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BIO-FABRICATION OF SILVER NANOPARTICLES USING *FICUS MOLLIS* VAHL. AQUEOUS LEAF EXTRACT, CHARACTERIZATION, AND EVALUATION OF THE ANTIBACTERIAL EFFICACY

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Abstract

Aim: The present work pursues to synthesize Silver Nanoparticles (AgNPs) from aqueous leaf extract of *Ficus mollis* Vahl. and their microbial efficacy. **Methods:** The synthesized nanoparticles were characterized by using UV-Vis spectroscopy, FT-IR (Fourier transform infrared spectroscopy), XRD (X-ray diffraction), SEM (Scanning Electron Microscope) with EDAX, and TEM (Transmission Electron Microscope). **Results:** The colour change from light brown to dark brown is observed upon synthesis and the peak obtained at 444 nm with UV-Vis Surface Plasmon resonance (SPR) analysis confirmed the synthesis of nanoparticles are silver nanoparticles. FTIR analysis confirms that phenols and proteins of leaf extract are generally responsible for the capping and stabilization of green synthesized silver nanoparticles. Crystallographic analysis of XRD denotes, the AgNPs are crystalline. SEM revealed that the nanoparticles are spherical and their size ranges from 22.0 to 38.6 nm. EDAX pattern of biosynthesized nanoparticles showed a 1.44 weight percentage of Ag metal. TEM microscopic analysis divulges that the nanoparticles are spherical with sizes ranging from 8.36 to 23.11 nm. Antimicrobial analysis of the synthesized AgNPs exhibited a toxic effect on selected Bacteria. **Conclusion**: It denotes that aqueous leaf extract of *Ficus mollis* is preferable for synthesizing stable AgNPs and to formulate eminent antibacterial agents.

Key Words: Biofabrication, Silver nanoparticles, *Ficus mollis* Vahl, Characterization, Antibacterial efficacy.

Introduction

Nanotechnology is the growing science of application nanoparticles in the diverse sectors of technology; it has transfigured many sectors of technology like medicine and industry, particularly in the pharmaceutical and energy industry, food safety and environmental science, agriculture, and among many others [1]. Throughout human history, medicinal plants were provided with ointments and cured many health problems. Plants can produce a manifold range of chemical compounds that are responsible for various biological actions [2]. Even at the rise of the twenty-first century, about 90% of developing drug molecules have been isolated directly or indirectly from plants [3]. Nanoparticles are prepared using metals such as gold, silver, and platinum, etc. through diversification of chemical and physical methods [4, 5]. But these methods are not eco-friendly, lengthy, and involve the use of dangerous chemicals [6]. There is a very need to develop an eco-friendly, cost-effective process for the synthesis of nanoparticles that does not execute toxic chemicals. Currently, methods of biosynthesis of Nanoparticles (NPs) utilizing different biological systems such as Yeast, Fungi, Bacteria, and plant extracts become significant [7, 8, 9]. Current reports included the synthesis of silver nanoparticles using *Syzygium alternifolium* [10], *Adansonia digitata* [11], *Azadiracta indica* and *Ocimum sanctum [12]*, *walsura trifoliata* [13], *Terminalia pallida* [14], and *Shikakai [15]*. The biological method is a single-step process for large-scale production of SNPs, eco-friendly moreover environmentally non-hazardous and there is no need to use high pressure, energy, temperature, and toxic chemicals. The synthesis of SNPs by using plant sources is better than the other biological methods [16]. Various compounds like alkaloids, amines, amides, flavonoids, terpenoids, proteins, phenolics, and pigments are present in the plant extract; serve in the reduction and stabilization of metal ions during the green synthesis of nanoparticles [17, 18, 19].

Ficus mollis Vahl. Belongs to the family Moraceae. Distribution is very common on rocks, foothills up to 900 m in India and Srilanka [20, 21]. The genus *Ficus* comprises about 1000 species from pantropical and subtropical origins [22]. The plant is used for diversified diseases in traditional medicine. Leaves of *Ficus mollis* were used to relieve the pain in the ear [23]. Alcoholic extracts of bark and leaves of *Ficus mollis* expressed good antimicrobial activity [24].

Material and Methods

Plant material collection

The fresh leaves were collected from the kapilateertham of Tirupati, Andhra Pradesh, India, in July 2019. The leaves were washed, cleaned and shade dried for 15 days and ground into a fine powder with the help of an electric blender, and stored in an airtight container.

Synthesis of AgNPs

5 ml of plant extract was taken in to cleaned 250 ml conical flask and then 100 ml of 1mM Ag solution was added to this. It was heated in a water bath for 30 minutes at 60 ^{0}C. The visible colour change indicated the synthesis of AgNPs, then contents were centrifuged for 15 minutes at 15000 rpm, later pellet and supernatants were collected separately and these were further used for the characterization and antibacterial activity.

Characterization of Nanoparticles

The eco-friendly synthesized nanoparticles were characterized by using various advanced spectroscopic and microscopic instruments. The synthesized nanoparticles are conformed as silver nanoparticles by using a UV-vis spectrophotometer (JASCO V-670 PC), between the wavelength scan range of 190-750 nm. The FT-IR analysis was carried out using FT-IR spectrophotometer (CAVY 630 Agilent) from the scan range of 500 cm^{-1} to 4000 cm^{-1}. X-ray powder diffraction analysis was done by the Bruker, D8 Advance. The size, shape of the particles, and the percentage of presence of appropriate metal nanoparticles were analyzed with Scanning Electron Microscope (FEI Quanta, 200 FEG HR-SEM) appended with an energy-dispersive X-ray spectroscope (EDAX). Size, shape, agglomerated pattern, and dispersed nature of nanoparticles were done by using Transmission Electron Microscopy (FEI-Techai G2 20 Twin).

Antibacterial activity

The effect of synthesized AgNPs was carried out against four selected Gram-positive and Gram-negative bacteria i.e. *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTTC-731), *Escherichia coli* (MTTC-443), *Klebsiella pneumonia* (MTTC-741) by using the disc diffusion assay method [25]. Streptomycin (20 µl/disc) was used as a standard control for bacteria, 20µl/disc of plant extract, $Ag(NO_3)_2$, AgNPs were applied on separate filter paper discs (Whatman No.1 Filter paper with 6 mm diameter), and allowed to dry before existence placed on the agar medium. Triplicates of each extracted sample were tested and incubated at 37 ^{0}C for 24 h in an incubator chamber. The zone of inhibition (Diameter of the zones) was measured with the use of scale and the results were tabulated (Figure 6, graph 1, Table 1).

Results and Discussion

UV-Visible spectroscopy

After the addition of $AgNO_3$ solution to the plant extract, the colour was changed from light brown to dark brown in the reaction mixture and the typical peak was obtained at 444 nm, (Figure 1) due to SPR (Surface Plasmon Resonance). The same results were obtained in *Abrus precatorius* leaf mediated synthesis [26].

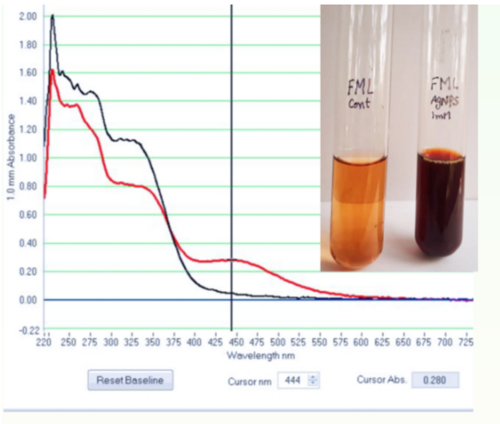


Fig.1: UV- Vis spectrum of synthesized AgNPs shows a broad peak at 444 nm. The inset figure shows colour change pattern from light brown to dark brown

FTIR Analysis

To find out the possible bio-molecules accountable for the capping and stabilization of nanoparticles as the reaction mixture was analyzed under the scan range from 4000 to 500 cm^{-1} of IR spectra by FT-IR (Figure 2). The broad peaks were obtained at 3290 cm^{-1} corresponds to O-H alcohols and phenols with a high amount of concentration and 1635 cm^{-1} corresponds to C=C stretching conjugated alkenes. Thus consequences confirm that alcohols, phenols, and conjugated alkenes are mainly responsible for capping and stabilization of AgNPs. A similar result was observed in *Syzygium alternifolium* [10].

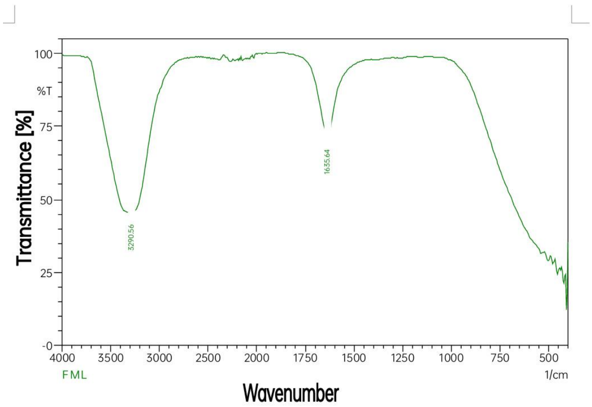
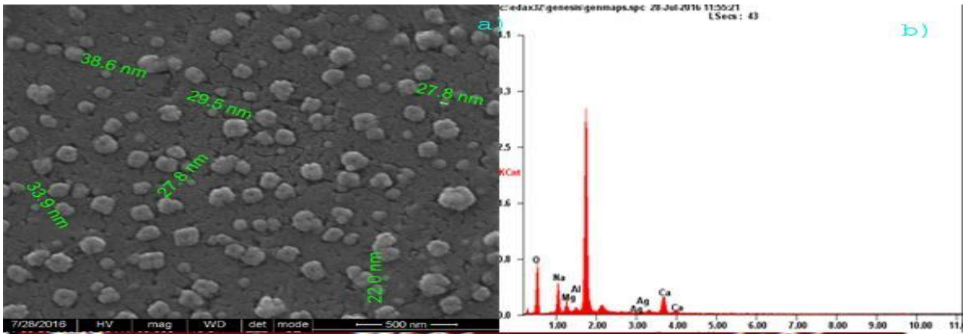


Fig. 2: FT-IR spectrum of AgNPs shows broad peaks at 3290 cm^{-1} and 1635 cm^{-1}

SEM-EDAX Analysis

Morphology and size of SNPs characterization were observed in SEM at 500 nm (Figure 3). The micrographs exhibit uniform diffusion of AgNPs on the surface, these are spherical in shape and size range from 22 to 38.6 nm and EDAX spectra revealed 01.44 weight percentage of Ag metal along with different elements with their weight percentage like Calcium (12.17%), Oxygen (54.00%), Sodium (22.33%), Magnesium (07.41%), and Aluminium (02.65) in the prepared sample. Similar results were found in *Acalypha indica* leaf-assisted synthesis of silver nanoparticles [27].



Element	Wt%	At%
OK	54.00	66.62
NaK	22.33	19.17
MgK	07.41	06.02
AlK	02.65	01.94
AgL	01.44	00.26
CaK	12.17	05.99
Matrix	Correction	ZAF

c)

Fig. 3: SEM image of synthesized AgNPs,

a) 500 resolution studies show spherical shape with 22 nm – 38 nm size, spherical shaped particles b) EDAX analysis of AgNPs shows a 1.44 weight Percentage of Ag metal in the reaction mixture c) Percentage of different elements present in the reaction mixture

XRD Analysis

The nature of the nanoparticles was confirmed with the peaks at 2θ values of X-axis shows 38.20°, 44.28°, 64.57°, and 77.38° corresponding to 111, 200, 220, and 311 Brag reflections of Y-axis respectively it may be indicated based on the face-centered cubic formation of silver (Figure 4). X-ray diffraction of silver nanoparticles formed by the reduction of Ag^+ ions by the leaf extract is crystalline. This type of result was reported in the leaf extract of *Carissa carandas* L. [28].

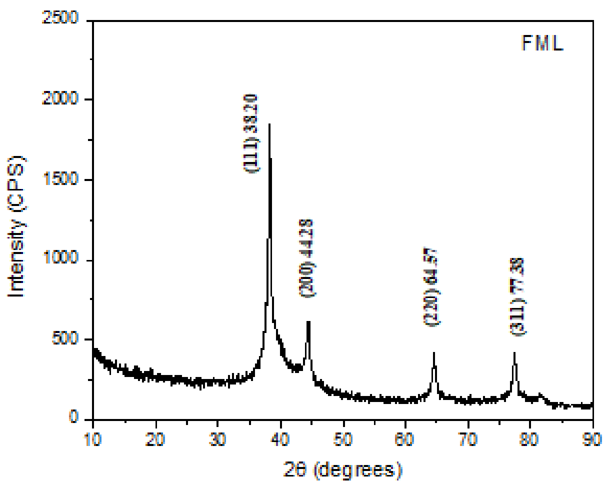


Fig. 4: XRD pattern of AgNPs shows four intensive peaks

TEM Analysis

TEM micrograph reveals that the size of biosynthesized AgNPs ranges from 8.36 nm to 23.11 nm at 50 nm resolution with spherical shape (Figure 5). TEM analysis shows higher magnification due to these green synthesized nanoparticles and exhibited interface between two lattice fringes with small-sized nanoparticles belonging to attachment of AgNPs between the surface sheets of the TEM micrographs. The same results were found in *Adansonia digitata* [29].

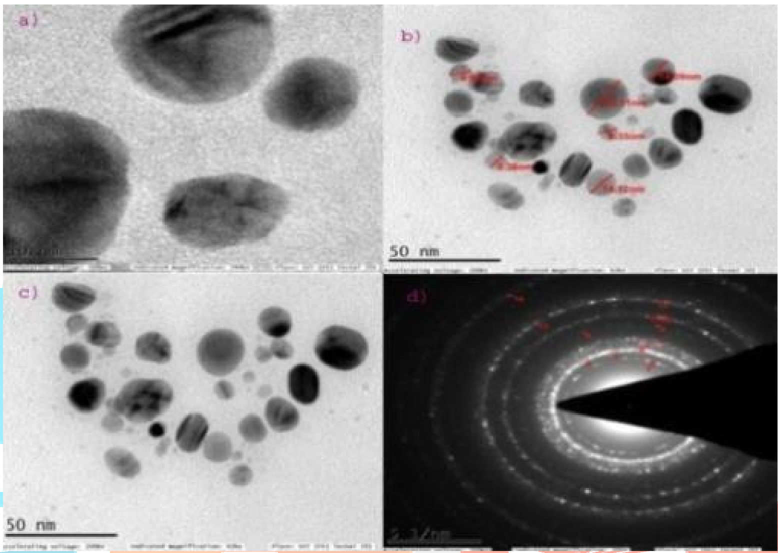


Fig. 5: TEM images of synthesized AgNPs using the leaf extract of *Ficus mollis*

a) 10 nm Resolution studies of AgNPs are spherical in shape, b&c) 50 nm Resolution studies of AgNPs shows 8 nm – 23 nm size, d) selected area electron diffraction (SAED) shows characteristic crystal spots of elemental silver

Bacterial activity

The effect of synthesized silver nanoparticles (AgNPs) on Gram-positive bacteria (*B. subtilis, S. aureus),* and Gram-negative bacteria (*E. coli* and *K. pneumonia)* showed significant activity. AgNPs have shown a lesser zone of inhibition on two gram-positive bacteria than gram-negative bacteria (Fig. 6, Table. 1 & Graph. 1). This is because gram-negative bacteria are not having a thick layer that is made up of Peptidoglycon like Gram-positive bacteria. Due to this penetration of AgNPs by cell wall is easy in the case of gram-negative bacteria and inhibition of growth is possible. The results were similar to those previously reported in Olive leaf extract (30).

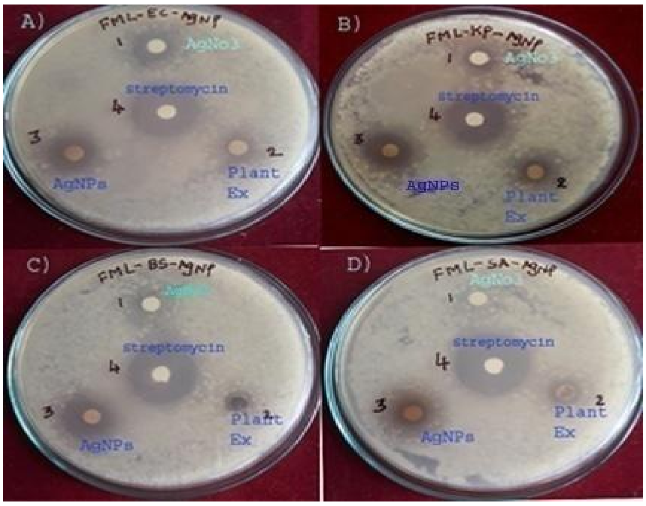


Fig. 6: Antibacterial activity *Ficus mollis* leaf mediated silver nanoparticles on

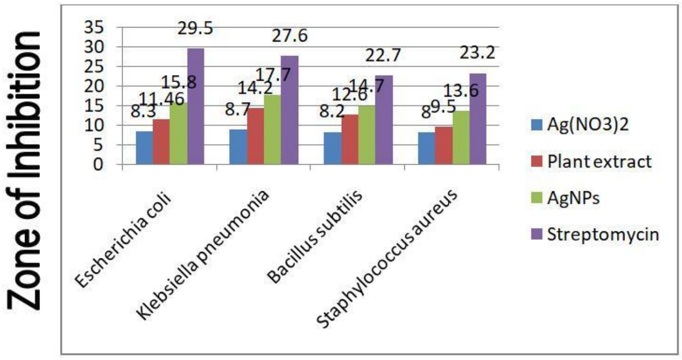
A) E. coli, B) *K. pneumonia,* C) *B. subtilis,* D) *S. aureus*

Different bacterial species show varying levels of antibacterial activity when subjected to bio-engineered nanoparticles. The bacterial cells with higher levels of peptidoglycan are more likely to inhibit the use of the nanoparticles. The results were similar to those previously reported in Olive leaf extract [30].

Table.1: Antibacterial efficacy of leaf AgNPs from *Ficus mollis*

S.NO	**Bacteria name**	**Zone of inhibition**			
		1 mMAg $(NO_3)_2$	Plant extract	AgNPs	Streptomycin
1.	*Escherichia coli*	8.3±0.115	11.46±0.066	15.8±0.057	29.5±0.033
2.	*Klebsiella pneumonia*	8.7±0.173	14.2±0.088	17.7±0.066	27.6±0.088
3.	*Bacillus subtilis*	8.2±0.057	12.6±0.033	14.7±0.088	22.7±0.057
4.	*Staphylococcus aureus*	8.0±0.033	9.5±0.1	13.6±0.033	23.2±0.066

mM- Milli Molar, mm- millimetre, AgNPs- silver nanoparticles



Graph.1: This graph represents the antibacterial activity of *Ficus mollis* leaf-mediated silver nanoparticles. The X-Axis refers to concentration in µl and Y-axis refers to the zone of inhibition of bacteria in mm.

Conclusion:

In the present study, we developed an eco-friendly, fast, and cost-effective technique for the production of silver nanoparticles (AgNPs) in a green route from an aqueous leaf extract of *Ficus mollis*. Plant source eco-friendly technique has a pivotal role and avoids the disadvantages of chemical, physical and bacteria mediated approaches instead of plant sources as reducing agents. These silver nanoparticles showed better antibacterial efficacy against clinically isolated pathogenic bacteria. Consequently, this type of green method for the synthesis of AgNPs at a low cost with natural sources is ultimately crucial to the industrial scale due to its high importance in the medical sector.

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