

Synthesis, characterization and evaluation of silver nanoparticles through leaves of *Abrus precatorius* L.: an important medicinal plant

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Abstract Biologically synthesized nanoparticles have been widely used in the field of medicine. The present study reports the green synthesis of silver nanoparticles using *Abrus precatorius* leaf extract with silver nitrate solution as reducing agent. The synthesized silver nanoparticles were analyzed through UV–Visible spectroscopy, X-ray diffraction, scanning electron microscopy, energy-dispersive X-ray analysis, atomic force microscopy and Fourier transform infrared. The synthesized silver nanoparticles were disk shaped with an average size of 19 nm. These silver nanoparticles were evaluated for antibacterial activity. The diameter of inhibition zones around the disk of *Pseudomonas aeruginosa* and *Staphylococcus aureus* are resistant to silver nanoparticles, whereas *Escherichia coli* and *Bacillus thuringiensis* are susceptible when compared with the other two species. The results were compared with the ciprofloxacin-positive control and silver nitrate. It is concluded that the green synthesis of silver nanoparticles is very fast, easy, cost-effective and eco-friendly and without any side effects.

Keywords *Abrus precatorius* · Antibacterial activity · Biological synthesis

Introduction

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. The most important and

distinct property of nanoparticles is that they exhibit larger surface to volume ratio. Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes (Mostafa et al. 2011). The most widely used and known applications of silver and silver nanoparticles include topical ointments and creams containing silver to prevent infection of burns and wound (Murphy 2008). Many attempts have been made to use silver nanoparticles as an anti-cancer agent and they have all turned up positive (Vaidyanathan et al. 2009). The role of silver nanoparticles as an anti-cancer agent should open new doors in the field of medicine. Production of nanoparticles can be achieved through different methods, for example reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds (Plante and Zeid 2010), radiation-assisted (Cheng et al. 2011), electrochemical (Hirsch and Zharnikov 2005) and recently via green chemistry methods (Sivakumar 2012). Biological synthesis is cost-effective, environmental friendly and easily scaled up for large-scale synthesis. In this method there is no need to use high pressure, energy, temperature and toxic chemical that may have adverse effect in medical applications. Biosynthesis of silver nanoparticles has been performed using a number of plants: *Svensonia hydrobadensis* (Linga Rao and Savithamma 2011), *Shorea tumbuggaia* (Venkateswarlu et al. 2010) and *Thespesia populnea* (Bhumi et al. 2013). The potential of the plants as biological materials for the synthesis of nanoparticles is currently under exploitation.

Abrus precatorius Linn. belongs to the family Fabaceae, commonly known as rosary pea and ratti is a medicinal plant used for various diseases. The plant parts are purgative, emetic, toxic, antiphlogistic, aphrodisiac

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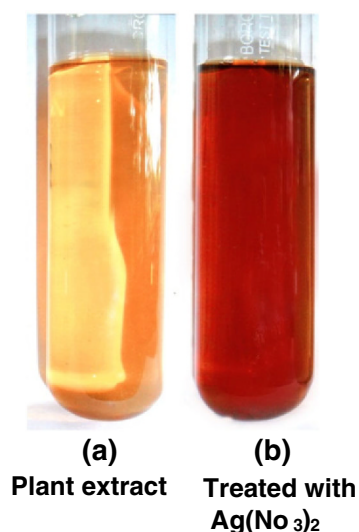


Fig. 1 Synthesis of SNPs (color change) using leaf extract of *Abrus precatorius*

and anti-ophthalmic (Manoharan et al. 2010). In India hot water extract of dried leaves and roots are applied to treat eye diseases. In Brazil, water extract of dried leaves and roots are taken orally as nerve tonic (Ivan 2003).

In the present study, we have explored the synthesis of silver nanoparticles and characterized them using UV–Visible spectroscopy, XRD, SEM, EDAX, AFM and FI-IR. Furthermore, the antibacterial activity of synthesized silver nanoparticles was evaluated against *E. coli*, *Bacillus*, *Pseudomonas* and *Staphylococcus*.

Materials and methods

All the chemicals and reagents used in the present study were of analytical grade. Silver nitrate was purchased from Sigma-Aldrich Chemicals. The glassware was washed with dilute nitric acid, thoroughly washed with double-distilled water and dried in hot air oven.

Preparation of plant extract

Abrus precatorius leaves were collected from S.V.U. Botanical Garden, Tirupati, Andhra Pradesh, India. The leaves were washed thoroughly thrice with distilled water and shade dried for 10 days. The fine powder was obtained from dried leaves by using kitchen blender. The leaf powder was sterilized at 121 °C for 5 min. 5 g of powder was taken into a 250-ml conical flask and 100 ml of sterile distilled water and boiled for 15 min at 100 °C. Then the leaf extract was collected in a separate conical flask by a standard filtration method.

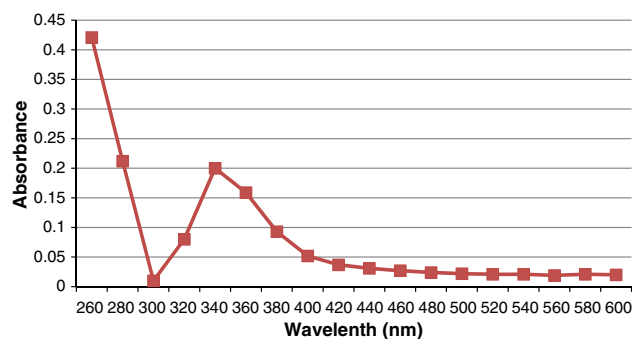


Fig. 2 UV–Vis spectra of silver nanoparticles synthesized from leaf extract of *Abrus precatorius*

Synthesis of silver nanoparticles

1 mM AgNO_3 solution was prepared and stored in amber color bottle. The leaf extract was added to 1 mM AgNO_3 solution. The color change of the solution from yellow to brown indicated that the silver nanoparticles were synthesized from the leaf for characterization and antibacterial activity.

Antibacterial assay

The following bacterial strains were used in this study, viz., *Bacillus thuringiensis* (ATCC10792) *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC6538) and *Pseudomonas aeruginosa* (ATCC15442). Disc diffusion assay method was carried out by using standard protocol (Anonymous 1996). Overnight, bacterial cultures (100 μl) were spread over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 100 μl of each extract were applied to each filter paper disc, Whatman No. 1 (5 mm dia), and allowed to dry before being placed on the agar. Each extract was tested in triplicate and the plates were inoculated at 37 °C for 24 h after incubation, the diameter of inhibition zones was measured with the help of MIC scale and the results were tabulated.

UV–Vis spectra analysis

The reduction of pure silver ions was monitored by measuring the UV–Vis spectrum of the reaction medium at 5 h after diluting. A small aliquot analysis was done using UV–Vis spectrophotometer UV-2450 (Shimadzu).

SEM analysis of silver nanoparticles

Scanning electron microscope (SEM) analysis was carried out by using Hitachi S-4500 SEM Machine. Thin films of the sample were prepared on a carbon-coated copper grid

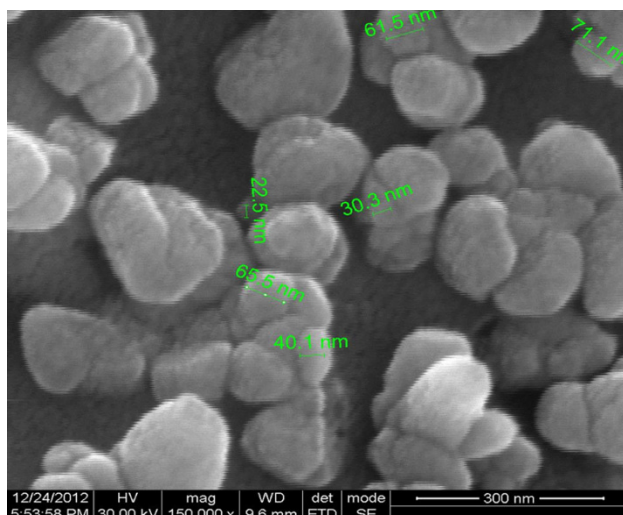


Fig. 3 SEM images of SNPs synthesized using leaf extract of *A. precatوريوس*

by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry.

EDAX measurements

The drop of leaf extract with reduced silver nanoparticles was dried on a film coated with carbon and EDAX analysis was performed on Hitachi S-3400N SEM instrument equipped with thermo EDAX attachments.

AFM measurements

The silver nanoparticles extracted through the above protocol were visualized with an atomic force microscope. A thin film of the sample prepared on a glass on the slide was allowed to dry for 5 min and the slides were then scanned with the AFM (Nano Surf® AG, Switzerland, Product: BTO 2089, BRO).

X-ray diffraction (XRD) analysis

The practice size and nature of the silver nanoparticles were determined using XRD. This was carried out using Shimadzu XRD-6000/6100 model with 30 kV, 30 mA with $\text{CuK}\alpha$ radiations at 2θ angle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation:

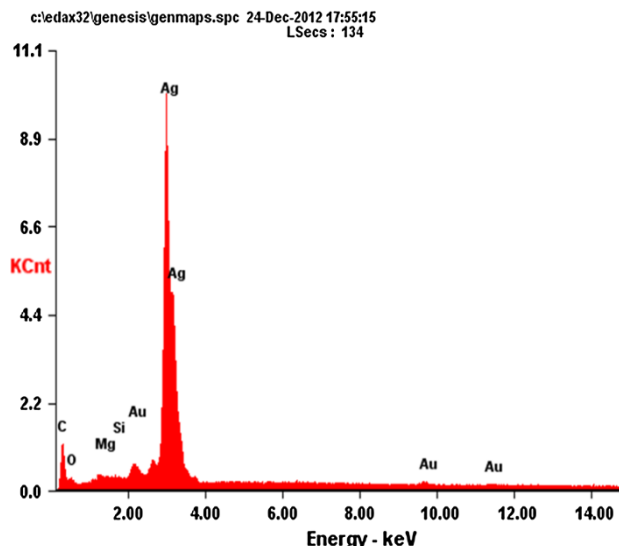


Fig. 4 EDAX images of SNPs of leaves of *A. precatوريوس*

Table 1 EDAX of synthesized element during the formation of silver nanoparticles through the leaves of *Abrus precatوريوس*

Plant name	<i>Abrus precatوريوس</i>	
Element	Weight	Atomic (%)
CK	22.60	62.68
OK	06.75	14.07
MgK	1.54	2.12
SiK	0.19	0.23
AgL	66.22	20.45
AuL	2.70	0.46

$$D = k\lambda/\beta(\cos \theta)$$

FT-IR

The functional group of silver nanoparticles was identified by FT-IR using (Thermo Nicolet-nexus 670 spectrometer of resolution 4 cm^{-1}) one drop of sample placed between the plates of sodium chloride. The drop forms a thin film between the plate and sodium chloride is transparent to infrared light.

Results and discussion

Green synthesis of silver nanoparticles using 1 mM AgNO_3 is shown in Fig. 1. The fresh suspension of leaves of *A. precatوريوس* was yellow in color. However, after addition of $\text{Ag}(\text{NO}_3)_2$ and accelerated the reaction at 60°C for 5 min, the suspension turned dark brown, roughly indicating that the formation of silver nanoparticles was confirmed. The time duration of change in color and

Nanosurf Easyscan 2 - Measurement Report

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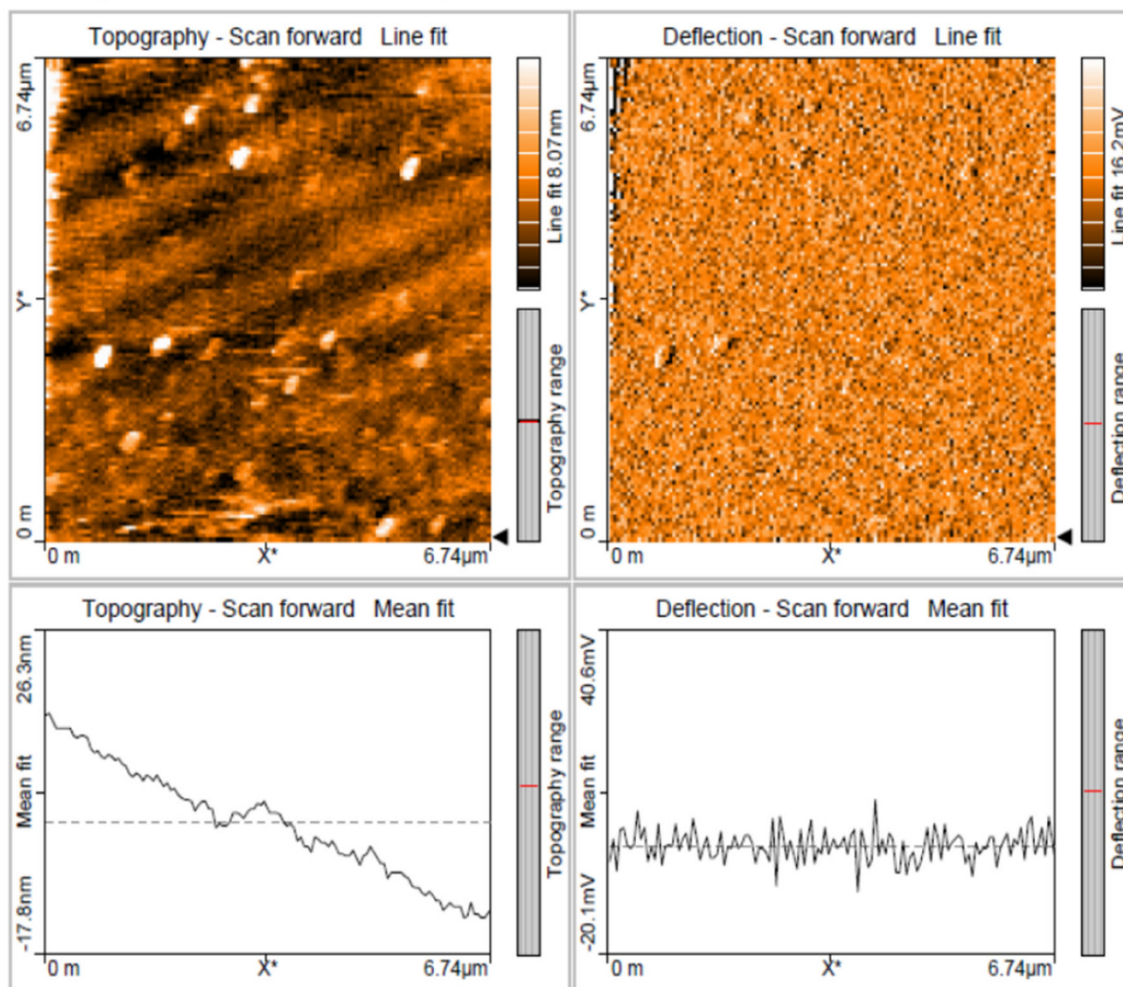


Fig. 5 AFM image of SNPs of *A. precatorius*

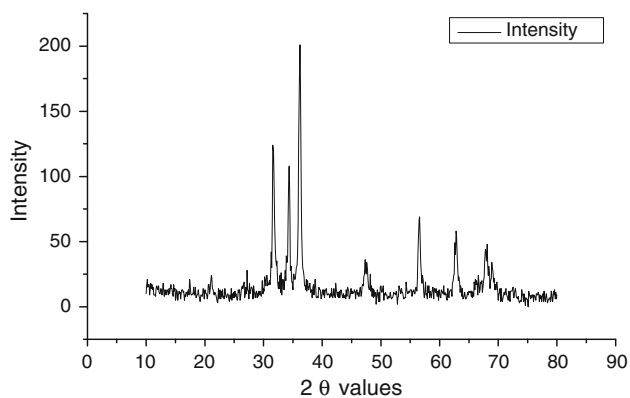
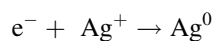
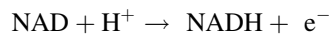
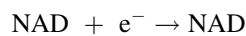
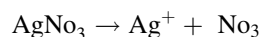


Fig. 6 XRD pattern of SNPs of *A. precatorius*

thickness of the color varies from plant to plant. The reason could be that NAD^+ is a coenzyme found in all living cells. NAD is a strong reducing agent. NAD^+ is involved in

redox reactions, carrying electron from one reaction to another. The coenzyme is therefore found in two forms in cells. NAD^+ is an oxidizing agent. It accepts electrons from other molecules and becomes reduced. This reaction results in NADH, which can donate electrons. These electrons transfer reactions are the main function of NAD:



NAD continues to get reoxidised and constantly regenerated due to redox reactions. This might have led to the transformation of Ag ions to Ag^0 (zero-valence state). Ascorbic acid is further responsible for the reduction

of Ag-NPs. Ascorbic acid is present at high levels in all parts of plants. Ascorbic acid is a reducing agent and can reduce, and thereby neutralize reactive oxygen species, leading to the formation of ascorbate radical and an electron. This free electron reduces the Ag^+ ion to Ag^0 . The formation and stability of silver nanoparticle in aqueous colloidal solution were confirmed using UV–Vis spectral analysis. The UV–Vis spectrum of colloidal solution of SNPs has the characteristic absorbance peaks ranging from 320 to 400 (Fig. 2). The broadening of the peak indicated that the particles were polydispersed. The

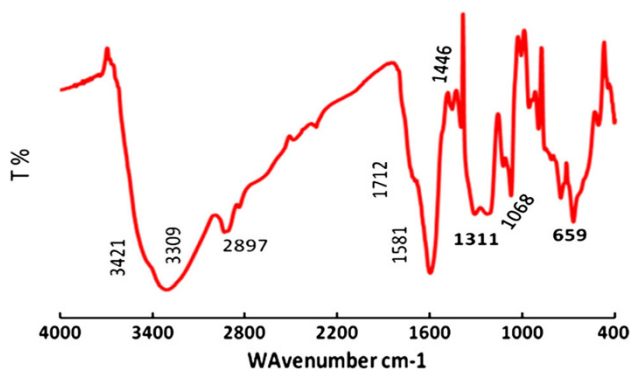
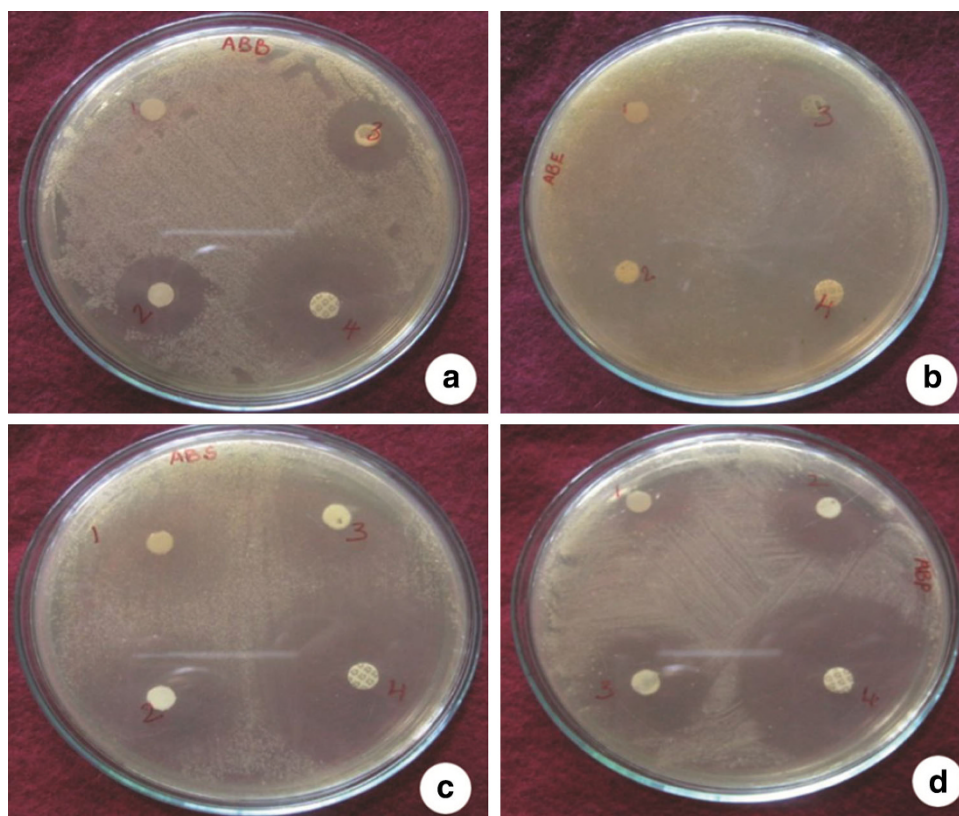


Fig. 7 FT-IR spectra of SNPs of leaf extract of *A. precatorius*

Fig. 8 Antibacterial activity of leaf extract of *A. precatorius*



weak absorbance peaks at shorter wavelengths are due to the presence of several organic compounds which are known to interact with silver ions. The SEM analysis was used to determine the structure of the reaction products that were formed. The SEM image shows individual silver particles as well as a number of aggregates. The SEM image showed relatively spherical-shaped nanoparticles formed with diameter ranging 19–35.4 nm. Aggregated molecules were formed in the range of 20 nm, as shown in Fig. 3.

The EDAX spectrum (Fig. 4) reveals that various elements are identified, such as C, O, Mg, Si, Ag and Au with different percentages (Table 1). The AFM analysis of silver nanoparticles showed that the silver nanoparticles agglomerated and formed distinct nanostructures. The topographical image of irregular silver nanoparticles and agglomeration are clearly observed in Fig. 4.

The particle size of the silver nanoparticles ranged from 35 to 40 nm. The XRD pattern showed a number of Bragg reflections that may be indexed on the basis of face-centered cubic structure of silver. XRD analysis confirmed that the silver particles formed in the experiments were in the form of nanostructures, as evidenced by the peaks at 2θ values of 38.28° , 44.04° , 64.34° and 77.28° , corresponding to (111), (200), (220) and (311) Bragg reflections,

Table 2 In vitro antibacterial activity of SNPs of leaf extract of *A. precatorius*

S. no.	Name of the tested bacteria	Zone of inhibition (mm)		
		Positive control	Negative control	Treated
1.	<i>Pseudomonas</i>	43	21	24
2.	<i>Staphylococcus</i>	40	18	24
3.	<i>E. coli</i>	36	20	22
4.	<i>Bacillus</i>	28	18	19

respectively, in Fig. 5, related to crystalline and amorphous organic phases. It was found that the average size from XRD data and using the Debye–Scherrer equation was approximately 38 nm.

The FT-IR spectra in Fig. 6 shows that sharp absorption peaks located at 1,311, 1,446 and 1,581 cm^{-1} . The absorption peak at 1,331 may be assigned to the amide I bond of proteins arising from carbonyl stretching in proteins, and the peak at 1,446 is assigned to OH stretching in alcohols and phenolic compounds. The absorption peak at 1,581 cm^{-1} is close to that reported for native proteins, which suggests that proteins interact with biosynthesized silver nanoparticles and also their secondary structure is not affected during reaction with Ag^+ ions or after binding with Ag nanoparticles. This FT-IR spectroscopic study confirmed that the carbonyl group of amino acid residues had a strong binding ability with silver, suggesting the formation of a layer covering silver nanoparticles and acting as a capping agent to prevent agglomeration and provide stability to the medium (Table 2). These results confirm the presence of possible proteins as reducing stabilizing agents (Fig. 7).

Biosynthesized silver nanoparticles of *A. precatorius* were analyzed for their antibacterial activity against two Gram-negative bacteria (*E. coli*—ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442) and two Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Bacillus thuringiensis* ATCC 10792) by disk diffusion method (Fig. 8). The results showed that the highest antibacterial activity was observed against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*, *E. coli* and *Bacillus thuringiensis*. Among the four bacterial species, *P. aeruginosa* and *S. aureus* showed higher zone of inhibition, 24 mm each, respectively. The present study revealed that leaves of *Abrus precatorius* are ideal material for the

synthesis of silver nanoparticles and also to extract novel biochemical compound.

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