pure error. Such a large “Missing F-value” due to noise has a probability of occurrence of 94.02%. An insignificant lack of fit is interpreted positively, because it is a desirable for the model to fit or be compatible [18, 19].

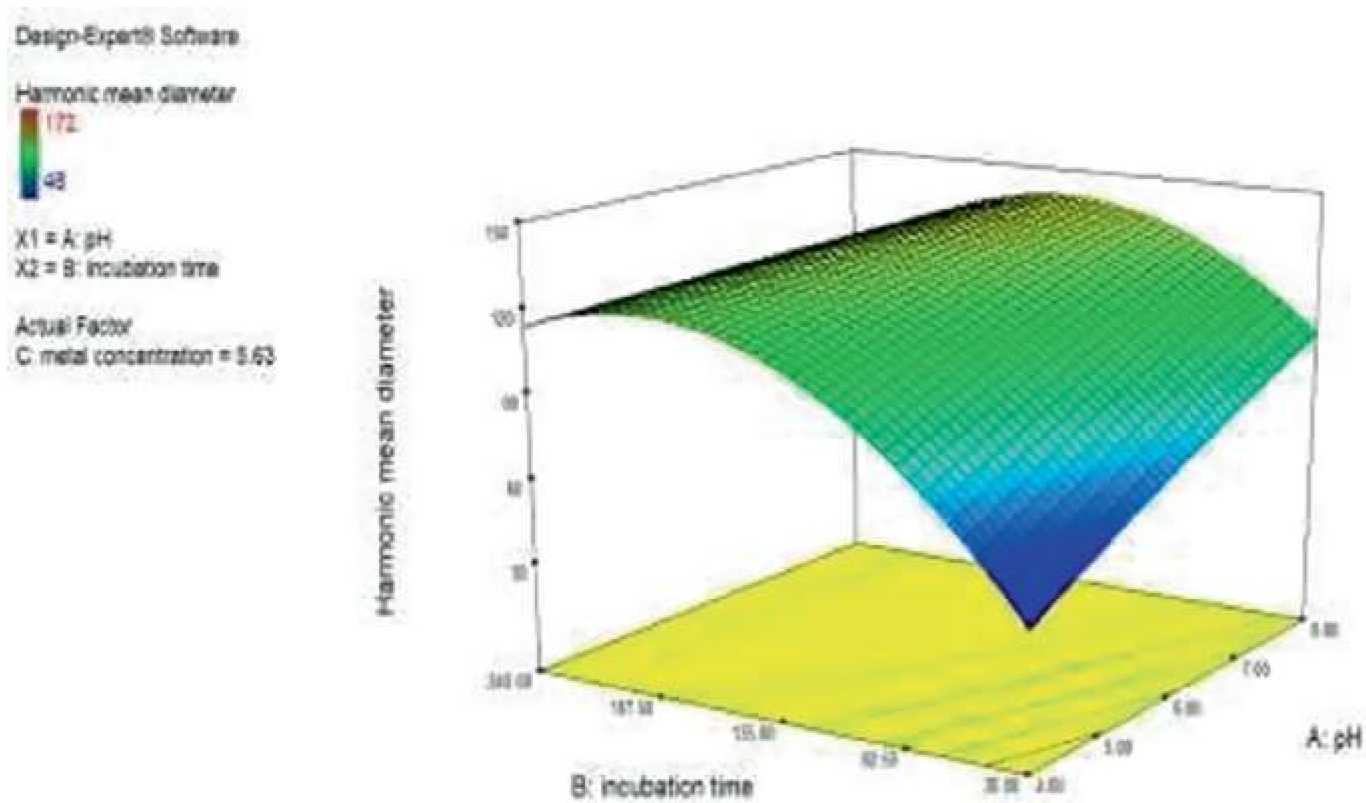
Table 1. Results of the ANOVA

Full size table

The “Pred R-Square” value of 0.9359 is in reasonable agreement with the “Adj RSquare” of 0.9477. “Adeq Precision” measures the signal-to-noise ratio. It is expected to have a ratio greater than 4. The obtained ratio of 24.277 is an indication of a sufficient signal, and therefore, the optimization success [20]. These results are shown in Table 1.

Appropriate trial patterns were examined at the points where the harmonic mean particle diameter (nm) was optimized to a minimum. As can be seen from Fig. 4, when both the pH and the incubation time increased, the harmonic mean particle diameter decreased. The three-dimensional (3 D) visual of the model also indicates that 8 mM silver concentration is suitable to diminish the nanoparticle diameters in the solution.

Fig. 4.



A 3 D interaction plot of harmonic mean particle diameter of AgNPs

Full size image

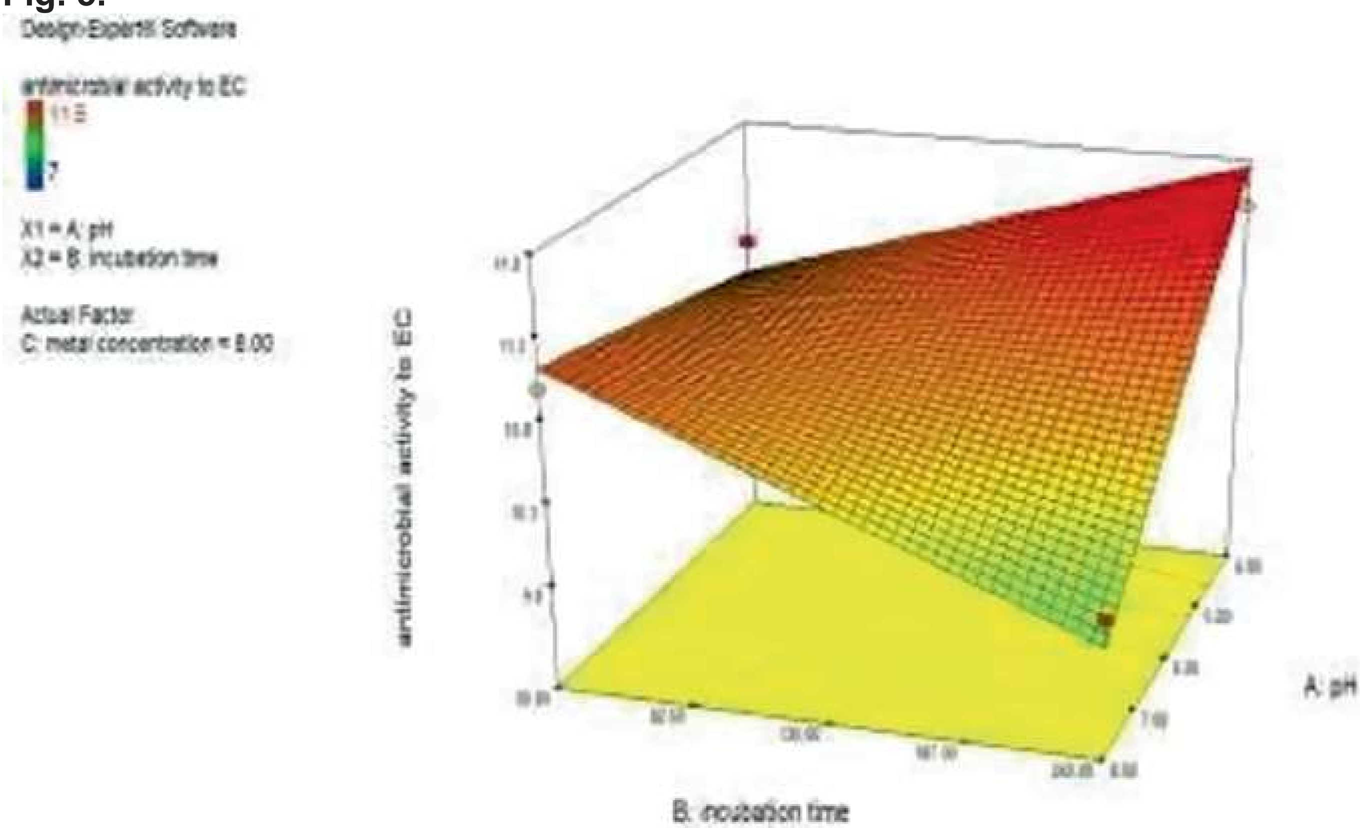
Based on the overall characterization results, it can be said that metal concentration is the most important determinant of success in nanoparticle synthesis, followed by small size, low p-value, volume, and other important values.

3.2 The Results from Antimicrobial Activity Studies

The first part of the study involves the optimization of the harmonic mean particle diameter of plant-based nanoparticles. The second part presents an optimization of the antimicrobial activity of these nanoparticles. Design expert results of antimicrobial activities against the *E.coli* and *S.aureus* are shown in Figs. 5 and 6, respectively. In this set of experiments, the highest desirability to achieve the maximum antimicrobial activity for EC was optimized and obtained as 11.06 mm for EC, where the metal concentration was 8 mM, the incubation time was 30 min, pH at 6.46 and the harmonic mean diameter was 99 nm (Fig. 5). After the statistical analysis of the model was conducted with analysis of variance (ANOVA), the F value of the model was found as 20.57, which indicates that the model is significant ($p < 0.05$). According to the ANOVA test, the metal concentration and pH and the interactions of these two variables were found to be significant ($p < 0.05$). The “Pred R-Squared” of 0.7812 is in reasonable agreement with the “Adj R-Squared” of 0.8607.

In this set of experiments, the highest desirability to achieve the maximum antimicrobial activity for SA was optimized and obtained as 12.5 mm for SA, where the metal concentration was 8 mM, the incubation time was 30 min, ph at 6.5 and the harmonic mean diameter was 98 nm (Fig. 6). The statistical analysis of the model was made with an analysis of variance (ANOVA). The model F value of 14.98 implies that the model is significant ($p < 0.05$). According to the ANOVA test, the metal concentration and pH and their interactions were found to be significant ($p < 0.05$). The coefficient of determination (R^2) was used to check the fitness of the model. An R^2 value closer to one implies a better correlation between experimental and predicted responses, such as more fit model explanation. In this model, the correlation coefficient (R^2) value of 0.8736 is in reasonable agreement with the adjusted determination coefficient (R^2Adj) value of 0.8153 in terms of the high significance of the model.

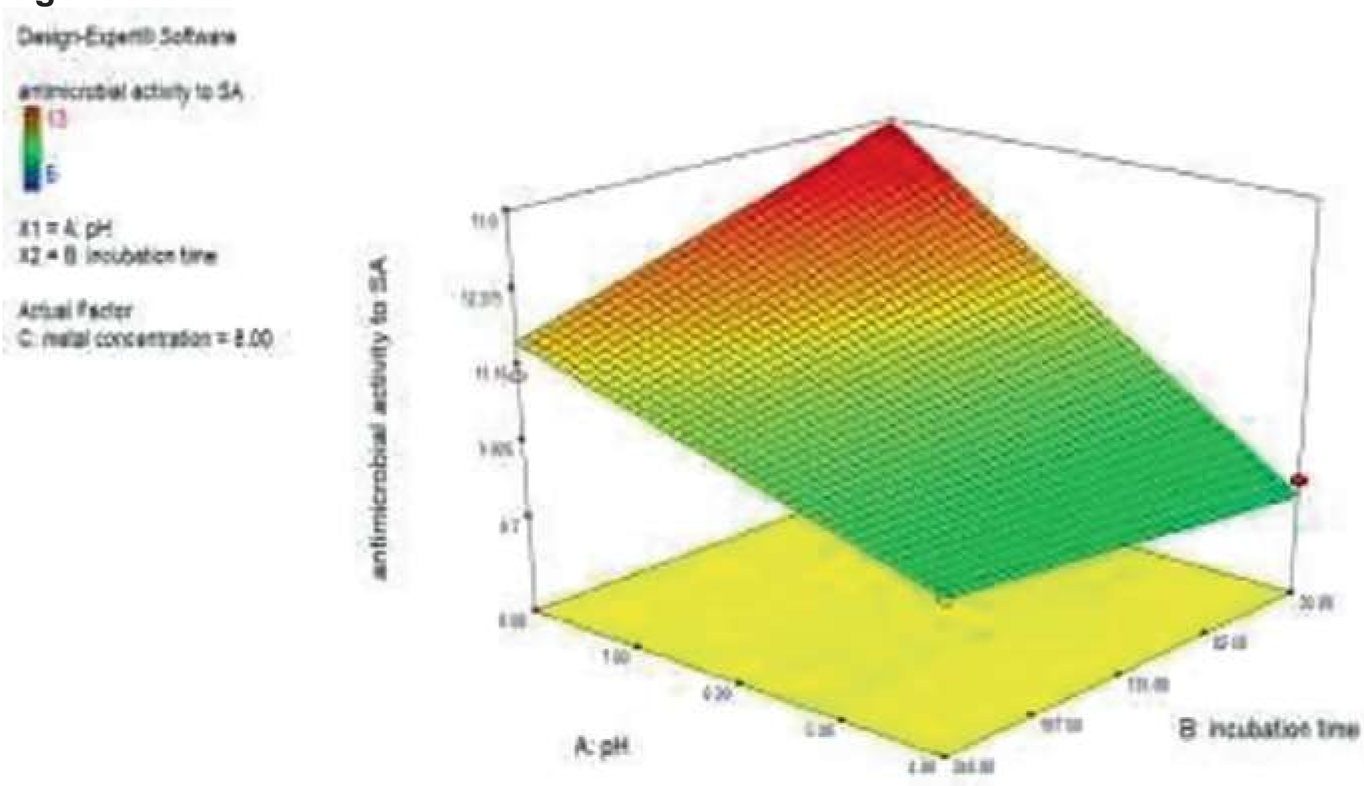
Fig. 5.



Design expert results of antimicrobial activities against the *E.coli*

Full size image

Fig. 6.



Design expert results of antimicrobial activities against the *S.aureus*

Full size image

Therefore, the antimicrobial activity showed a reasonable effect on these plant- based nanoparticles. Also, the green synthesized nanoparticles produced showed, interestingly, 99% antistatic properties.

Consequently, it is clear that advanced characterization methods are suitable for investigating the optimization potential of nanoparticle production specific to *Inula Viscosa (L.)* plant, and this approach should be developed in further studies.

3.3 Conclusions

The biological synthesis of nanoparticles from plants has been extensively studied, yet no study has focused specifically on the potential of biological components of *Inula viscosa (L.)* as anticancer, antimicrobial reagent in the synthesis of silver nanoparticles. The present work is a model study using optimization of both synthesis and antimicrobial activity of silver nanoparticles.

Moreover, in these efforts, it is essential to choose endemic plants, and understand the effects of different parameters such as incubation time, metal concentrations, pH level and their interactions on both biosynthesis of nanoparticles and antimicrobial effects, and also, to conduct eco-friendly green synthesis. AgNPs of *Inula viscosa (L.)* indicated significant antibacterial effect against *E. coli* and *S. aureus* bacteria in this study, which will guide future studies on the pharmaceutical and biomedical applications of biogenic AgNPs. Nanoparticles were identified with certain characterization features in our study; however, stabilization and further characterization studies are still needed. Future studies should also consider challenges related to biosynthesized AgNPs such as safety profile, genotoxicity, pharmacokinetics, and antibacterial resistance. Eventually, coating techniques with biocompatible chitosan etc. may be employed to increase stabilization. Another possible innovative direction is the evaluation of these nanoparticles in drug delivery systems and anticancer research. In short, these special nanoparticles are material components with great potential for development. This present study shows the potential for applications of synthesized AgNPs as a novel antibacterial and cytotoxic agent for biomedical applications such as drug delivery, biosensor, and hyperthermia.

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