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Production of Silver Nanoparticles Through Eco-Friendly Approach from Stem Bark of *Ficus Mollis vahl* Characterization and Evaluation

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Abstract
Background: Nanoparticles have great importance due to their wide range of applications in various fields such as biology, pharmaceutical, biotechnology, medical, physics, chemistry, food industry, and textile industry, etc. Green synthesis is the safe and eco-friendly method to synthesize silver nanoparticles, these nanoparticles were found to have antibacterial efficacy. **Aim/objective**: The present study aimed to the synthesis of stable silver nanoparticles (AgNPs) using aqueous stem bark extract of *Ficus mollis* Vahl. a traditional medicinal tree taxon. **Methods**: The synthesized nanoparticles were characterized by using UV-Vis spectroscopy with a scan range of 190-750 nm, FT-IR spectroscopy, XRD (X-ray diffraction pattern), SEM with EDAX (Scanning Electron Microscope), and TEM (Transmission Electron Microscope). **Results**: The colour change from light brown to extreme essence brown was observed based on the peak at 438 nm obtained from UV-Vis spectroscopic analysis. FT-IR spectroscopic data confirmed that alcohol and amine of stem bark aqueous extract were responsible for the capping and stabilization of the AgNPs. Crystallographic studies through XRD indicated the AgNPs are crystalline. The TEM reveals that nanoparticles are spherical and contain a size range from 8.06 to 18.20 nm. EDAX patterns of synthesized AgNPs showed a 04.26 weight percentage of Ag metal in the sample indicate the purity of the reaction mixture. The anti-bacterial efficacy of synthesized AgNPs showed toxicity on four selected (two gram-negative and two-gram positives) bacteria. **Conclusion**: These studies revealed that the selected traditional medicinal plant contains the potentiality towards the synthesis of tiny-sized nanoparticles and can inhibit the growth of bacterial strains.

Keywords
Ficus mollis Vahl, Characterization of AgNPs, Anti-bacterial activity.

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INTRODUCTION:
Nanotechnology earning enormous attention as a new area of research dealing with the development of nanomaterials and nanoparticles (NPs) for their application in various fields such as biomedicines, catalysis, cosmetics, food technology, sensors, and agriculture [1,2]. Green synthesized nanoparticles involve finding cost-effective, rapid, and harmless solutions for the synthesis of nanoparticles, particularly silver and gold nanoparticles [3,4]. Towards all types of nanoparticles, metal, and metal oxide nanoparticles have been thoroughly monitored

using science and technology due to their marvellous properties such as high surface to volume ratio, high dispersion in the reaction mixture, and exhibit increased antimicrobial properties [5]. Metal nanoparticles like silver, gold, copper, zinc, and platinum show different properties like bio-sensing, drug delivery, cell labeling, and imaging, antifungal, and antibacterial [6]. There are various physical, chemical, and biological methods for the synthesis of metal nanoparticles. However, the physical and chemical methods are time consuming, laborious, not eco-friendly, and are producing toxic chemicals that contaminate the environment. Hence, green synthesis i.e., using plant extracts is the most preferred and desirous method and can be easily scaled up for large production. Plants possess different phytochemicals like Alkaloids, flavonoids, polyphenols, etc. which are responsible for the reduction of metal ions into metal nanoparticles and may act as capping and stabilization agents for synthesized metal nanoparticles. These green synthesized nanoparticles have wide applications in biomedical fields such as antibacterial, antifungal, antioxidants, and anticancer agents [7]. Silver nanoparticles act as effective lethal agents against a wide range of Gram-positive and Gram-negative bacteria and even against antibiotic resistance strains [8]. Numerous botanical species have been reported for these purposes like algae-*Sargassum muticum* [9], *Shorea tumbaggaia* [10], *Azadirachta indica* [11], *Svensonia hyderabadensis [12], Boswellia ovalifoliolata* [13], *Ficus* [14], *Nelumbo tetragona* [15], *Syzygium alternifolium* [16], *Adansonia digitata* [17].

Ficus mollis belongs to the family Moraceae comprises shrubs, vines, or rarely herbs, frequently with milky latex Trees, sometimes spiny. Leaves alternate, rarely opposite, petiole frequently present and explicated, simple leaf blade, sometimes with cystoliths, margin entire or palmately lobed, venation pinnate or palmate. Figs occur in leaf axils, in pairs of the cluster. Fig wall is somewhat fleshy, brownish, velvety [18]. The distribution of plants is very common on rocks, foothills up to 900 m in India and Srilanka [19]. Plants have been used to treat hepatoprotective [20], antibacterial [21], and antioxidants [22]. Owing to all this information on *Ficus mollis* the present work was undertaken to synthesize silver nanoparticles and to characterize with advanced microscopic techniques and evaluate the antibacterial efficacy of AgNPs.

MATERIAL AND METHODS:

Collection of plant material

The fresh Stem Bark of *Ficus mollis* Vahl. was collected from Kapilateertham, the forest of Seshachalam hill range of Andhra Pradesh, India. Then the stem bark was washed with tap water and followed by distilled water then the material was shade dried for 15 days and ground to a fine powder with the electric blender.

Synthesis of AgNPs

10 ml of bark extraction was taken into the sterile conical flask and added 1mM $Ag(NO_3)_2$ solution for 1 hour at 60-80 c°, the reaction mixture was visibly changed from light brown to dark brown and it was centrifuged at 15,000 rpm for 20 min to remove biological impurities, then supernatant and pellet were collected separately, and the supernatant was used to characterize and for the evaluation of antimicrobial efficacy.

Characterization

First and for most confirmation of nanoparticles (NPs) was analysed with UV-vis spectroscopy (Nanodrop range from 190-750 nm) to know whether nanoparticles were truly reduced or not; to find out the phytochemicals involved in the reduction of nanoparticles (NPs) through surface Plasmon resonance (SPR) and to understand the unique Phyto-compounds were involved in capping and stabilization of nanoparticles (NPs). The FT-IR spectroscopy was carried out using range from 4000-500 cm^{-1}ALPHA interferometer, ECO-ART, Bruker, Ettlingen, Karlsruhe, Germany. The crystalline nature of AgNPs was carried out by an X-Ray Diffractometer (Shimadzu, XRD-6000). The size, shape of nanoparticles (NPs), and percentage of selected metal nanoparticles were analysed using Scanning Electron Microscope (SEM-FEI Quanta, 200 FEG HR-SEM) adjoined with an energy-dispersive X-ray spectroscope (EDAX). Size, shape, dispersed nature, and agglomeration pattern of nanoparticles (NPs) analysed with TEM (HF-3300, 300 kV TEM/ STEM, Hitachi).

Antibacterial Studies of AgNPs

Biosynthesized AgNPs were analysed for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* both Gram-positive and Gram-negative bacteria respectively. Bacterial cultures were procured from the Department of Microbiology, Sri Venkateswara University, Tirupati. Disc diffusion assay method was performed using a standard protocol (Cruickshank 1986) [23]. 20 µl of $Ag(NO_3)_2$, plant extract, AgNPs, and streptomycin was applied on separate sterile filter paper discs (Whatman No. 1filter paper with 6 mm diameter) and allowed to dry before being placed on nutrient agar medium. Triplicates of each solution were examined and incubated at 37 c° for 24 h in the incubation chamber. Zone of inhibition diameter was measured with the help of scale and the results were tabulated.

RESULTS AND DISCUSSION:

UV- vis Spectroscopy

The stem bark extract can reduce silver nitrate solution into the ionic form. The extract reacts with 0.001 mM Ag $(NO_3)_2$ solution and visually colour changes from light brown to dark brown colour (Fig.1). It is initial confirmation of reduction of silver ions and synthesis of silver nanoparticles, which were observed the SPR spectrum peak at 438 nm. Similar results were found in the bark-mediated synthesis of silver nanoparticles from *Cochlospermum religiosum* [24].

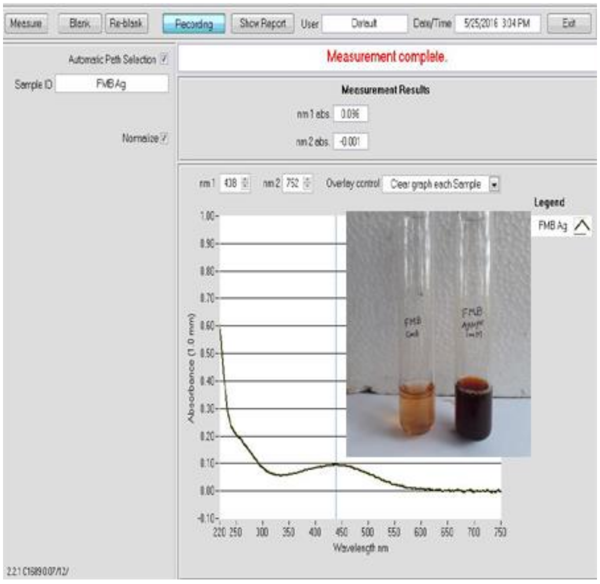


Figure. 1: UV-Vis spectroscopic analysis absorbance peak at 438 nm with colour change.

FT-IR

These phytochemicals are accountable for capping and stabilization. The synthesized silver nanoparticles (AgNPS) showed broad peaks assigned at 3292cm^{-1} represented strong broad O-H stretching alcohol and 1635cm-1 indicated amine N-H medium bond bending (Fig. 2). The phytoconstituents present in the plant extract are responsible for the synthesis of silver nanoparticles. This can be concluded from *Ficus mollis* formed strong capping on the nanoparticles. These kinds of results were reported from *Walsura trifoliate (A.Juss.) Harms.* aqueous bark extracts [25].

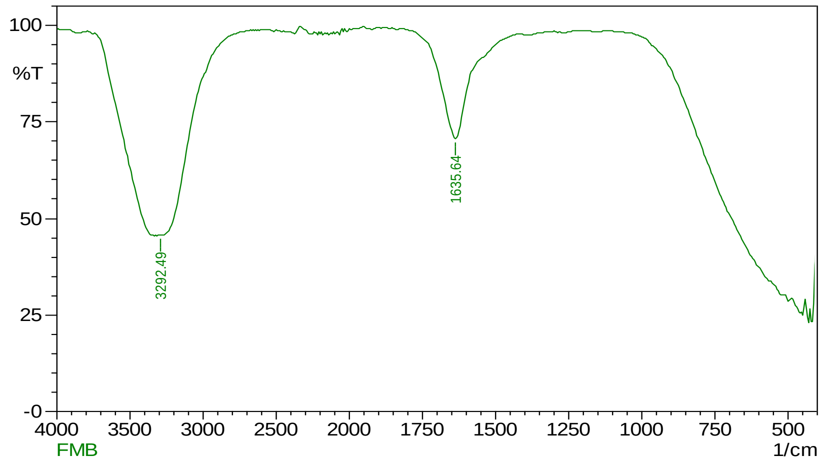
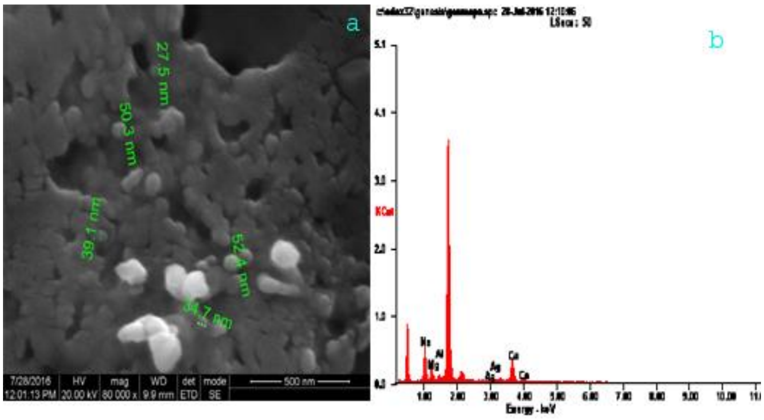


Figure. 2: FT-IR spectrum of AgNPs from bark extract of *Ficus mollis*

SEM with EDAX

The SEM revealed the average size of AgNPs was 40.8 nm at 500 nm resolution and all the AgNPs have spherical shapes (Fig. 3). EDAX results revealed the percentage of elements present in the prepared reaction mixture Na (37.34%), Mg (19.05%), Al (08.41%), Ag (04.26%), and Ca (30.94%). These results confirmed that the reduced nanoparticles belong to silver nanoparticles (AgNPs). Similar results were observed in *Syzygium alternifolium* [26].



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Element	Wt%	At%
NaK	37.34	45.99
MgK	19.05	22.19
AlK	08.41	08.83
AgL	04.26	01.12
CaK	30.94	21.87
Matrix	Correction	ZAF

Figure. 3: SEM image of bark AgNPs of *Ficus mollis* along with EDAX; a) 500 nm resolution studies show 27-52 nm size; b&c) EDAX spectrum of synthesized AgNPs shows 04.26 weight percent of Ag metal in the sample.

XRD

The crystalline molecular formation and particle size of synthesized nanoparticles exhibited the peaks at 2θ values of x-axis showed 111, 200, 220, 311 and the corresponding to 38.15, 44.45, 64.57, and 77.38. Similar results were observed in *Cochlospermum religiosum* [24] (Fig. 4).

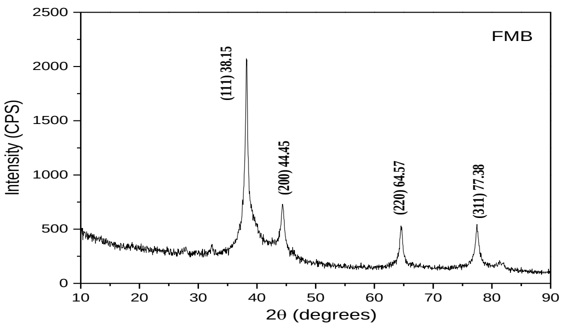


Figure. 4: XRD patterns of synthesized AgNPs represent four Bragg reflections corresponding to the crystalline nature of nanoparticles.

TEM

TEM is valuable, often used and an important tool for the characterization of nanoparticles at high magnifications (20 nm) done by the Transmission Electron Microscope, used to acquire quantitative measures of particle size and size distribution, and morphology of nanoparticles (NPs) (Fig.5). In TEM analysis, green synthesized AgNPs were coated on copper grids and analysed by Hitachi HF- 3300 advanced with 300 kV. 20 nm scale bar of TEM studies revealed AgNPs denote that the synthesized nanoparticles are polydispersed, predominantly spherical, due to the 8. 06 to 18.20 nm size and are not in physical touching with each other i.e., agglomeration free of the nanoparticles (NPs) were found. A similar kind of result was observed in the stem-bark extract of *Terminalia pallida* Brandis [27].

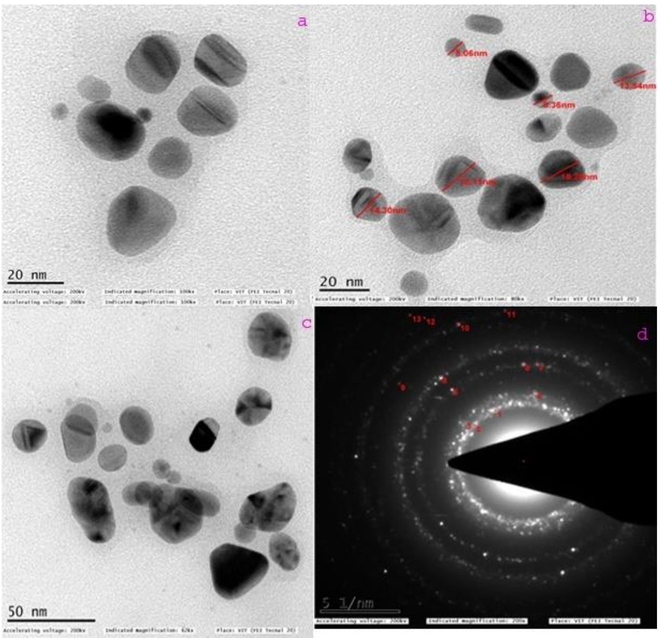


Figure. 5: TEM images of bark AgNPs of *Ficus mollis*. a & b) 20 nm resolution studies; c) 50 nm resolution studies; d) SAED of green synthesized AgNPs.

Antibacterial activity

The acquired zone of inhibition of diverse extracts was compared with standard drug streptomycin for bacteria maximum inhibition zones of synthesized AgNPs bacteria were observed in *K. pneumoniae*, *E. coli, S. aureus, and B. subtilis* (Fig. 6, Table. 1 & graph. 1). Gram-negative bacteria are too permitting when compared to Gram-positive bacteria. Among the bacterial cultures, the passage of nanoparticles by the cell membrane is easy in the case of gram-negative bacteria when compared to gram-positive bacteria due to the disparity in manufacturing of cell membrane. Among the activity zone of inhibition was larger in gram-negative bacteria when compared with gram-positive bacteria. Similar results were found in stem bark AgNPs of *Boswellia ovalifoliolata and Shorea tubaggaia* [28].

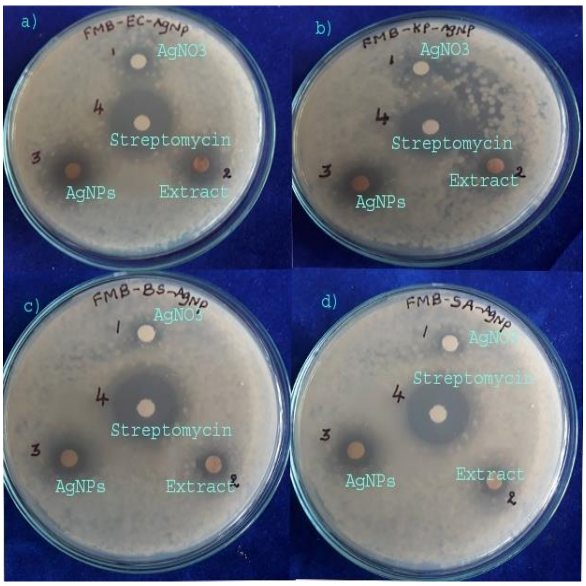
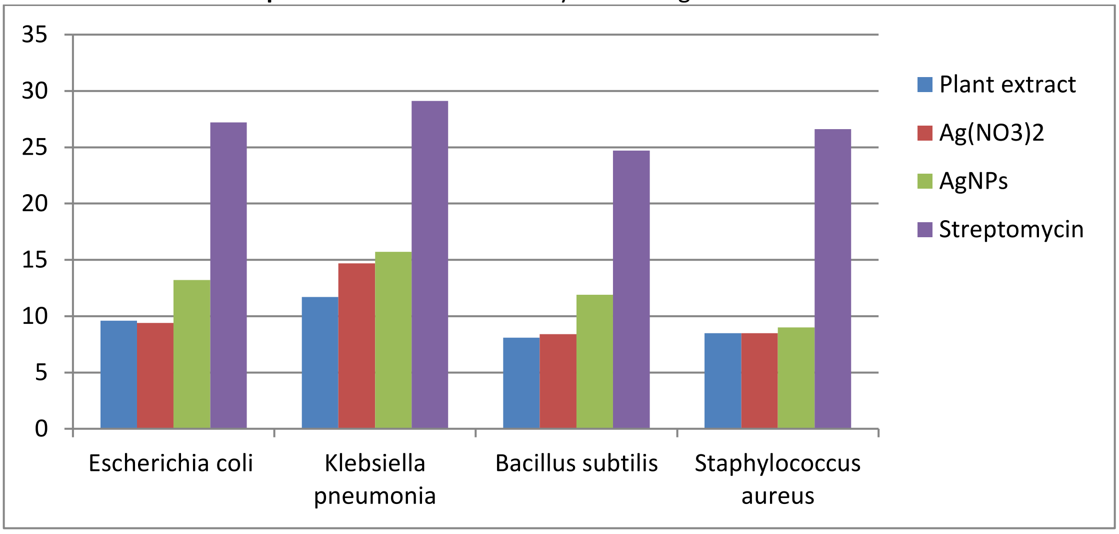


Figure. 6: Antibacterial efficacy of stem bark AgNPs of *Ficus mollis. a) Escherichia coli; b) Klebsiella pneumonia; c) Bacillus subtilis d) Staphylococcus aureus*

Table. 1: Antibacterial efficacy of bark AgNPs of *Ficus mollis*

Sno	Name of the Bacteria	Zone of inhibition (mm)			
		Plant extract	Ag $(NO_3)_2$	AgNPs	Streptomycin
1.	*E. coli*	9.6±0.066	9.4±0.088	13.2±0.033	27.2±0.055
2.E	*K. pneumonia*	11.7±0.057	14.7±1.256	15.7±0.120	29.1±0.115
3.	*B. subtilis*	8.1±0.088	8.4±0.066	11.9±0.033	24.7±0.057
4.	*S. aureus*	8.5±0.033	8.5±0.057	9.0±0.088	26.6±0.066

Graph. 1: Antibacterial efficacy of bark AgNPs of *Ficus mollis*



CONCLUSION:

Due to serious disadvantages through physical, chemical, and micro-mediated methods of silver nanoparticles (AgNPs). The present study was aimed to grow up a fast, cost-effective, and environmentally friendly method of synthesis of AgNPs from *Ficus mollis* stem bark. FT-IR studies revealed the presence of alcohol and amines are mainly responsible for the capping and stabilization of AgNPs in the sample. These green synthesized AgNPs are polydispersed without any agglomeration and possess sizes ranging from 27.5 nm to 52.4 nm were analysed through SEM, whereas TEM analysis reveals the size of AgNPs from 8.06 to 18.20 nm at 20 nm resolution. Spherical-shaped AgNPs exhibited significant antibacterial activity. A high quantity of tiny-sized nanoparticles was produced by a low amount of plant extract through a traditional medicinal plant is beneficial. Based on the above-mentioned results, we conclude that the *Ficus mollis* stem bark is an effective source for the synthesis of silver nanoparticles (AgNPs), which are effective antibacterial products.

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