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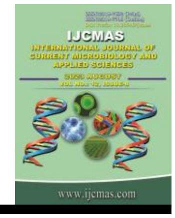

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Eucalyptus gunni Mediated Fabrication of Silver Nanoparticles and their Biomedical Applications

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A B S T R A C T

Nanobiotechnology in the development of nanoparticles is of considerable prominence to enlarge their biological solicitations. At present, a wide array of metal nanoparticles has been synthesized by using biogenic enzymatic methods. In the present study, Ag nanoparticles were green synthesized by using the aqueous leaf extract of *Eucalyptus gunni*. Further, the NPs were characterized by UV-Visible spectroscopy, FT-IR spectroscopy, X-ray diffraction (XRD), and Scanning Electron Microscopy (SEM). The antibacterial activity of the biosynthesized Ag nanoparticles was tested against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Maximum antagonistic activity was observed against *Escherichia coli*. Thus the biogenic nanoparticles can be sight seen in biomedical applications devoid of adverse side effects.

Introduction

Nanoscale materials frequently present properties dissimilar from their bulk counterparts, as their high surface-to-volume ratio results in an exponential upsurge of the reactivity at the molecular level. Such properties include electronic, optical and chemical properties, while the mechanical characteristics of the nanoparticles (NPs) may also differ comprehensively (Thanh *et al.,* 2014). Nanotechnology is still undergoing constant growth, and the scientific community is rather aware that there may be certain differences between the way analytical characterization methods operate for nanomaterials, in comparison with their more ‘traditional modes of use for more ‘conventional’ (macroscopic) materials (Mourdikoudis *et al.,* 2018). Nanobiotechnology combines biological principles with physical and chemical approaches to produce nano-sized particles with specific functions, representing an economic substitute for chemical and physical methods of nanoparticles formation (Durairasu *et al.,* 2017). Nanotechnology enables us to achieve sustainable development goals by addressing major problems of third-world countries like poverty, malnutrition and food scarcity. Despite having innumerable implications, nanotechnology is not exploited to its fullest scale. It is yet to reach every nook and corner of the globe (Saritha *et al.,* 2022).

Different practices have been intended for the fabrication of metallic nanoparticles. Presently, two leading approaches are being used to synthesize nanoparticles, referred to as the Top-Down and Bottom-Up approaches. Briefly, in the top-down approach, nanoparticles production involves the size reduction of bulk material by mechanical techniques, while, in bottom-up approach, small building blocks are collected into a larger structure, e.g., chemical synthesis (Karthika *et al.,* 2015). However, the most appropriate and effective approach for nanoparticle preparation is the bottom-up approach, where a nanoparticle is "grown" from simpler molecules known as reaction precursors. In this way, it is likely possible to control the size and shape of the nanoparticle depending on the subsequent application through variation in precursor concentrations and reaction conditions (temperature, pH, etc.) (Kavitha *et al.,* 2013). Microbial drug resistance (MDR) has developed as a world-wide health concern, as microorganisms obtain resistance by altering their metabolic events and genetic edifice. Nanotechnology is anticipated to open certain novel aspects to combat and preclude diseases using atomic scale tailored materials (Afreen *et al.,* 2011). Nanotechnology is presently labouring as a tool to target the darkest avenues of medical fields to control diseases induced by MDR microbes (Singh *et al.,* 2014). The present study focuses on the biosynthesis, characterization and antibacterial activity of silver nanoparticles (Ag NPs) produced in the presence of aqueous leaf extract of *Eucalyptus gunni.*

Materials and Methods

Collection of Plant Materials

Fresh healthy leaves of *Eucalyptus gunni* were collected from in and around Pondicherry. The fresh and mature leaves *Eucalyptus gunni* were used for the present study. Leaves were separated from the tree and washed thoroughly with running water to remove any dirt or debris on the surface and finally rinsed briefly in deionized water before use.

Chemicals used

Silver nitrate, a Silver precursor was procured from Sigma Aldrich chemicals Ltd, Bangalore India. All the solutions were freshly prepared using sterile distilled water. All the glass wares used in experimental procedures were properly sterilized before use.

Preparation of leaf extract

30 g of fresh *Eucalyptus gunni* leaves were thoroughly washed in running tap water. Further the leaves were cut in to small pieces using sterile scalpels. The leaves along with 20 ml sterile water were crushed with a pre-sterilized pestle and mortar to get a paste like consistency. The extract was then filtered through whatman filter paper No. 1 and stored for further work.

Biosynthesis of Ag NPs from aqueous leaf extract of *Eucalyptus gunni*

15 ml aqueous leaf extract of *Eucalyptus gunni* was added into 200 ml of aqueous solution of 1 mM silver nitrate and kept for 15-20 min for aiding the synthesis of Ag NPs. aqueous leaf extract acts as reducing and stabilizing agent. The prepared Ag NPs were further characterized (Karthika *et al.,* 2015).

Characterization of synthesized Ag NPs

The bioreduction of ions was scrutinized by intervallic sampling of the aqueous solution after 20 times dilution and quantified using UV–Vis spectra. Samples were observed as a function of time of reaction using Shimadzu spectrophotometer in the 100– 800 nm range operated at a resolution of 1 nm. The condensed solution was centrifuged at 8000 rpm for 30 min and resulting supernatant was discarded, while the pellet acquired was redispersed in deionized water. Centrifugation was repeated three times to wash off any adsorbed substances on the surface of the synthesized NPs. Thus obtained purified and dried pellet of synthesized Ag NPs were subjected to X-ray diffraction (XRD) analysis.

The particle size and morphology of Ag NPs were examined using Electron microscopic observations. SEM measurements were performed on a JEOL JSM 6390 instrument operated at an accelerating voltage at 15kV.

Antibacterial activity of Ag NPs nanoparticles

The antibacterial effect of Ag NPs nanoparticles were examined by disc diffusion method against some selected pathogens viz., *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa* collected from lab stock. Muller Hinton agar was prepared and poured onto the sterile petriplates.

After solidification, 2 wells were cut (for test and control) and each culture was swabbed individually on the respective plates. The synthesized Ag NPs nanoparticles were diluted with distilled water (15µg/ml) and placed onto each wells and incubated for 24 h. Following incubation, the zone of inhibition against Ag NPs was observed and measured (Tharanya *et al.,* 2015).

Results and Discussion

Nanobiotechnology combines biological principles with physical and chemical procedures to generate nano-sized particles with specific functions. Nanobiotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation.

Green Synthesis of AgNPs

Green synthesis of silver nanoparticles was carried out by using the aqueous leaf extract of *Eucalyptus gunni*. On mixing aqueous leaf extract of *Eucalyptus gunni* with silver nitrate solution (1mM), a change in the color from green to dark brown was observed. The obtained result was in concurrence with the results reported by many researchers (Kartika *et al.,* 2015; Durairasu *et al.,* 2017). The brown color confirms the reduction of Ag^+ indicating the formation of Ag nanoparticles. Similarly many researchers reported the synthesis of Nanoparticles using plant sources (Rajamohamed *et al.,* 2022; Kabeerdass *et al.,* 2022a; Kabeerdass *et al.,* 2022b).

Characterization of Ag Nanoparticles

UV Spectroscopic Analysis

The onset wavelength of the optical absorption for uncapped Ag NPs was observed at 280 nm in UV–vis spectroscopy, representing the development of nanoparticles (Fig.1). The free-electron oscillates and produces charges over the surface of nanoparticles under electromagnetic radiations as a result of the SPR effect (Silva *et al.,* 2019).

X-ray Diffraction Analysis

XRD is a characterization methodology for measuring the crystallinity of the AgNPs. The crystal structure of the Ag NPs was analyzed by X-ray diffractometer. The biosynthesis of Ag nanoparticles using the aqueous leaf extract of *Eucalyptus gunni* was sustained by X-ray diffraction dimensions. XRD analysis for the synthesized Ag NPs showed distinct diffraction peaks at 25.46°, 31.91°, 37.16°, 38.72°, 48.13°, 54.07°, and 62.86° indexed to the planes 110, 101, 111, 211, and 220 respectively (Fig. 2). Similarly, XRD characterization method has been used by different researches to determine the crystallinity of Ag NPs.

FTIR of TiO_2 Nanoparticles

FTIR spectroscopy is highly explored to investigating the chemical configuration of the NPs. FTIR analysis of synthesized Ag NPs using aqueous leaf extract of *Eucalyptus gunni* is represented in Fig. 3.

The synthesized Ag NPs displayed the presence of bands due to hydroxy group, H-bonded OH stretch (3271 cm^{-1}), methylene C-H asym./sym. stretch (2916 cm^{-1}), secondary amine, NH bend (1629 cm^{-1}), phenol or tertiary alcohol, OH bend (1336 cm^{-1}), cyclic ethers of large rings, C-O stretch (1026 cm^{-1}) and thioethers, CH_3-S-, C-S stretch (823 cm^{-1}). AgNPs characterization through FTIR is done to identify the molecules which act as coating and stabilizing agents and also to detect the reduction of silver ions (Silva *et al.*, 2019).

Table.1 Antibacterial activity of Ag nanoparticles against the selected bacterial isolates

S. No.	Bacterial strains	Zone of Inhibition
1.	*Staphylococcus aureus*	16 ± 0.2 mm
2.	*Bacillus subtilis*	11 ± 0.4 mm
3.	*Pseudomonas aeruginosa*	12 ± 0.6 mm
4.	*Escherichia coli*	19 ± 0.2 mm

Fig.1 UV-Vis absorption spectrum of Ag NPs

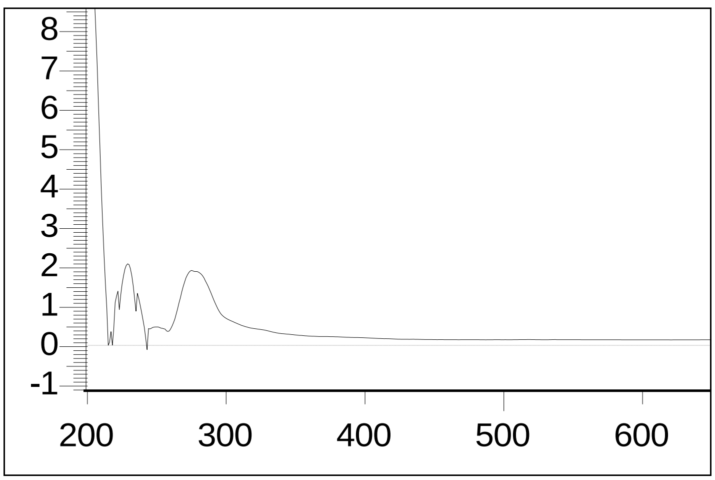


Fig.2 FTIR analysis of Ag NPs

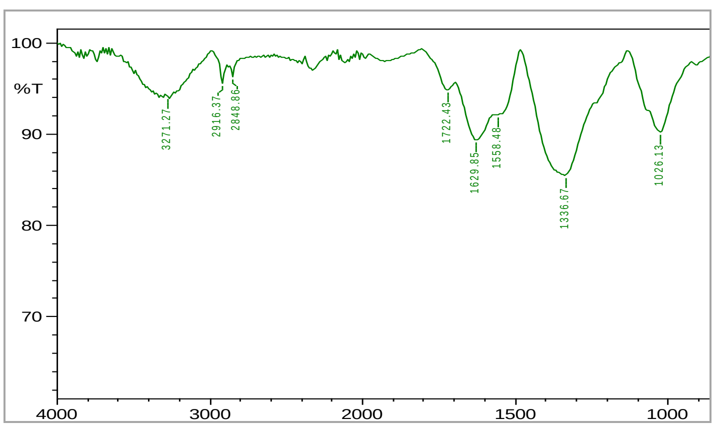


Fig.3 XRD analysis of Ag Nanoparticles

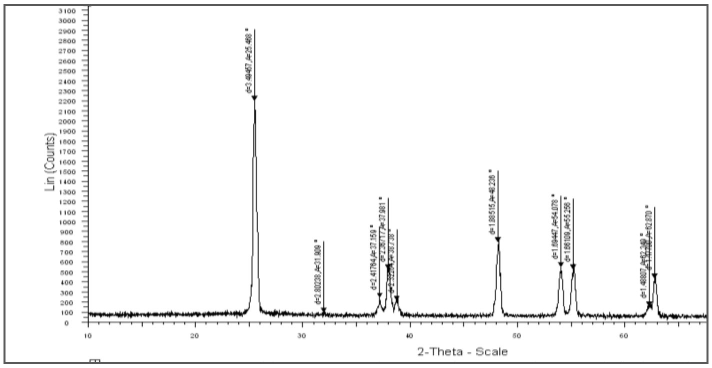


Fig.4 SEM analysis of Ag Nanoparticles

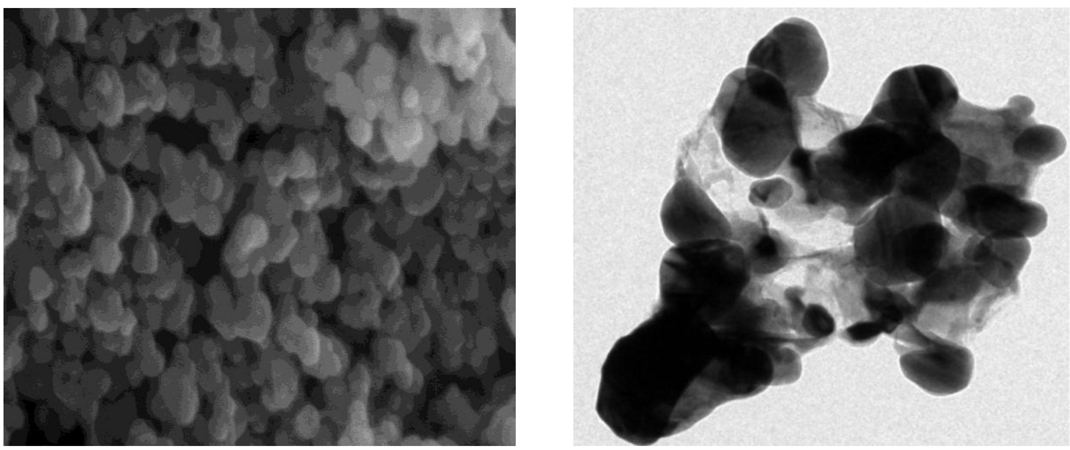
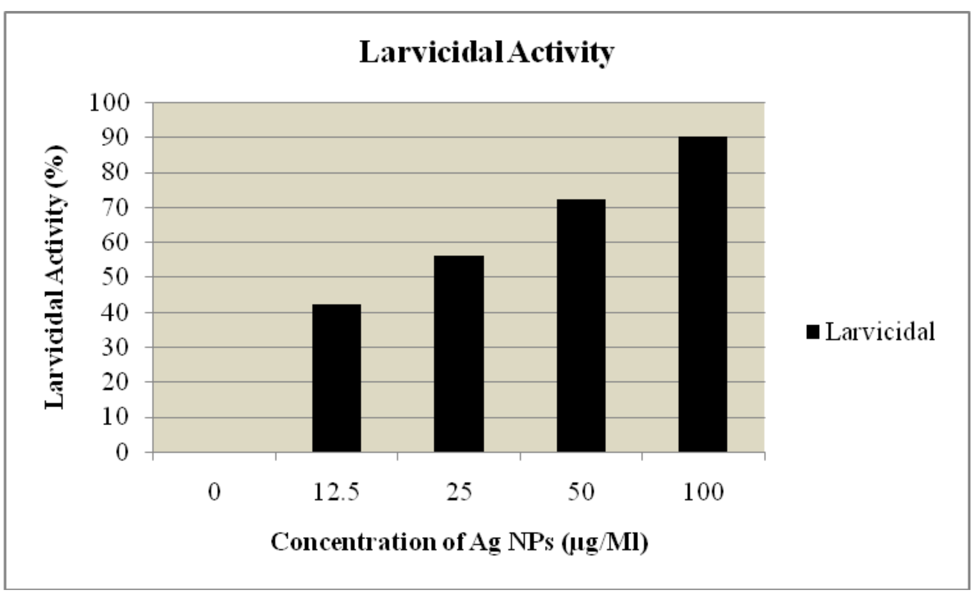


Fig.5 Larvicidal activity of Ag nanoparticles



SEM Analysis

The SEM images of Silver nanoparticles obtained with the aqueous leaf extract of *Eucalyptus gunni* are represented in (Fig. 4). The formation of Silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the shapes were uniformed spherical and ellipsoidal. The nanoparticles were arranged overlapping each other. Similar reports were presented by Durairasu *et al.*, (2017).

Antibacterial activity of Silver Nanoparticles

Silver nanoparticles (AgNPs) are well known for its antagonistic effects towards microbial pathogens in the past decades (Cho *et al.*, 2005). Antibacterial activity of AgNPs can be explored in biomedical applications such as ailment of various infectious diseases, prevention of bacterial colonization on catheters (Singh *et al.*, 2014). Antibacterial activity of biogenic silver Nanoparticles was investigated (Table 1).

Among the above mentioned, biogenic Ag NPs exerted remarkable antagonistic activity against *Escherichia coli* (19 mm) which was followed by *Staphylococcus aureus* (16 mm), *Pseudomonas aeruginosa* (12 mm), and *Bacillus subtilis* (11 mm). Silver ions cause the release of K^+ ions from bacteria; thus, the bacterial plasma or cytoplasmic membrane, which is associated with many important enzymes and DNA, is an important target site of silver ions (Kim *et al.*, 2011). Velmurugan *et al.*, (2022) reported the usage of *B. subtilis* and *P. aeruginosa* as model bacterial strains to examine the anti-bacterial property of AgNPs, AuNPs and Ag-AuBNPs.

Larvicidal activity of Silver NPs against C*ulex* mosquito

Increasing concentrations of silver NPs synthesized using aqueous leaf extract of *Eucalyptus gunni* ranging from 10 µg/ml – 100 µg/ml was investigated for their larvicidal activity against C*ulex* mosquito. Larvicidal activity amplified with the elevation in concentration of nanoparticles, reaching maximum level (90 %) in the presence of 100 µg/ml of Ag NP s. In contrast, 12.5 µg/ml of the silver nanoparticles exhibited only 20 % of larvicidal activity (Fig. 5).

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