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Biosynthesis and Optimization of AgNPs Yield from *Chromolaena Odorata* Leaf Extract Using Response Surface Methodology (RSM)

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Using plant bioresources to produce nanoparticles has emerged as a low-cost, eco-friendly process and an ethically sound way of protecting and preventing the environment from releasing toxic and hazardous substances associated with other synthetic methods. In this study, silver nanoparticles were prepared by using *Chromolaena odorata* leaf extract bio-reduction of silver nitrate. The bio-reduced nanoparticles were characterized by UV-Vis, FTIR, and SEM coupled with EDS. The effects of the volume ratio of plant extract to silver nitrate solution, time, and concentration were examined on the yields of the nanoparticles using a central composite factorial design and a response surface model. The FTIR spectrum of the synthesized silver nanoparticles showed the possible involvement of conjugated C-C and C-O-H groups in the bio-reduction process. The nanoparticles showed some antimicrobial properties against some tested pathogens. The nanoparticles appeared clustered and aggregated together in morphology. The optimum nanoparticle yields were obtained using low values of the ratio of plant extract to silver nitrate solution (1:1) and high values of time (45 min) and concentration of silver nitrate (3 mM), respectively. These are possible reaction conditions for maximizing nanoparticle yields from *Chromolaena odorata* leaves.

Keywords: Nanoparticles, Plant extract, Biosynthesis, Operational variables, *Chromolaena odorata*

INTRODUCTION

Nanoparticles are particles in the nano dimensions (1-100 nm). They constitute the building blocks for nanotechnology and nanoscience. Particles in the nano sense have distinct and scientifically interesting behavior and characteristics compared to the bulk or starting materials from where they are derived. They are highly desirable and particularly indispensable in many applications such as medical, material chemistry, water treatments, effluent detoxication, catalysis, electronics, pharmaceuticals, and drug delivery [1-5], due to their peculiar size, shape, and distribution. They also possess in addition to their unique size, optical, electrical, and magnetic properties which have found applications in photonic, electronic, and magnetic fields [6,7].

Nanoparticles have been synthesized using a variety of methods, such as grinding, pulsed laser ablation, wire discharge, chemical vapor deposition, chemical reduction, ionized cluster beam deposition, sol-gel, sonochemistry, co-precipitation, inert gas condensation, and solvothermal process [8,9]. There are, however, significant disadvantages to many of these methods, including the use and dispensation of toxic reagents (organic and inorganic), while others are associated with huge costs and the release of high energy into the environment. It is therefore necessary to look for other methods that are environmentally benign, energy efficient, and relatively inexpensive. Biological routes provide a better alternative to other synthetic methods. It involves the use of microbes (algae, yeast, fungi, and bacteria) and various plant

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materials (leaves, stems, and roots) to prepare nanoparticles [9,10]. However, the use of plant material is preferred to microbes because the latter requires the additional cost of an aseptic environment for the maximum performance of the microorganisms [11]. In addition, the plant-mediated synthetic process is fast, safe to handle, cost-effective, and energy-efficient, and eliminates the requirement for an aseptic environment for the maintenance of cell cultures.

Various plant materials have been used for the preparation of different metallic nanoparticles including silver, gold, copper, nickel, silica, and bimetallic nanoparticles [4,12-17]. The silver nanoparticle is distinct and more effective in its antimicrobial activities compared to other metallic nanoparticles [18]. It is applicable in various processes such as water treatment operations, creams for wound healing, anti-bacterial agents in surgical operations, and textiles due to its non-toxicity to animal cells [19,20]. It also possesses anti-fungal, anti-inflammatory, anti-angiogenic, and anti-permeability activities [19,21].

Chromolaena odorata, also known as Akintola and Awolowo by the Yorubas of South West, Nigeria is an extremely common plant/weed with many medicinal properties, including antipyretic, anti-inflammatory, analgesic, anti-diarrheal, antihypertensive, diuretic antimicrobial, and antipyretic [22-24]. These other uses of the plant are a consequence of the presence of phytochemicals, including alkaloids, tannins, flavonoids, and phenolic compounds, among others. It is viewed as a weed and is readily available. Farmers spend a great deal of money clearing and burning these plants. According to [28], converting this weed into an efficient plant for nanoparticle synthesis will have a significant economic impact.

In recent studies, copper, cobalt, magnetite, and silver nanoparticles were synthesized from *Chromolaena odorata* [4,16,17,26,27,29,30]. Many of these studies focused largely on the biological and antimicrobial properties of the reduced nanoparticles from the plant extract, without much consideration of the impact of operational variables on the yield of nanoparticles. In the present study, silver nanoparticles were obtained by the reduction of $AgNO_3$ solution with the leaf extract of *Chromolaena odorata*. The influence of some of the operating parameters on the yield of the synthesized AgNPs was examined using a factorial experimental design and a response surface methodology.

EXPERIMENTAL PROCEDURES

Plant Material

Chromolaena odorata leaves were obtained at the University of Ibadan premises. The leaves were washed thoroughly with tap water and then shredded into small sizes. About 60 g of the leaves were boiled with 600 ml distilled water inside a cleaned 1000 ml beaker for about an hour. The extract was then filtered, cooled, and stored in a refrigerator for further use.

Synthesis of Silver Nano-Particles

Silver nanoparticles were prepared by the reaction of the leaf extract of *Chromolaena odorata* and $AgNO_3$ as described in our previous work [14]. An equal volume of plant extract was added to the 1 mM solution of $AgNO_3$. The mixture was heated on the hot plate at 70 °C with a magnetic stirrer for 30 min. The bio-reduction of silver nitrate to silver ions was confirmed by a colour change. The formation of silver nanoparticles was monitored by UV-Vis spectrophotometer at the wavelength range of 300 to 900 nm.

Effects of Process Variables

The volume of extract to silver nitrate solution. The ratio of leaf extract to silver nitrate solution was monitored by heating an equal volume of extract and the silver salt at 70 ºC until a color change was observed which signaled the formation of AgNPs. Other ratios were similarly carried out by varying the volume of silver salt.

The concentration of silver nitrate. An equal volume of different concentrations (1, 2, and 3 mM) of silver nitrate was reacted with the same volume of leaf extract at 70 ºC for about an hour. A change in the color of the reaction was used to confirm the formation of nanoparticles.

Duration of Reaction

The effect of time on the formation of silver nanoparticles was studied by adding 60 ml of *Chromolaena odorata* plant extract to 60 ml of 1 mM $AgNO_3$. The mixture was heated and an aliquot sample of the mixture at time intervals of 0, 15, 30, and 45 min. of the reaction was taken and scanned using UV-Visible spectroscopy.

Characterization of Silver Nanoparticles

The progress of the nanoparticles formed was monitored with the UV-Vis spectrophotometer (Perkin-Elmer, Lamda 25, Germany), at a wavelength range of 300 to 900 nm. The functional groups involved in the bio-reduction process were identified by taking the FTIR of the plant extract and AgNPs respectively. The Scanning Electron Micrograph (SEM) equipped with the Energy Dispersive Spectroscopy (EDS) detector was used to determine the morphology and the elemental composition of the AgNPs.

Antimicrobial Effects of AgNPs

The agar diffusion plate method was used for the antimicrobial screening of the AgNPs as described in [28,31]. The AgNPs were tested against 5 pathogenic micro-organisms: bacteria (*Escherichia coli, a-H.Streptococcus*, Staphylococcus *aureus*) and fungi (*Aspergillus candideus, Aspergillus niger*). Ciprofloxacin was used as a positive control [32].

The Central Composite Experimental Design

The effect of the operation variables, namely, the ratio of leaf extract to silver salt, the concentration of the $AgNO_3$ solution, and time, was investigated using an experimental factorial design together with the response surface methodology (RSM). In this design, some of the results of the experiment were grouped to form a first-order full factorial design, with variables at two levels (2^3) [33]. Part of the experimental data was fitted to a first-order polynomial regression equation as implemented in the Design Expert 12 statistical package. Individual and second-order interaction effects over the response surface of the independent variables were evaluated [33-35].

Below is the mathematical model:

$$Y = a_0 + \sum_{i=1}^{3} b_i X_{ni} + \sum_{i=1}^{3} c_i X_{ni}^2 + \sum_{i=1;j=1}^{3} d_{ij} X_{ni} X_{nj} (i < j) \quad (1)$$

The response variable Y represents the silver nanoparticle yield. The independent variables, X_1, X_2 and X_3 correspond to the ratio of extract to silver nitrate solution, the concentration of silver nitrate solution, and time, respectively. The ranges of values for each independent variable were: ratio, 1:1 to 1:3, concentration, 1 to 3 mM solution and time, 15 to 45 min. The normalization of the values of the independent variables was done, from -1 to +1, using the equation:

$$X_n = 2\frac{X - \bar{X}}{X_{max} - X_{min}} \quad (2)$$

Where:

X_n is the normalized value of extract/nitrate ratio, time, and concentration,

X is the absolute experimental value of the variable.

$\bar{X}$is the average of all the experimental values for the variable in question

X_{max} and X_{min} are the maximum and minimum values respectively, of such a variable.

Statistical Analysis

The experimental design and RSM analysis were conducted using Design Expert Version 12. The data analyses were performed with SPSS software, while the analysis of variance (ANOVA) was applied to determine whether there were significant differences between the values under the experimental conditions examined.

RESULTS AND DISCUSSION

Biosynthesis of the Silver Nanoparticles

The complete bioreduction of the silver nitrate solution by the aqueous extract of the plant was achieved within 45 min of the reaction. The reaction was monitored progressively by the UV-Vis spectrum and was accompanied by a change in the color of the reaction medium from light to dark brown (Fig. 1). The colour change signified the formation of the nanoparticles as a consequence of the excitation of the surface plasmon vibrations in the silver nanoparticles. The UV-Vis spectra of the extract and the synthesized silver nanoparticles were shown in Fig. 2. The synthesized AgNPs have an absorption maximum at 380 nm while that of the aqueous extract appeared at 330 nm.

The rate of formation of AgNPs concerning time was depicted in Fig. 3. The rate increased with an increase in time (from 0 to 45 min), though with a slight shift in the absorbance maxima. As contact time increases, the intensity

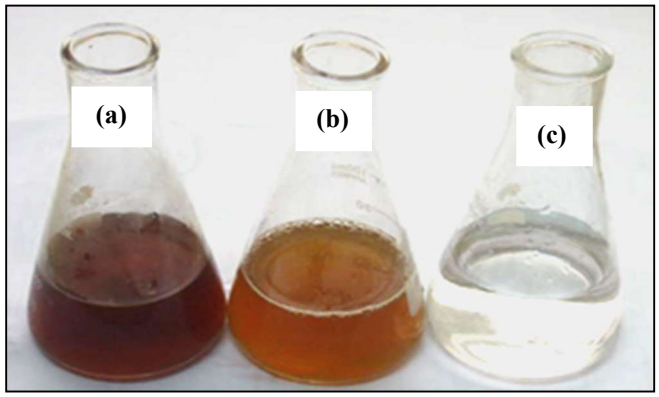


Fig. 1. Solutions of silver nanoparticles (a), leaf extract (b), and silver nitrate (c).

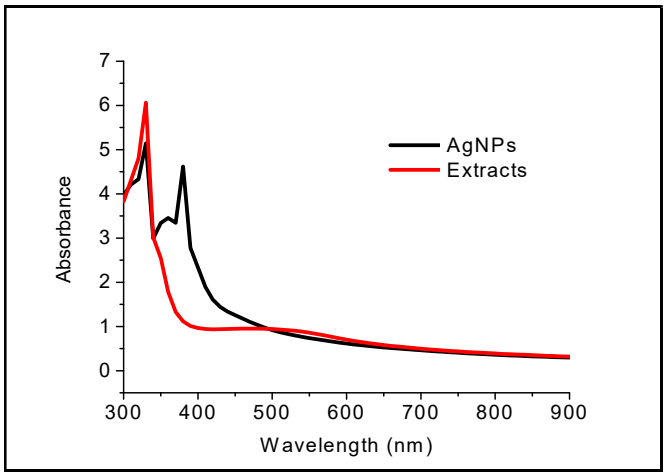


Fig. 2. UV-Vis spectral of the AgNPs and extract obtained from *Chromolaena odorata*.

of the peak increases. Longer contact periods will result in better plasmon bands since a large amount of Ag^{+} will be converted into Ag^{0} [36]. A minimum time of 15 min. was sufficient for the formation of AgNPs at 70 °C.

FTIR Spectra of Extract and Silver Nanoparticle

The FTIR of the extract and AgNPs (Fig. 4) were taken to identify the potential functional group(s) that are possibly involved in the bioreduction of the $AgNO_3$ solution. The spectrum of the plant extract was similar to that of the AgNPs with the presence of a broad rounded peak of the O-H group around 3400 cm^{-1}, conjugated C-C bond at 1600 cm^{-1}, and a C-O-H absorption band about 1400 cm^{-1} [15].

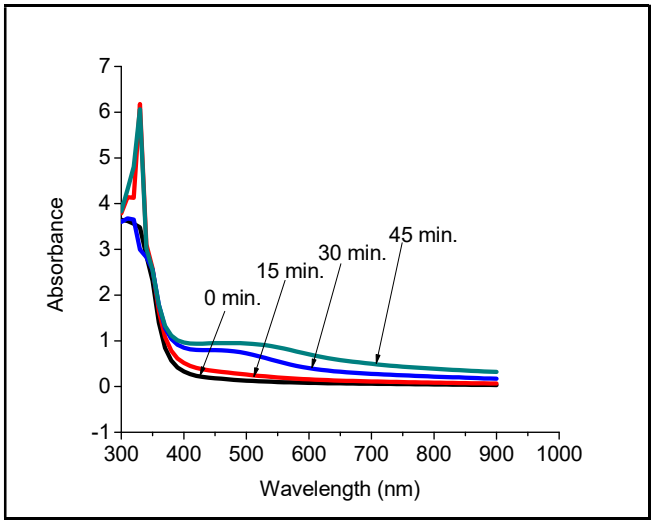


Fig. 3. UV-Vis spectral of AgNPs from *Chromolaena odorata* as a function of time.

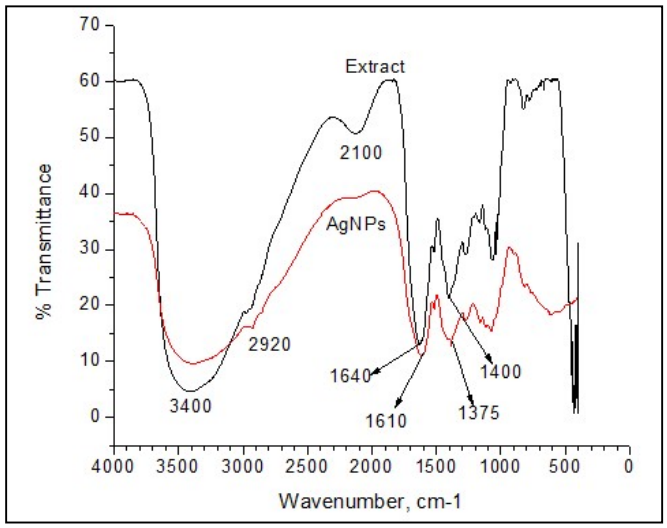


Fig. 4. FTIR spectra of AgNPs and extract obtained from *Chromolaena odorata*.

However, there was a shift in the absorption band from 1610 to 1640 cm^{-1} and 1370 to 1400 cm^{-1} as observed in the AgNPs spectrum, indicating their involvement in the bio-reduction process. The disappearance of a weak band around 2100 cm^{-1} (attributed to a triple-bonded carbon such as an alkyne or nitrile) possibly showed its involvement in the capping and stability of the synthesized AgNPs.

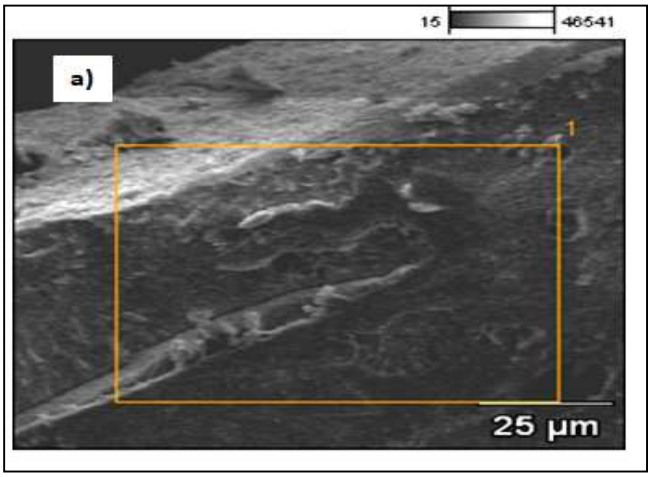
SEM of Silver Nanoparticle

The morphology of the synthesized silver nanoparticles and their elemental composition were observed using the Scanning Electron Micrograph (SEM) equipped with the Energy Dispersive Spectroscopy (EDS) detector. The nanoparticles appeared aggregated and clustered together in a dense form (Fig. 5a). There was a strong optical absorption peak around 3 keV (Fig. 5b) which is typical of metallic silver, due to surface plasmon resonance [37], thus confirming the formation of silver nanoparticles.

Antibacterial Assessment of Silver Nanoparticles

The antimicrobial activity of the biosynthesized nanoparticle was investigated, at different plant extract to Ag ion ratios, against five microorganisms (three bacteria and two fungi); and compared with Ciprofloxacin -a standard antibiotic. All the bacteria *(E. coli, a-H.Streptococcus, S.aureus*) isolates were highly sensitive to ciprofloxacin (Table 1). The synthesized AgNP showed more antimicrobial activity against bacteria than against fungi [15]. While there was no noticeable zone of inhibition of AgNP at ratios 1:1 and 1:3 for *A. candideus* (fungus), there is a zone of inhibition of 5 mm at ratio 1:5. The contrary was the case for *A. niger* (fungus) as the AgNP exhibited antifungal activity against it at the three concentrations (ratios) with the inhibition zones ranging from 6 mm to 7 mm. This shows that *A. niger* was more sensitive to the AgNP than *A. candideus*. The sensitivity of the *A. candideus* to the synthesized AgNP might be dependent on the ratio of the plant extract to the Ag ion.

For the bacteria, the AgNP showed antimicrobial activity with zones of inhibition ranging from 7 mm to 11 mm at different ratios. While *a-H. streptococcus* was most sensitive to AgNP at a 1:5 ratio, and *S. aureus* was most sensitive at a 1:3 ratio.



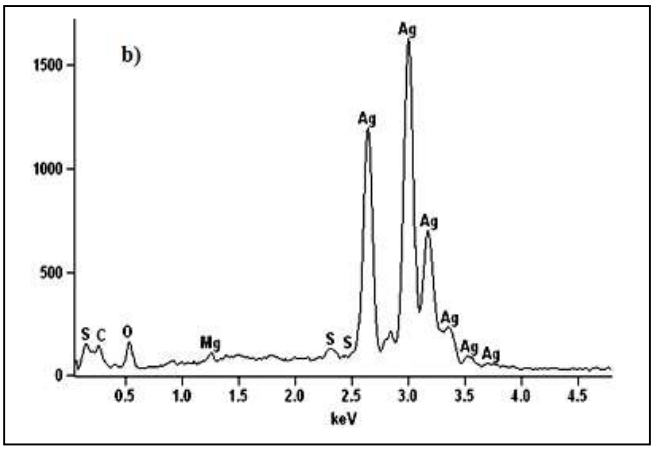


Fig. 5. (a) SEM image (Accelerating Voltage: 15.0 kV; Magnification: 1000) and (b) EDS profile of the AgNPs.

Table 1. Zones of Inhibition of Green-synthesized AgNPs Against Strains

	Zones of inhibition (mm)				
Samples	*E. Coli*	*α-H. streptococcus*	*S. aureus*	*A. niger*	*A. candideus*
AgNP-1:1	7	8	8	7	-
AgNP-1:3	8	9	10	6	-
AgNP-1:5	7	11	9	6	5
Ciprofloxacin	20	25	22	-	-

The low values of the zones of inhibition of our biosynthesized AgNP compared to similar studies on silver nanoparticles are the consequence of the low concentrations used in the present work [28,38,39]. It is evident from our results that the ratio of the plant extract to the Ag ion plays a critical role in the sensitivity of AgNP against microorganisms, and the insensitivity recorded might be attributed to the low concentrations of the synthesized nanoparticles.

Experimental Design and Optimization of Process Variables

The experimental design (2^3) together with the AgNPs yield is presented in Table 2. Experiments 1-11 allowed the calculation of different parameters in the regression equations a_i and a_{ij}. These were subsequently subjected to a T-test to check their significance at a 90 to 95% confidence level using the experimental error estimated from the replication at the central point of the design, that is, experiments 9, 10, and 11 of Table 2.

The design central points correspond to the following reaction conditions:

The ratio of the volume of extract to silver salt solution = 1:5

Time = 30 min.

Concentration = 2 mM

All normalized independent variables for the central points of the design are zero.

The dependent variable (*i.e.* AgNPs yield) was related to the independent variables through the equation below [40]:

$$Y = 0.0319 - 0.00434X_1 + 0.00386X_2 + 0.00129X_3 - 0.00249X_1X_2 - 0.00401X_1X_3 + 0.00429X_2X_3 \quad (3)$$

Equation (3) allows the estimation of the variation of the yield with changes in each independent variable over the range considered while the other two remain constant. Equation (3) revealed that all the operational parameters, *i.e.*, ratio, concentration, and time had significant effects on the AgNPs yield at a 95% confidence level ($p < 0.05$). Also, there were significant interactions among the three operational variables, *i.e.*, between ratio and concentration, ratio and time, and concentration and time, respectively, over the yield.

Table 2. Experimental Design and Result for AgNPs Yield

Experiment	X_1(ratio)	X_2(concn)	X_3(time)	Yield
1	-1	-1	-1	0.0288
2	-1	-1	1	0.0313
3	-1	1	-1	0.0334
4	-1	1	1	0.0521
5	1	-1	-1	0.0336
6	1	-1	1	0.0191
7	1	1	-1	0.0273
8	1	1	1	0.0309
9	0	0	0	0.0319
10	0	0	0	0.03
11	0	0	0	0.0324

According to Eq. (3), the highest AgNPs yield (0.052 g) was obtained at a low value of the ratio of plant extract to $AgNO_3$ salt (1:1) while the lowest obtainable yield (0.019 g) occur at a low value of the concentration of $AgNO_3$. This fact was also evident from experiments 4 and 6 of Table 1. The maximum variation in the highest nanoparticles yield was caused by changes in ratio and the minimum by changes in time, while changes in concentration lie in between.

Values obtained from the polynomial equation above were correlated with the experimental results for the response variables as shown in Fig. 6. The results showed good agreement between actual and predicted values by the model. The level of significance for the coefficients of the polynomial model was determined through analysis of variance (ANOVA). The result was summarized in Table 3. The P-values less than 0.0500 indicated that the model terms were significant. In this case, A, B, C, AB, AC, and BC, that is ratio, concentration, time, the products of ratio and concentration, ratio and time, and concentration and time, respectively, were significant model terms. The high values of the coefficient of multiple determination (R^2) and adjusted coefficient of multiple regression (Adj. R^2) revealed the adequacy and accuracy of the regression model. The values of the predicted R^2 (0.9482) and adjusted R^2 (0.9816) were much close (<0.2) which is an indication of a reasonable

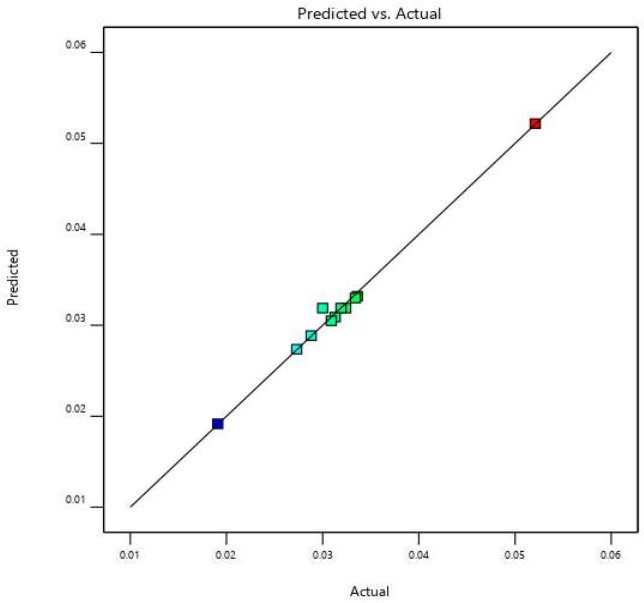


Fig. 6. Correlational graph of predicted versus experimented values of the synthesized AgNPs.

agreement. Also, the measure of the signal-to-noise ratio (adeq. precision) gave a value of 38.909 which is desirable because it is higher than 4 and can be used to navigate the design space. A value of 0.41 was obtained from lack of fit. This showed that relative to the pure error, it was not significant. This is good and indicated that the model is statistically accurate.

Optimization of AgNPs Yield Using RSM

The surface plots for the yield of AgNPs versus the process parameters were shown in Figs. 7-9. It was observed that there were no quadratic terms generated in equation 3 because they were not significant. Thus, the predicted surfaces are flat with no maxima or minima values in the curves. The effect of concentration and ratio on the yield of the synthesized nanoparticles was shown in Fig. 7. It revealed that the lowest yield occurs at the lowest time (15 min.) and the highest ratio (1:9). The yield increased as the concentration increased and the ratio decreased. A similar phenomenon was also observed in the surface plot of Fig. 8 showing the effects of time and ratio on the yield. However, the surface plot showing the influence of time and concentration on the yield (Fig. 9) indicated that the AgNPs yield increases with the two variables. It then followed that for a maximum yield of AgNPs from *Chromolaena odorata* weed, a minimum ratio of 1:1, a high value of time (45 min), and concentration of $AgNO_3$ (3 mM) must be used.

Table 3. ANOVA of the Regression Coefficients of the Fitted Equations for AgNPs Yield

Variables	Mean square	F-Value	p-Value
Main effect			
A-Ratio	0.0002	133.15	0.0003
B-Concentration	0.0001	105.58	0.0005
C-Time	0	11.73	0.0267
Interaction effect			
AB	0	43.79	0.0027
AC	0.0001	113.94	0.0004
BC	0.0001	130.1	0.0003
Model	0.0001	89.72	0.0003
Lack of fit	0.000000658	0.4101	0.7092
R^2		0.9926	
Adjusted R^2		0.9816	

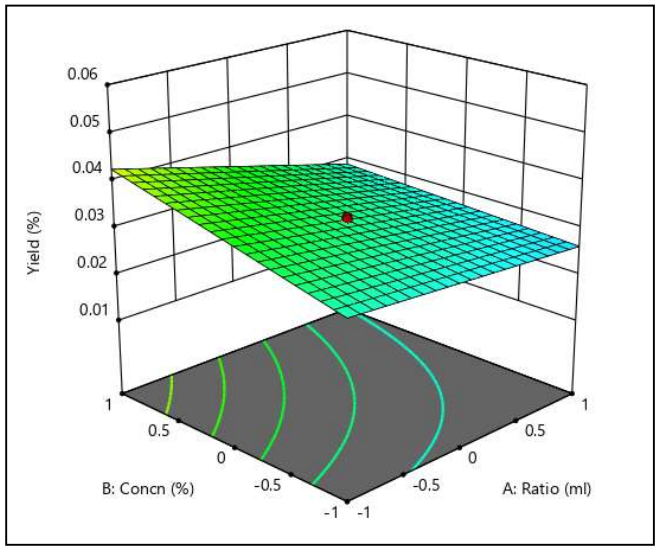


Fig. 7. Response surface plot depicting the influence of concentration of silver salt and ratio of extract to the silver salt solution on the yield of the silver nanoparticle.

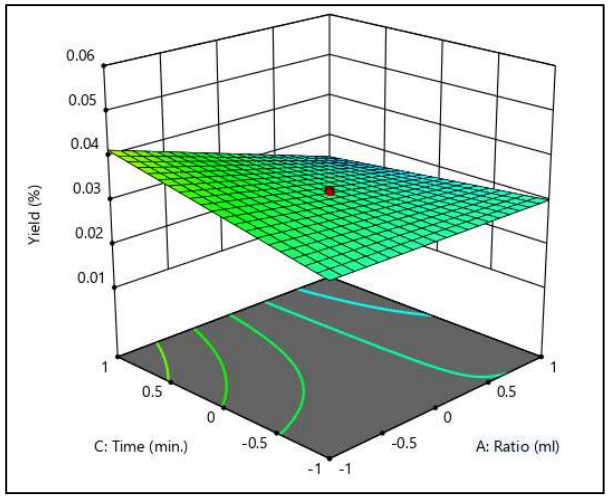


Fig. 8. Response surface plot depicting the effects of time and ratio of extract to the silver salt solution on the yield of the silver nanoparticle.

CONCLUSIONS

The study revealed the synthesis of silver nanoparticles through bio-reduction of silver nitrate solution. The FTIR spectrum indicated the possible involvement of conjugated C-C and C-O-H groups in the bio-reduction process. The

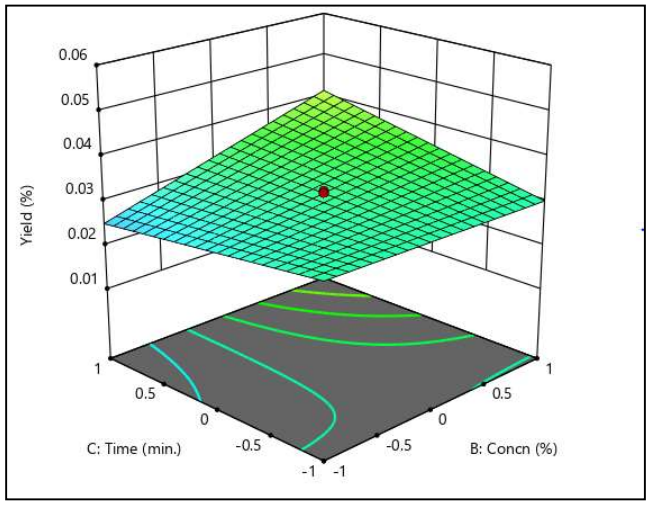


Fig. 9. Response surface plot showing the effects of time and concentration of the silver salt on the yield of the silver nanoparticle.

nanoparticles appeared aggregated and clustered together in a dense form with a strong optical absorption peak typical of metallic silver. The synthesized AgNP showed more antimicrobial activity against bacteria than against fungi. Optimization of operating variables revealed that for a maximum yield of AgNPs from *Chromolaena odorata* weed, a minimum ratio of 1:1, a high value of time (45 min), and concentration of $AgNO_3$ (3 mM), must be used.

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