

Synthesis of Silver Nanoparticles by Bioreduction Method using Leaves Extracts of *Tecoma capensis* and Study of their Antibacterial Properties

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ABSTRACT: In this study the silver nanoparticles were synthesized from leaves extract of *Tecoma capensis* plant by bioreduction method. Silver has long been known to have effective bactericidal properties. The synthesized AgNPs was elucidated using various instrumentation techniques including UV-Vis, FTIR, XRD & SEM. The synthesized AgNPs have shown an effective antibacterial activity when compared against standard antibiotic (Taxim) by *E-coli*, *Staphylococcus aureus*, *Klebsiella aerogenes* and *Pseudomonas aeruginosa* bacterial strains.

KEYWORDS: *Tecoma-capensis*, AgNPs, bioreduction, SEM, FT-IR, XRD.

I. INTRODUCTION

Nanotechnology is the most active area of research in modern material science. Nanoparticles exhibit completely new or improved properties based on their specific characteristics such as size, morphology and distribution [1]. Nanotechnology means scaling down to nano level starting from the bottom [2]. Numerous methods of physical, chemical and biological methods have been established and reported for the synthesis of silver nanoparticles which play a major role in the control of their size and shape [3-5]. Among these methods, the biological methods utilize bacteria, fungus, bio-derived chemicals, and plant extracts for the synthesis of nanoparticles [6]-[8]. Bioreduction methods of synthesizing silver nanoparticles has increasingly become a topic of interests as conventional chemical reduction methods are expensive and requires the use of chemical compounds and organic solvents as reducing agents which causes environmental issues. Many researchers have reported the bio-synthesis of silver nanoparticles using plant extracts such as pine, persimmon, ginkgo, magnolia, and platanus leaves [9], *Acalypha indica* [10], *Chenopodium album* leaf [11] and *Murraya koenigii* [12].

Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities, which has been used for centuries to prevent and treat various diseases, most notably infections [13]. Antibacterial activity of the silver containing materials is used in medicine to reduce infections in burn treatment [14], arthroplasty [15], preventing bacterial colonization on prosthesis [16], catheters [17], dental materials [18], stainless steel materials [19], and human skin [20].

Plant description: *Tecoma capensis* common called as Cape honeysuckle (Fig.1) is a species of flowering plant in the family *Bignoniaceae*, and native to southern Africa. Despite its common name, it is not closely related to the true honeysuckle. The classification of *Tecoma capensis* is given below.

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Kingdom	Plantae	
Order	Lamiales	
Family	Bignoniaceae	
Genes	Tecoma	
Species	T. capensis	
Synonyms	<i>Tecoma capensis</i>	

Fig.1 *Tecoma capensis*.

II. EXPERIMENTAL

Collection and preparation of stem extracts:

Fresh leaves *Tecoma capensis* plant, free from diseases were collected from S.I.E.T college campus and then washed thoroughly 2-3 times with tap water and once with sterile water. 20 g of fresh leaves was finely chopped and added to 100 ml of distilled water and stirred at 60⁰ C for 1h. After boiling, the mixture was cooled and filtered with Whatman filter paper number 1. Filtrate was collected and used for further studies.

III. CHARACTERIZATION

UV-Vis spectroscopy: UV-Visible spectroscopy analysis was carried out on a systronic UV-Visible absorption spectrophotometer (model Shimadzu) with resolution of +1nm between 200 and 1000nm processing a scanning speed of 200 nm/min. Equal amounts of the suspension were taken and analyzed at a room temperature. The progress of the reaction between metal ions and the leaf extract was monitored by UV-Visible spectra of silver nanoparticles in aqueous solution with different wavelength in nanometers from 340 to 800 nm. The reduction of silver ions and formation of silver nanoparticles occurred within an hour of reaction. Control was maintained by using AgNO₃.

FT-IR analysis: FT-IR analyses were performed using Shimadzu FT-IR model number 8400 to identify probable biomolecules present in the leaves extracts of *Tecoma capensis* which may be responsible for the reduction of metal ions and even for the NPs stabilization. Approximately 3 mg of lyophilized leaves extract under study was mixed with 300 mg of dried KBr, crushed well in mortar and pestle to prepare thin pallet for analysis, 16 scans per sample were taken in range of 400-4000 cm⁻¹.

XRD analysis: The sizes of particle and nature of silver nanoparticles were resolved by XRD employing a Rigaku diffractometer at a voltage of 40 keV and a current of 30 mA with Cu-K α radiation with a wavelength of 1.5418 Å. A thin film of the dried silver nanoparticles was coated on an XRD grid and carried out for X-ray diffraction studies. The obtained data which is used for analysis having peak corresponding to different planes of crystal was compared with the data in JCPDS card. The average size of crystalline silver nanoparticles was calculated from the width of the XRD peaks and the average size of the nanoparticles can be calculating by using the Debye-Scherrer equation,
 $D=0.9\lambda/\beta\cos\theta$.

where, λ is wavelength, θ is Bragg's diffraction angle, β is full width at half maximum of peak and D is average particle size.

SEM analysis: A drop of aqueous solution containing purified silver nanoparticles obtained after repetitive centrifugation was placed on the carbon coated copper grids and dried under infrared lamp for characterization of their morphology using FEI Quanta 200 Scanning electron microscope at accelerating voltage of 20 Kev.

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Antimicrobial activity: The silver nanoparticles were mixed with deionized water and were tested for their antibacterial activity by the disc diffusion method. Four bacterial strains, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Staphylococcus aureus* and *E-coli* were used for these analyses which were collected from the Department of Microbiology, Shridevi Institute of Medical Sciences and Research Hospital of Tumakuru, Karnataka, India. These bacteria were grown in nutrient broth (NB) media for 24 hours prior to the experiment and seeded on agar plates by the pour plate technique. The plates were incubated at 37°C overnight. Next day the inhibition zones formed around the discs were measured.

IV. RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles using *Tecoma capensis* leaves extracts:

5 ml of *Tecoma capensis* leaves extracts were added to the 45 ml of AgNO₃ solution at room temperature and stirred continuously for 15 min by using magnetic stirrer. Bio-reduction of Ag⁺ into AgNPs was observed by the color change and kept for 24 h for the complete bio-reduction (Fig. 2).

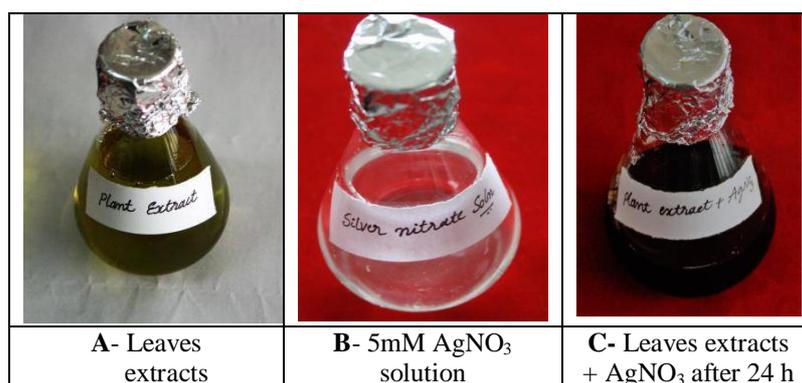


Fig: 2 Formation of AgNPs.

UV-Vis-spectroscopy analysis:

The aqueous solution of synthesized AgNPs was observed by recording the absorption spectra at a wavelength range of 340–900nm (Fig. 3). It was noticed that solution of silver nitrate turned brown on addition of leaves extract. It indicated the formation of AgNPs. In the UV-Vis spectrum; a single, strong and broad Surface plasmon resonance (SPR) peak was observed at 382nm that confirmed the synthesis of AgNPs.

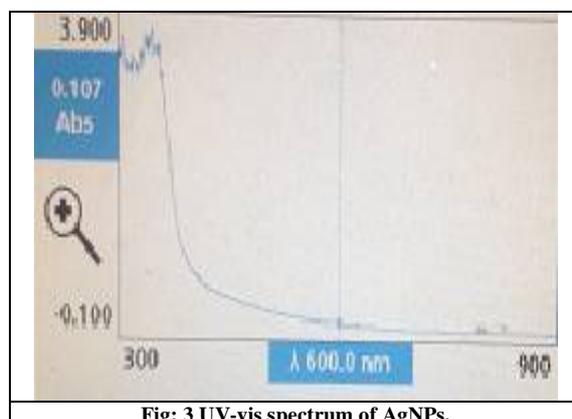


Fig: 3 UV-vis spectrum of AgNPs.

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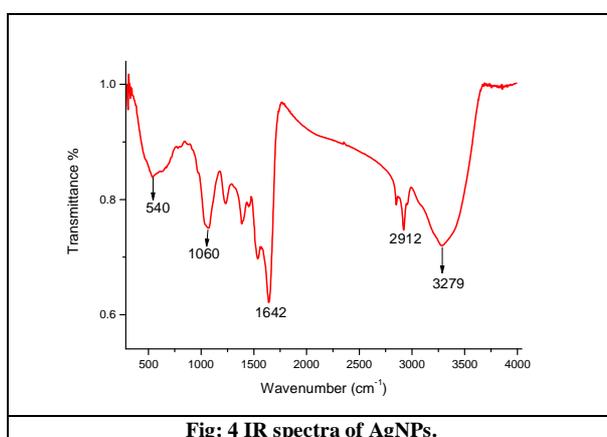
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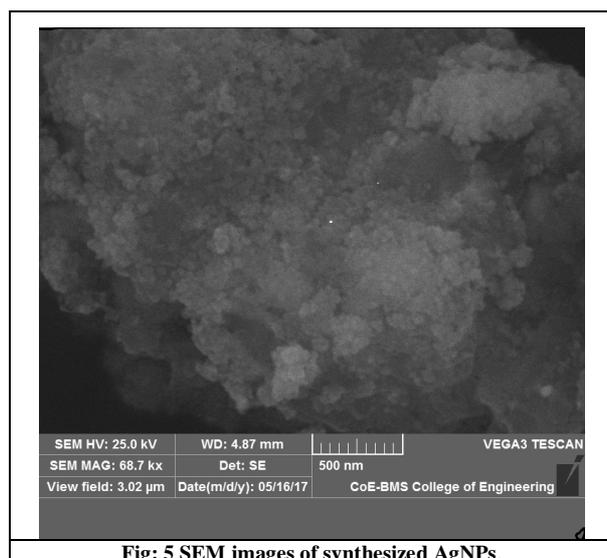
FT-IR analysis.

FTIR measurements were carried out to identify the possible biomolecules responsible for the capping and efficient stabilization of the AgNPs synthesized by the plant extract. Fig. 4 show the IR spectra of AgNPs synthesized using *Tecoma capensis* leaves extracts, respectively. Absorbance bands of AgNPs were observed at 3279 cm⁻¹ Alcohol O-H, 2912 Alkane C-H, 1642 Imine C=N, 1060 Primary alcohol C-O, 758 and 540 Halo compound C-I.



SEM analysis:

The scanning electron microscopy (SEM) image (Fig. 5) further ascertains that the AgNPs are pre-dominantly spherical in morphology with their sizes ranging from 23 to 47 nm with average diameter of 31.9 nm.



X-ray diffraction:

The silver oxide molecules formed are necessarily subjected to XRD analysis for the measurement of size of these particles. Fig. 6 shows the XRD pattern obtained for the silver nanoparticles. The intense peak of nanoparticles 38, 44, 64 and 77° in *Tecoma capensis* appeared which are indexed as crystalline Ag. The sharpening of the peaks clearly

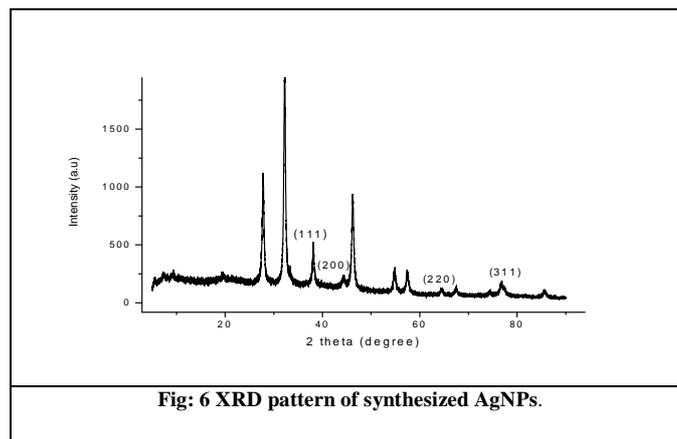
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indicates that the particles are the spherical NPs. The average size of the AgNPs is estimated by using Debye Scherrer's formula. The average of silver nanoparticles synthesized by *Tecoma capensis* leaves extract is 31.64nm.



Antibacterial Assay:

The synthesized AgNPs by the leaves extracts of *Tecoma capensis* have a significant antibacterial activity against *Klebsiella aerogenes* followed by *E-coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Fig. 7: Table 1).

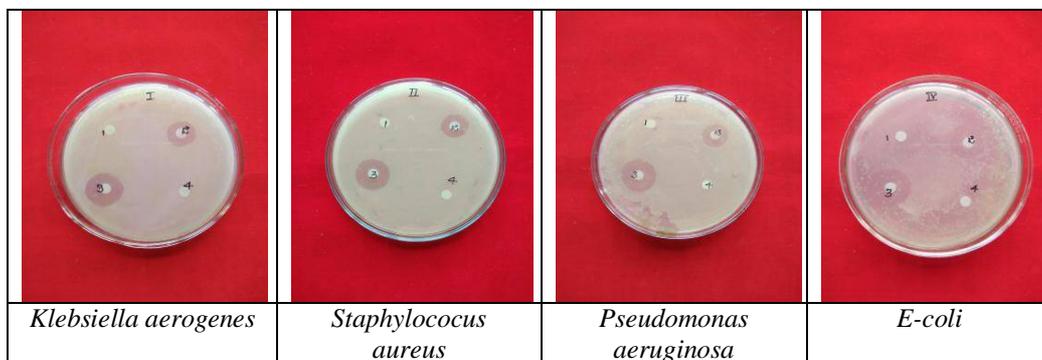


Fig.7 Antibacterial activity of AgNPs.

Table:1 Antibacterial zone formation.

Zone of Inhibition (mm)					
S.No	Strains	Control	Standard	AgNPs	Leaves Extracts
1	<i>Klebsiella aerogenes</i>	—	11	18	—
2	<i>Staphylococcus aureus</i>	—	10	16	—
3	<i>Pseudomonas aeruginosa</i>	—	9	16	—
4	<i>E-coli</i>	—	12	17	—

1. Control - Double distilled water, 2. Standard -Taxim, 3. AgNPs - Silver Nanoparticles, and 4. Leaves Extracts - *Tecoma capensis*.

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V. CONCLUSION

In the present study, it is demonstrated that the use of a natural, low cost *Tecoma capensis* leaves extracts as biological reducing agent for the production of AgNPs. The bioreduction method excluded the use of toxic solvents and chemicals. The bio-synthesized AgNPs using *Tecoma capensis* leaves extract provided to be excellent against nosocomial pathogens. The antimicrobial activity is well demonstrated by disc diffusion method. The present study showed a simple, rapid, and economical route to synthesize silver nanoparticles. UV-Visible spectrum the maximum absorbance peak was observed at 382 nm. FT-IR analysis confirmed that the bio-reduction of Ag⁺ ions to AgNPs is due to the bioreduction by plant leaves extracts. The XRD studies reveal that AgNPs are poly dispersed and the average size 31.64nm. SEM micrograph, it was observed that the spherical NPs were in the size ranging from 23 to 47 nm with average length of 31.9 nm (spherical shape). The obtained AgNPs have great zone of inhibition on *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Staphylococcus aureus* and *E-coli*. Further the research work has scope of studying the applications of prepared silver nanoparticles as bacterial, in wound healing, water purification, and in the field of medicine.

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