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One-step green synthesis of silver nanoparticles using flower extract of *Tabebuia argentea* Bur. & K. Sch. and their antibacterial activity.

Vinay SP[1], Chandrashekar N[1]*, and Chandrappa CP[2].

[1]Research and Development center, Department of Chemistry, Sridevi Institute of Engineering and Technology, Sira Road, Tumkur-572106, Karnataka, India. .
[2]Department of Biotechnology, Shridevi Institute of Engineering and Technology, Sira Road, Tumkur- 572106, Karnataka, India.

ABSTRACT

Green synthesis is one of the rapid, reliable, and best methods for the synthesis of silver nanoparticles at ambient temperature without application of hazardous agents. The present study described the formation of silver nanoparticles by the flower extracts of *Tabebuia argentea*. Synthesized silver nanoparticles were characterized by UV-vis spectroscopy, Scaning electron microscopy and X-Ray diffraction studies. Silver nanoparticles synthesized have shown significant antibacterial effect and the outcome of our study propose that the produced silver nanoparticles bestow superior substitutes in drug development.

Keywords: *Tabebuia argentea*, flower extract, UV-vis, SEM, XRD, antibacterial.

Corresponding author

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INTRODUCTION

Nanoparticles are the particles of size between 1 and 100 nanometers. Nanoparticles are more active and exhibit unexpected properties because of high surface to volume ratio and quantum size effect. Nanoparticles (NP's) of metals like Ag and Cu are found to exhibit enhanced optical and catalytic activity due to the quantum size effect. They have distinct physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical and biological properties [1]. Metal NP's are considered as building blocks for the next generation optoelectronics [2], electronics [3], and various chemical and biochemical sensors [4] as they have advantages over bulk materials.

Green synthesis is the use of plants, bacteria, and fungi to synthesize nanoparticles during which the metal ions are reduced to corresponding metal nanoparticless [1]. Many reports have been published about the green synthesis of silver nanoparticles using various plants such as *Glycyrrhiza glabra* root extract [5], Pineapple (*Ananas comosus* L. var. queen) fruits extract [6], leaf extract of *Moringa oleifera* [7], leaf extract of Neem (*Azadirachta indica* L.) [8,9], Soybean Seeds Extract [10], plant extracts of *Actaea racemosa, Magnolia grandiflora, Aloe* sp, *Eucalyptus angophoroides, Sansevieria trifasciata, Impatiens balsamina, Pelargonium graveolens* [11], *Lens culinaris* seeds [12], leaves extract of *Jatropha gossypifolia*, *Euphorbia tirucalli*, *Pedilanthus tithymaloides* and *Alseuosmia macrophylla* [13], and leaf extracts of *Asclepias curassavica* [14].

Tabebuia is a genus of flowering plants in the family Bignoniaceae. *Tabebuia argentea* consists almost entirely of trees, but a few are often large shrubs. A few species produce timber, but the genus is mostly known for those that are cultivated as following trees.

Fig 1: *Tabebuia aurgentia*

Cortex preparations of this tree are used for the elimination of parasites, malaria and uterine malignancy and decoction to treat anemia and constipation. Flowers, leaves and roots of *Tabebuia argentea* has been used as a therapy to reduce fevers and pains, sweating, tonsil inflammation and other untidiness [15]. Lapachol, a natural organic compound has been isolated from *Tabebuia argentea* which is being used to treat some cancers and also as anti-malarial and antitrypanosomal agent [16-22]. Saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins are present in the crude extract of *Tabebuia argentea* [23]. Currently, there are a few reports on phytochemical analysis, isolation of endophytes and preparation of crude extracts and their biological evaluation in *Tabebuia argentea.* Hence, here we are aimed at the synthesis of silver nanoparticles and to screen for their antibacterial activity by using flower extract of *Tabebuia argentea*.

EXPERIMENTAL

Collection and preparation of flower extract:

Flowers of *Tabebuia argentea* were collected from Shridevi Institute of Engineering and Technology Campus, Tumakuru of Karnataka, India in the month of September, 2016 was identified and authenticated by Dr. M.Govindappa, Taxonomist, Department of Biotechnology, Dayananda Sagar College of Engineering, Bangaluru, Karnataka of India. Collected flowers were washed thoroughly 2-3 times with tap water fallowed by distilled water. 20 gm of fresh flowers were added to 100 ml of distilled water and boiled at 60^0 C for 1 hour

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with stirring. The mixture was cooled and filtered using Whatman filter paper number 1. The filtrate was collected for further investigation.

Synthesis of Silver Nanoparticles using flower extract:

45 ml of freshly prepared 6mM aqueous silver nitrate ($AgNO_3$) was mixed with 5ml of flower extract of *Tabebuia argentea* for bioreduction process at room temperature in dark condition and allowed incubation for 24 hours.

a) *T. argentea* flower	b) Flower extract	c) 6 mM $AgNO_3$	d) Ag Nanoparticles

Fig 2: Synthesis of silver nanoparticles.

A reduction of Ag NPs was clearly observed when *Tabebuia argentea* flower extract was added with $AgNO_3$ solution within 20 min. The colorless solution was changed to brown color which indicates the formation of silver nanoparticles. The procured silver nanoparticles were purified by centrifugation in Remi CM-12 PLUS Cooling Micro Centrifuge at 10,000 rpm for 20 min. Supernatant was disposed and the obtained pellate was washed thoroughly with double distilled water to fling off unreacted $AgNO_3$ and flower extract. The refined pellate collected was air dried and conserved for further necessary characterization.

CHARACTERIZATION

UV-Vis spectroscopy: An aliquot of collected pellate containing silver nanoparticles was subjected to UV–Vis spectroscopy (Shimadzu 1601 model, Japan) at the resolution of 1 nm in range of 340 to 900 nm.

X-Ray diffraction 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-Kα radiation with a wavelength of 1.5418 Å.

FT-IR analysis: FT-IR analyses were performed using Shimadzu FT- IR model number 8400 to identify probable biomolecules present in the flower extracts of *T. argentea* which may be responsible for the reduction of metals and even for the nanoparticle stabilization. Approximately 3 mg of lyophilized flower extract under study was mixed with 300 mg of dried KBr, crushed well in mortar and pestle to prepare thin pallet for analysis, similar procedure was performed for synthesized Ag NPs using flowers extract, 16 scans per sample were taken in range of 400-4000 $cm^{-1.}$

Scanning Electron Microscopy (SEM) and EDX observation of silver nanoparticles:

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. Energy-dispersive X-ray (EDX) analysis was carried out by the same instrument and employed to confirm the presence of silver in the particles as well as to detect the other elementary compositions of the particles.

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Antimicrobial activity of silver nanoparticles :

The silver nanoparticles were mixed with deionized water and were tested for their antibacterial activity by the agar diffusion method. Four bacterial strains, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Staphylococus aureus* and *E-coli* were used for this analysis 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.

RESULTS AND DISCUSSION

The extracellular silver nanoparticle synthesis occurred during the exposure of leaf extract of *T. argentea* to 6mM aqueous silver nitrate solution. The complete reduction of silver ions was observed after 20 min. The change in color of the reacting mixture was observed during the incubation period because the formation of silver nanoparticles consequence in a specific, brownish colour. The impression of this dark (Fig. 2.d) distinctly affirm formation of silver nanoparticles succeeding addition of the leaf extract [23].

UV-Vis-spectroscopy and Fourier transform-infrared spectroscopy analysis

The aqueous solution of synthesized silver nanoparticles was observed by recording the absorption spectra at a wavelength range of 340–900 nm (Fig. 3). It was noticed that solution of silver nitrate turned brown on addition of flower extract; it indicated the formation of AgNPs, whereas no color change was observed in the absence of plant extract (Fig. 2 c). In the UV-Vis spectrum; a single, strong and broad Surface plasmon resonance (SPR) peak was observed at 340 nm that confirmed the synthesis of AgNPs.

Fourier transform-Infrared (FT-IR) analysis was performed to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the reduced Ag NPs synthesized using *Tabebuia argentea* flower extract, The strong IR bonds were observed at 3,701, 3380, 2,925, 3333, 1,618, 1,387, 1,070, and 601 cm^{-1}. The bands which appeared at 3,701 and 2,922 cm^{-1} corresponding to N-H, –OH stretching and aliphatic –C-H stretching, respectively. The bands at 2,333 and 1,618 cm^{-1} are due to the CO_2 and C=C stretching, respectively. The IR bands observed at 1,387 and 1,070 cm^{-1} may be ascribed to –C-O and –C-O-C stretching modes, respectively. The low band at 601 cm^{-1} corresponds to C-Cl stretching. The two new strong bands recorded at 820 and 601 cm^{-1} in the spectra of the synthesized material were assigned to C-H bending peak may be raised due to the reduction of $AgNO_3$ to Ag nanoparticles (Fig 4).

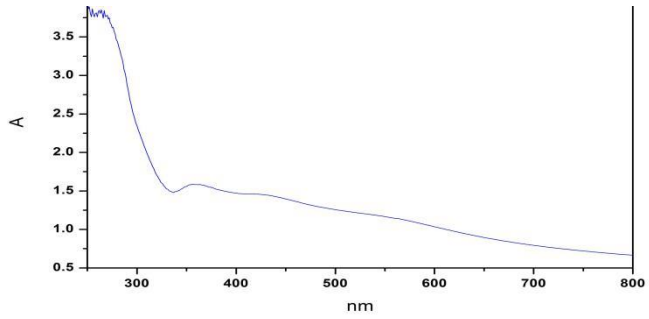

Fig 3: UV-vis spectrum of Ag NPs synthesized by *Tabebuia argentea* flower extract.

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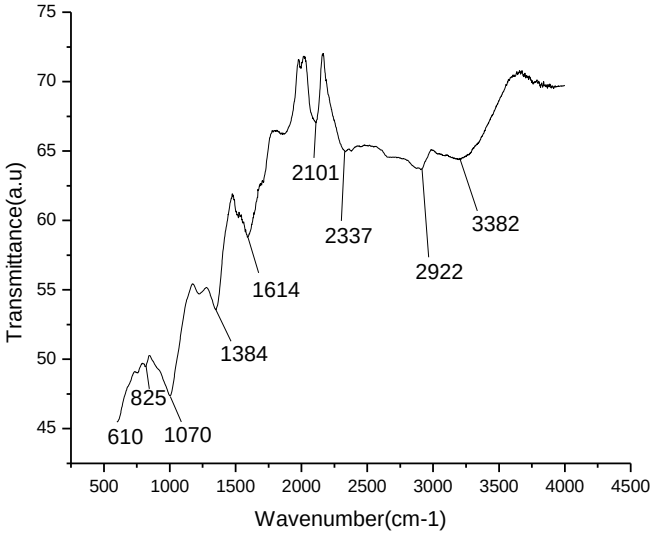

Fig 4: IR spectra of Ag NPs synthesized using *Tabebuia argentea* flower extract.

X-ray diffraction studies

X-ray diffraction pattern (XRD) was recorded for the synthesized Ag NPs (Fig 5). Three distinct diffraction peaks at 38^0, 44^0, and 64^0 were indexed with the planes (111), (200), and (220) for the face-centered cubic silver as per the JCPDS card no. 4-783.The well resolved and intense XRD pattern clearly showed that the Ag NPs formed by the reduction of Ag^+ ions using Tabebuia aurea flower extract are crystalline in nature. The low intense peak at 77^0 belongs to (311) plane. Average particle size (D) of synthsised NPs is found to be 22nm using Scherer's formula.

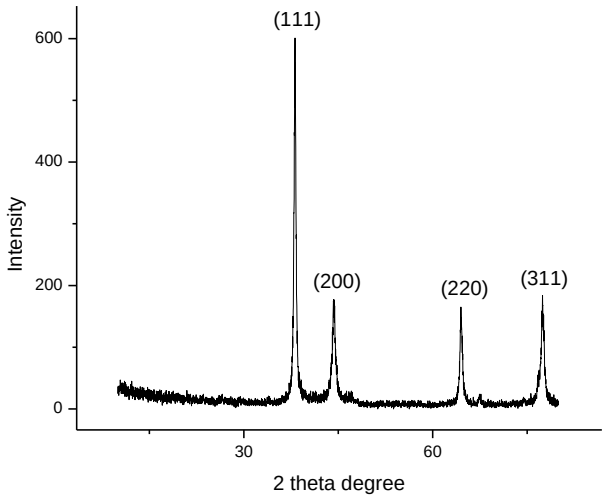

Fig 5: XRD pattern of synthesized silver nanoparticles

Scanning Electron Microscopy: Energy dispersive X-ray spectrometry (SEM:EDX) analysis

The scanning electron microscopy (SEM) image (Fig 6) further ascertains that the silver nanoparticles are pre-dominantly spherical in morphology with their sizes ranging from 20 to 30nm and have an average size of about 22.87nm. Energy-dispersive X-ray spectroscopy (EDX) (Fig 7) illustrated the chemical nature of synthesized silver nanoparticles using *Tabebuia argentia* flower extract. The peak was obtained at the energy of 3 keV, for silver, and also some of the weak peaks for C, O, Cl, Al, Mg, Si, P, S and K were found. The

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emission energy at 3 keV indicates the reduction of silver ions to element of silver. The quantitative analysis using EDX showed high silver content of 52.27%. The spectrum also showed the presence of carbon,oxygen, and silicon of 8.17% ,2.28% and 1.16 %, respectively (Fig 7).

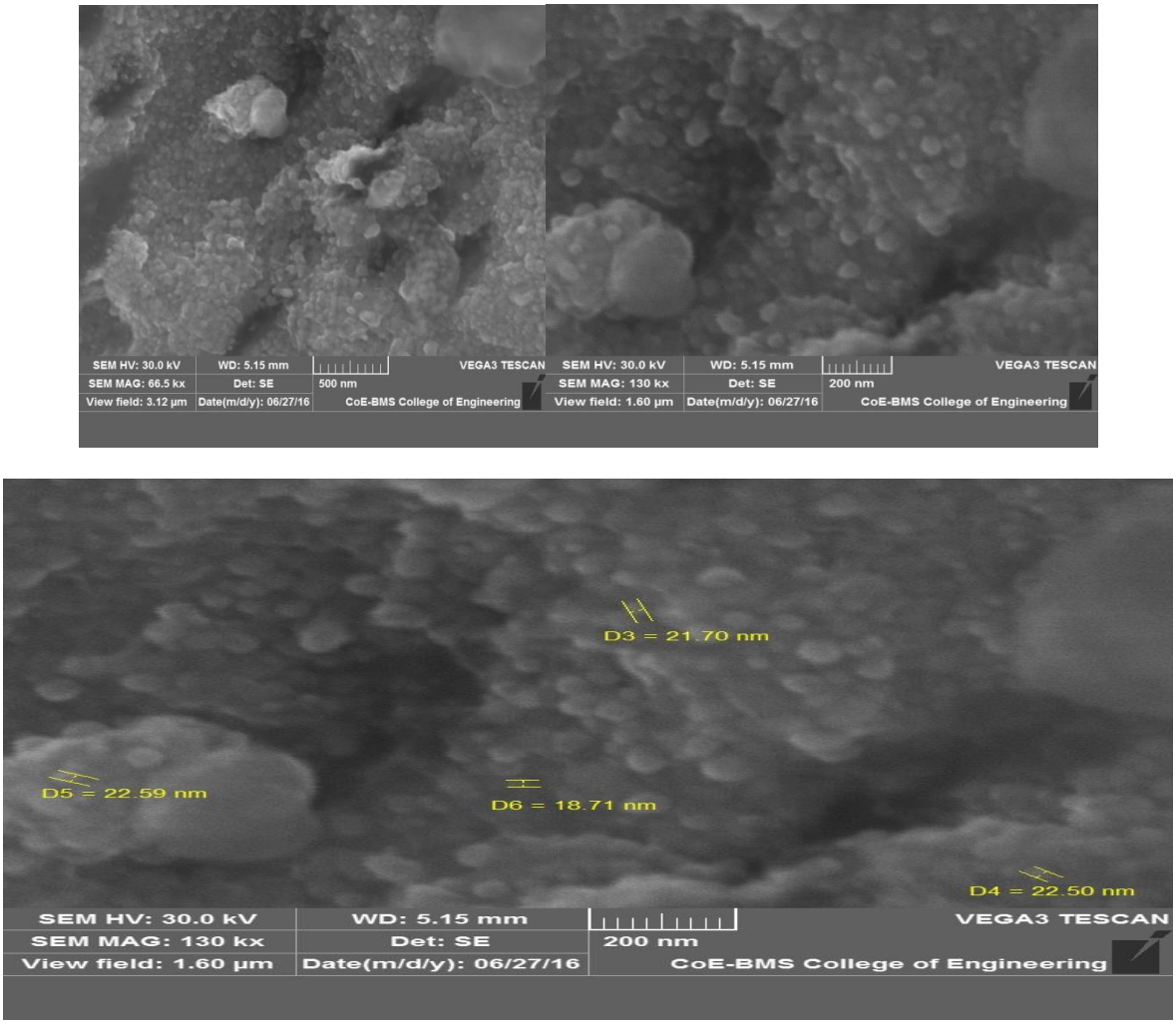

Fig 6: SEM image of Ag NPs using flower extract of *Tabebuia argentea*

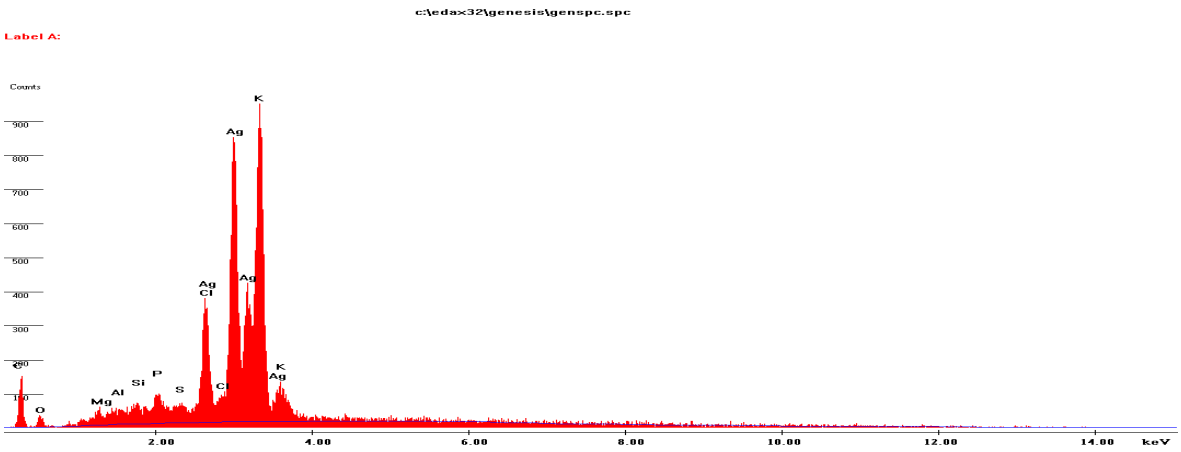

Fig 7: EDX results demonstrating the formation of silver nanoparticles.

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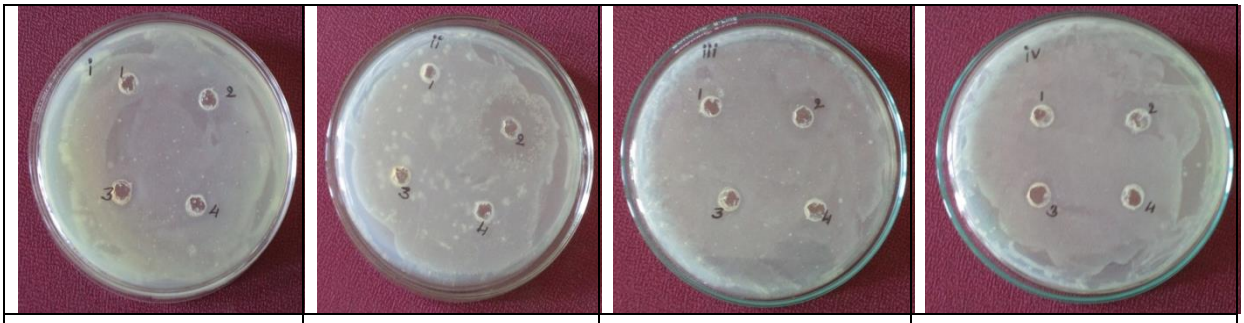
Antibacterial Assay:

The antibacterial assay was performed against bacterial pathogens like nosocomial pathogens such as *Pseudomonas aeruginosa, Klebsiella aerogenes, Staphylococus aureus* and *E-coli* by standard well diffusion method. 24 h broth culture was aseptically spreaded by sterilized cotton swab over solidified Mueller Hinton agar plates. Wells of equal distance and equal diameter (4 mm) were made by sterilized gel borer. Each well was filled with 50 µl of synthesized silver nanoparticles suspension (30µg/ml). Taxim and distilled water were maintained as positive and negative control respectively and flower extract alone). The plates were kept for incubation at 37°C for 24 h. The sensitivities of the test organisms to the different samples were indicated by clear zone around wells. Triplicates were maintained and for each replicates the diameter were measured in eight different directions and the average values were noted.

Investigation of the antibacterial properties demonstrated that antibiotics with silver nanoparticles synthesized by flower extract of *Tabebuia argentea* displayed a noteworthy zone of inhibition when contrasted with standard antibiotic taxim alone (Tab. 1; Figure 6), and the effects of the synthesized silver nanoparticles were analysed, based on the zone of inhibition around the microbial colonies.



E-coli	*Klebsiella aerogenes*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*

Fig 6: Antibacterial activity of Ag NPs synthesized by flower extract of *Tabebuia argentea.*

Table 1: Antibacterial activity of Ag NPs synthesized by flower extract of *Tabebuia argentea*

Zone of inhibition (mm)					
S.No	Species	Negative Control (Distilled water)	Synthesized AgNP	Positive Control (Taxim)	Flower extract
i	*E-coli*	0	18.4	6.6	10.0
ii	*Klebsiella aerogenes*	0	21.0	3.8	11.0
iii	*Pseudomonas aeruginosa*	0	15.2	4.4	8.0
iv	*Staphylococcus aureus*	0	9.4	5.2	5.0

CONCLUSION

In the present work silver nanoparticles were successfully synthesized by green-synthesis using *Tabebuia argentia* flower extract at room temperature. Formation of silver nanoparticles was confirmed by ultraviolet-visible (UV-vis), SEM-EDX and XRD. FT-IR study showed absorption bands corresponding to the main functional groups present in flower extracts. The antibacterial activity of Ag NPs to shows significant effect against the gram-positive and gram-negative bacteria. The green-synthesized method is convenient, eco-friendly and can be applied in various applications.

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