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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *PLUMBAGO ZEYLANICA* LEAF EXTRACT AND THEIR ANTIBACTERIAL, ANTIFUNGALAND LARVICIDAL ACTIVITIES

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ABSTRACT: The metal nanoparticles have attention among researchers who wish to use them for development of new generation antimicrobial compounds. Silver nanoparticles are one of the widely used nanoparticles due to their antimicrobial properties. The present work deals with the Eco-friendly green synthesis of silver nanoparticles using leaf extract of *Plumbago zeylanica,* Characterization and their biological applications. The synthesized silver nanoparticles characterized using techniques such as UV-visible spectrophotometry to determine the presence of silver nanoparticles, a peak at 420nm was obtained and scanning electron microscopy to determine its size and shape, it confirmed the shape of the nanoparticles are spherical. The synthesized AgNPs were tested for antibacterial, antifungal and larvicidal activities. The AgNPs showed significant biological properties. The results of this work suggest that the silver nanoparticles synthesized from the leaf extract of *Plumbago zeylanica* possess antibacterial activity on both gram-positive (*S. aureus)* and gram-negative (*E. coli*) bacterial strains, antifungal activity on *Aspergillus niger* and Larvicidal activity against *Culicidae Larvae*. In conclusion, the green synthesized AgNPs proved to be an excellent antimicrobial and larvicidal agent.

INTRODUCTION: Metal nanoparticles synthesis has emerged in material science due to its applications in medicine, drug delivery, agriculture environmental bioremediation, and information storage due to its unique optical, catalytic, magnetic, electronic, and antimicrobial properties [1]. Silver is preferred for the synthesis of nanoparticles due to its antibacterial and catalytic characteristics and is non-toxic to humans in comparison with other metals. Nanocrystalline silver particles are often used in high-sensitivity bimolecular detection, therapeutics, catalysis, antimicrobials, and microelectronics.

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Silver nanoparticles can be synthesized by physical, chemical, and biological methods [2]. There are different types of metals available for the synthesis of nanoparticles using plant extract such as gold, silver, copper, zirconium, titanium, and ferrous each metal has unique functions like antibacterial, antifungal, antiviral, larvicidal, dye decolourization, antioxidant, drug delivery, cancer detection, cancer treating activities [3].

The nanoparticles after synthesis, it is characterized using UV-*vis* spectrometer, and scanning electron microscopy to know the absorbance, size, shape, net surface charge, and nature of bonds [4].

In view of the biological importance of AgNPs, the present work was aimed to synthesize the silver nanoparticles from leaf extract of *Plumbago zeylanica* to assess their antimicrobial and larvicidal activities.

MATERIALS AND METHODS:

Collection of Plant: The plant *Plumbago zeylanica* was collected from the Nallamala forest Kurnool district, Andhra Pradesh, India.

Phytochemical Analysis of Plant Leaf Extract: The photochemical constituents in the leaf extract of *Plumbago zeylanica* were studied (Latheef *et al.* 2024) [5].

Preparation of Plant Leaf Extract: 10grams of *Plumbago zeylanica* leaves were grounded in motor and pestle, and made up to 100ml with distilled water. The prepared plant leaf extract was used for the synthesis of AgNPs.

Synthesis of Silver Nanoparticles: The silver nanoparticles were synthesized by using the green synthesis method [6-11]. One mM of $AgNO_3$was transferred to a flask containing 100mL of leaf extract and incubated in room temperature.

Characterization of AgNPs:

UV-visible Spectrophotometry: UV-Vis Spectrometer observed the absorbance peak of AgNPs. The readings were taken from 350 to 490nm and a graph was plotted with the optical density of the AgNPs [12].

Scanning Electron Microscopy: the AgNPs size and shapes were measured by scanning electron microscope [13].

Antibacterial Activity: The antibacterial activity of AgNPs was assessed by well diffusion method [14]. Both gram-positive and gram-negative bacteria such as *Staphylococcus aureus* and *E. coli* were used to determine the bactericidal activity of silver nanoparticles. For this 100µL of the active bacterial cultures were poured onto the nutrient agar medium and wells were made using a sterile borer. AgNP suspensions with different concentrations were loaded into the wells, later the plates were incubated in an incubator for 24–48 hours at 37°C. After incubation the zone of inhibition was measured [15, 16].

Antifungal Activity: The antifungal activity of AgNPs tested on the fungi *Aspergillus niger* grown on PDA media and activity was studied by well diffusion method [14]. *A. niger* spore suspension was poured on the potato dextrose Agar medium with various concentrations of AgNPs loaded into the wells that were made using a sterile borer and incubated at room temperature for 4-6 days, the zone of inhibition was measured [17, 18].

Larvicidal Activity: To evaluate the larvicidal activity of AgNPs, *Culicidae larvae* were collected from sewers around local area. The collected larvae were carefully transferred to glass plates containing fresh water with different concentrations of AgNP suspension and incubated in room temperature. After incubation the mortality rate was observed and noted the mortality rate was calculated by using the formula [19, 20].

Mortality Rate = (No. of Larvae Dead) / (No. of Larvae Incubated) ×100)

RESULT AND DISCUSSION:

Collection of Plant: The plant *Plumbago zeylanica* was collected from the Nallamala forest, Kurnool district, Andhra Pradesh, India **Fig. 1.**

FIG. 1: *PLUMBAGO ZEYLANICA*

Preparation of Plant leaf Extract: Ten grams of *Plumbago zeylanica* leaf were taken and washed with tap water and grounded in mortar and pestle and made up 100ml with distilled water **Fig. 2.**

FIG. 2: LEAF EXTRACT OF *PLUMBAGO ZEYLANICA*

Synthesis of Silver Nanoparticles: 1mM of silver nitrate was prepared in 100 mL of leaf extract and allowed to react at room temperature. After

incubation (30 minutes) the colour of the filtrate was changed from green to dark brown colour as shown in **Fig. 3**. Formation of the brown colour is an indication of presence of AgNPs. It may be due to the reduction of silver ions in the filtrate. Similar reports onmetallic nanoparticles synthesis from various plants Frankincense resin, Narasimha *et al.*, (2021) [7]. Aervalanata, Seku *et al.*, (2022) [8], microwave, Ganesh *et al.*, (2021) [9], *Sesbania grandiflora* leaf extract, Vaishnavi *et al.*, (2020) [10], *Pterocarpus santalinus*, Seku *et al.*, (2020) [11].

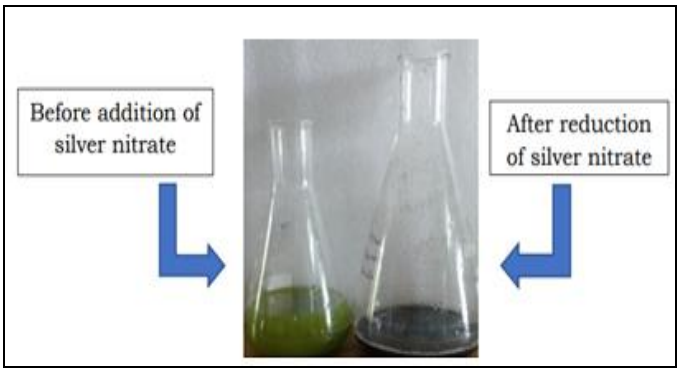

FIG. 3: BIO REDUCTION OF $AGNO_3$

Characterization of AgNPs:

UV-visible Spectrophotometry: The UV spectrum of AgNPs shows the maximum peak at 430nm, it is thought to be the absorbance range of AgNPs **Fig. 4.** The reduction of silver nanoparticles was able to be observed at this wavelength due to its surface Plasmon Resonance (SPR). Similar reports of UV spectrum were also observed in the work of Bhagavanth Reddy *et al.*, (2022) [12].

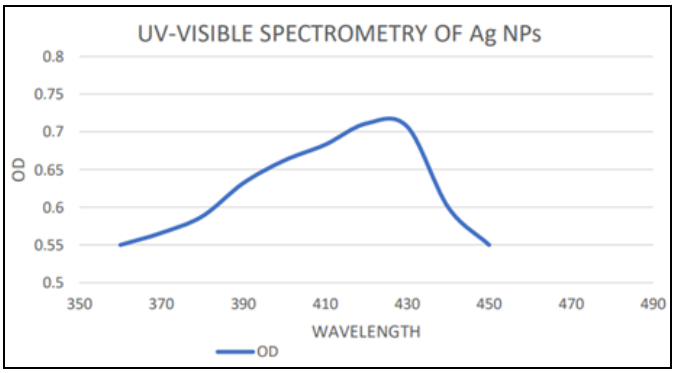

FIG. 4: UV SPECTRUM OF AGNPS

Scanning Electron Microscopy: The size and shape of the silver nanoparticles were confirmed by Scanning Electron microscopy. In this study, the size of the silver nanoparticles was observed from 91nm to 99nm and the shape was spherical **Fig. 5**. Some of them were well dispersed and some were clumped together. Similarly, Rahul *et al.*, (2021) [13], conducted the scanning electron microscopy of silver nanoparticles and reported that the size of AgNPs was ranging from 32-48nm.

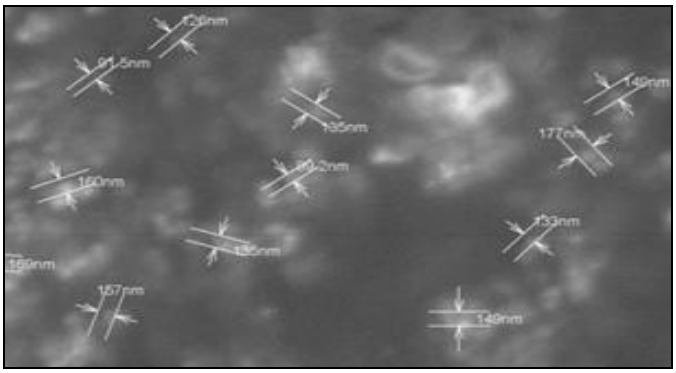

FIG. 5: SEM IMAGE OF AGNPS

Antibacterial Activity: The antibacterial activity of AgNPs was studied and the zone of inhibition was measured [14]. With increasing the concentration of AgNPs the size of the zone was also increased. At low concentration of AgNPs the zone of inhibition was 0.7 to 1.5 cm and at high concentration (75µl) it was 0.7 to 2.0 cm **Table 1**, **Fig. 6**. Similar results were reported by Enerelt *et al.*, (2021) [15,] Antibacterial activity of AgNPs from *Carduus crispus* and reported that silver nanoparticles can inhibit both $Gram^{-ve}$ and $Gram^{+ve}$ bacteria efficiently. Tamara Bruna (2021) [16] conducted antibacterial activity of AgNPs and concluded that combination of medicine and nanotechnology can bring the ability to prevent infections or eliminate selective pathogens.

TABLE 1: ANTIBACTERIAL ACTIVITY OF AGNPS

Sample (µl)	Zone of Inhibition (cm)	
	Staphylococcus aureus	***Escherichia coli***
25	1.5	0.7
50	1.7	1.0
75	2.0	0.7
Streptomycin (Control)	0.9	0.9

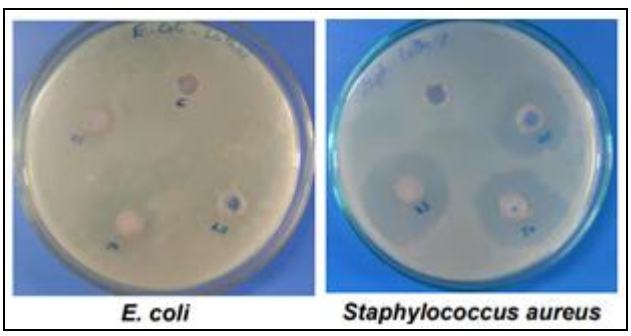

FIG. 6: ANTIBACTERIAL ACTIVITY OF AGNPS

Antifungal Activity: Fungicidal activity of AgNPs was determined on A. *niger* and the zone of inhibition was 0.2-0.4cm. As the AgNPs concentration increased the zone of inhibition also increased **Table 2**. Similar reports of fungicidal activity were observed by the reports of Hashem *et al.,* (2022) [17], performed antifungal activity of silver nanoparticles from *B. thuringiensis* MAE 6 culture and concluded that the AgNPs exhibited zone of inhibition from 1.6 to 1.9cm. Arsène *et al.,* (2023) [18], assessed the antifungal activity of silver nanoparticles from *Aloe vera*.

TABLE 2: ANTIFUNGAL ACTIVITY OF AGNPS

Conc. of Ag NPs (µl)	Zone of Inhibition (cm)
50	0.2
75	0.2
100	0.4

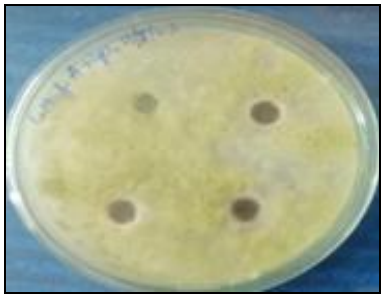

FIG. 7: ANTIFUNGAL ACTIVITY OF AGNPS

Larvicidal Activity: The AgNPs showed good larvicidal activity on *Culicidae larvae*. With increasing the concentration of AgNPs and incubation time the mortality rate of the larvae increased **Fig. 8, Table 3.** After 10 hours of incubation, most of the larvae were killed at the end of 24 hours of incubation all the larvae were killed. The percentage of mortality was 20 to 100%. Similar reports were Amarasingh *et al.,* (2020) [19], performed the larvicidal activity of AgNPs from *Annonaglabra* plant and reported that the larvicidal activity of silver nanoparticles increased as the concentration increased. Govindan *et al.,* (2020) [20], worked on larvicidal activity of silver nanoparticles synthesized from *Plumbago auriculata* and reported that the silver nanoparticles exhibited potential larvicidal activity.

TABLE 3: LARVICIDAL ACTIVITY OF AGNPS

No. of larvae	Conc. AgNPs (µl)	Mortality Rate (%)
10	20	20
10	40	50
10	60	70
10	80	100

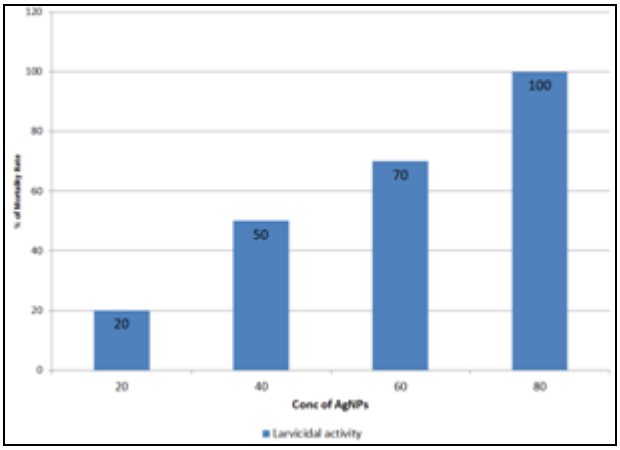

FIG. 8: LARVICIDAL ACTIVITY OF AGNPS

CONCLUSION: In the present work, the silver nanoparticles were synthesized using *Plumbago zeylanica* leaf extracts through an eco-friendly cost-effective method. The synthesized silver nanoparticles were characterized. The Silver nanoparticles exhibited antibacterial, antifungal, and anti-larval properties. The AgNPs could be used as antimicrobial and larvicidal agent.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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