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Green synthesis of ZnO nanoparticles using the leaf extract of Lavandula angustifolia and evaluation of their antibacterial activity against human pathogens

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***Abstract*---**The present study was aimed to the biosynthesis of Zinc oxide nanoparticles using leaf extract of *Lavandula angustifolia.* The nanoparticles were characterized by different techniques like UV-Visible spectroscopy, FESEM analysis, and X-ray diffraction. From the UV-Visible spectra analysis, it was observed the peak obtained at 350 nm confirmed the biosynthesis of LA-ZnONPs. The shape and size of LA-ZnONPs were confirmed by FESEM analysis, and from FESEM analysis it was found that the LA-ZnONPs were truncated and triangular in shape with an average size of 61.52 nm respectively. Further, the XRD analysis showed the LA-ZnONPs were crystalline in nature. By using well diffusion assay, the antibacterial activity of LA-ZnONPs was investigated against gram-positive *S. aureus (ATCC 25923)* and gram-negative *E. coli (ATCC 11229)* pathogens in a dose-

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dependent manner. The LA-ZnONPs exhibited excellent antibacterial activity against both the pathogens. The LA-ZnONPs thus possessed excellent antibacterial activity against human pathogens and can be used in drug development.

***Keywords*---**ZnONPs, L. angustifolia, well diffusion assay, FESEM, pathogens.

Introduction

Metal oxide nanoparticles have a wide range of applications due to their unique properties like electrical, thermal, optical, physical, and chemical (Chaudhuri et al.,2017). Among the metal oxide nanoparticles, Zinc oxide nanoparticles have been investigated extensively due to their wide range of uses in medical, cancer, antibacterial, antifungal, antidiabetic, and biosensors (Umar et al., 2019). Zinc oxide nanoparticles have a bandgap of 3.3eV, high excitation energy, catalytic, semiconducting, photochemical, and UV filtering properties (Rajendran et al.,2021). Zinc oxide nanoparticles are synthesized using a variety of processes, including physical, chemical, and biological. Physical and chemical procedures have drawbacks such as time-consuming, hazardous, expensive, and unsafe. (Agarwal and Shanmugam,2019). In recent years, researchers have shown a growing interest in the biosynthesis ZnONPs via a greener approach by utilizing plant extracts in comparison to physical and chemical methods. Nanoparticles made from plant extracts are more environmentally friendly, safer, and less harmful (Mahendiran et al.,2017). Nanoparticles synthesis from plant extracts is rich in secondary metabolites like phenols, alkaloids, and tannins which act as capping and reducing agents in the bio-reduction nanoparticles (Sana et al., 2020). There are various reports, in which leaf extract mediated synthesis ZnONPs were carried out, *Bergenia ciliate* (Rather et al.,2021), *Crotalaria verrucosa* (Sana et al., 2020), *Cynara scolymus* (Rajapriya et al.,2020), and *Bauhinia tomentosa (*Sharmila et al.,2018).

Lavandula angustifolia belongs to the family of *Lamiaceae*. It has been used for medicinal purposes since ancient times. It is a perennial evergreen plant. *Lavandula angustifolia* is commonly called as English lavender. *L.angustifolia* oil contains many essential compounds, and possesses significant antibacterial and antioxidant properties as well as beneficial effects on the nervous and digestive system (Prusinowska and Smigielski.,2014). The aim of the present study was to the biosynthesis of Zinc oxide nanoparticles using the leaf extract of *L. angustifolia* and evaluation of its antibacterial activity against human pathogens

Materials and methods

Zinc acetate dihydrate, sodium hydroxide pellets, and other chemicals were all procured from Hi-media Mumbai. All the chemicals were of high-grade purity. The bacterial strains were obtained from the Unit of Biocontrol and metabolites laboratory Centre for advanced studies at the botany University of Madras. The

plant leaves of *L. angustifolia* were collected from Srinagar, Jammu, and Kashmir, and authenticated at the University of Kashmir Department of Botany.

Preparation of leaf extract

The leaves of *L. angustifolia* were dried in the shade after being thoroughly washed with water and then double-distilled water.4 grams of leaf powder were accurately added into the 500 mL flask containing 300 mL distilled water and kept for boiling in a water bath at 60°C for 30 minutes. The solution was cooled and filtered by using Whatman's no.1 filter paper. The purified filtrate obtained was used for the biosynthesis of LA-ZnONPs.

Synthesis of LA-ZnONPs

The biosynthesis of LA-ZnONPs was carried out as per our already reported work with slight modifications (Ahmed Rather et al., 2021) ,60 mL of 0.01M zinc acetate dihydrate were added to the 2mL of aqueous extract of *L. angustifolia.* The pH of the solution was adjusted to12, by adding dropwise 2M sodium hydroxide solution. Later on, the solution was kept in a magnetic stirrer for two hours. The change in color from brownish red to yellowish white indicated the formation of ZnONPs. The solution was then centrifuged at 12,000 for 15 minutes. Finally, the pellet was washed with ethanol followed by Milli Q water to remove the impurities. The purified pellet was dried in a hot-air oven for 12 hours at 60°C. The dried pellet was then crushed into fine LA-ZnONPs powder and used for further analysis.

Characterization of LA-ZnONPs

The LA-ZnONPs synthesized from *L. angustifolia* were characterized by different techniques, like UV-Visible spectroscopy, Field Emission Scanning microscopy (FESEM) and X-ray diffraction (XRD) analysis. The absorption spectra of LA-ZnONPs were measured by using UV-Visible spectroscopy (Model SHIMADZU UV-1800 JAPAN) in the UV range from 200-800 nm. The size and the morphology of LA-ZnONPs were confirmed by using FE-SEM model FEI Quanta. The crystalline nature of LA-ZnONPs was carried out by using XRD (Malvern Pananalytical Ltd., UK) analysis

Antibacterial assay

According to Magaldi et al.,2004 the biogenic LA-ZnONPs were screened for their antibacterial studies against human pathogens by well diffusion assay. Two different strains of bacteria gram-positive *S. aureus (ATCC 25923) and gram - negative E. coli (ATCC 11229* were used. To determine the antibacterial activities of LA-ZnONPs Muller Hinton Agar (MHA) medium were prepared and poured into sterile Petri plates. After the solidification, both gram-positive and gram-negative strains at a concentration of 10^4 to 10^6 cfu/mL were uniformly inoculated into the petri plates by using sterile cotton. Four different concentrations of LA-ZnONPs were taken starting from 25 µl ,50 µl, 75 µl and 100 µl and poured into the respective wells. Amphilox and Ciprofloxacin were used as positive controls against gram positive and gram-negative pathogens. DMSO was used as a

negative control. After 24 h of incubation at 37°C the zone of inhibition was measured in mm. All the experiments were conducted in triplicates and the results were expressed as mean ± SD.

Results and Discussion

The green synthesis of LA-ZnONPs was observed by the change in color from brownish red to yellowish white when Zinc acetate dihydrate was added as shown in figure 1.

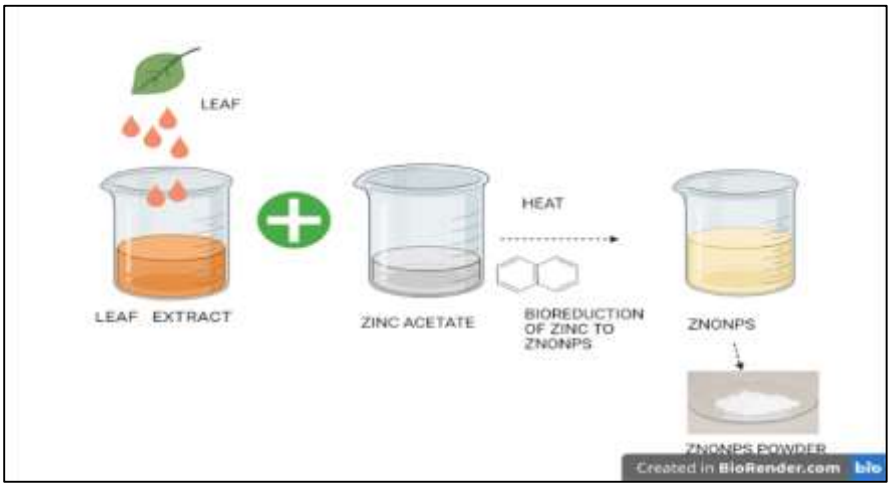

Figure 1. Process of biosynthesis of LA-ZnONPs from *L. angustifolia* leaf extract

The green synthesis of LA-ZnONPs were first characterized by UV-Visible spectroscopy in the UV range from 200-800 nm. From the UV-Visible spectra analysis, the SPR peak found at 350 nm, confirmed the biosynthesis of LA-ZnONPs (figure 2). The findings are consistent with previous research in which ZnONPs were found to have a UV Visible peak at 350 nm employing the leaf extract of *Calotropis gigantea*, indicating the formation of ZnONPs (Chaudhuri et al.,2017).

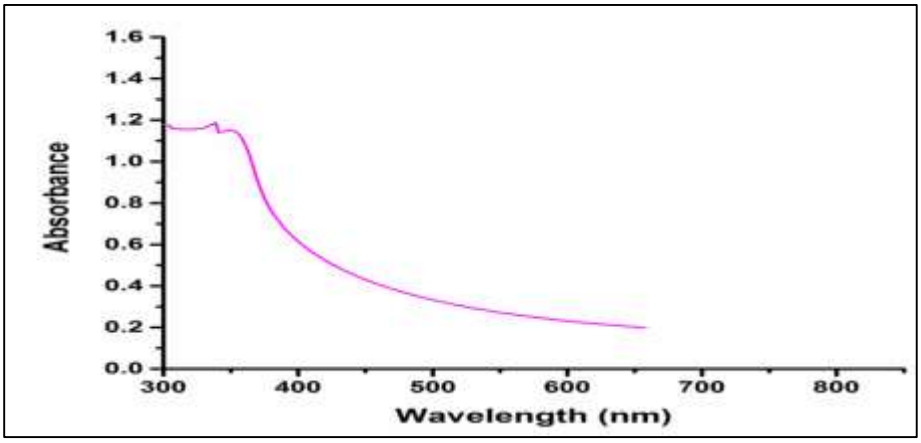

Figure.2 UV-Visible spectra analysis of LA-ZnONPs

From the FESEM analysis, it was observed that the LA-ZnONPs were formed in aggregates with truncated and triangular morphology (Rather et al.,2021). The size of the LA-ZnONPs was found to be 61.1 to 77.3 nm, with average size 61.52 nm (figure 3) respectively.

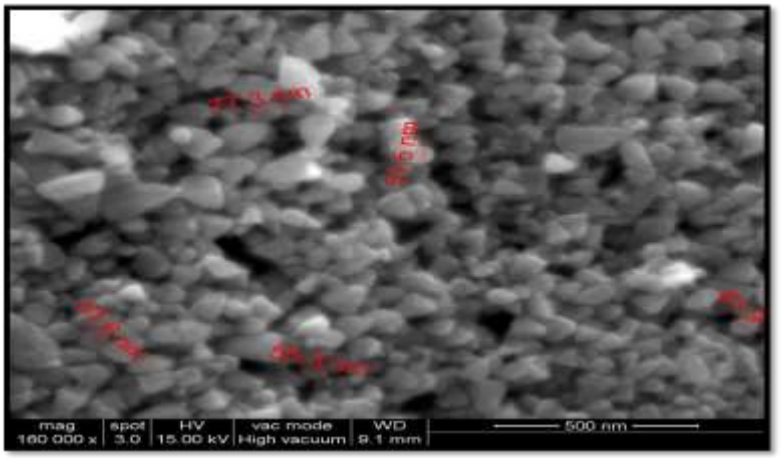

Figure 3 FESEM analysis of *L. angustifolia* mediated LA-ZnONPs

XRD analysis LA-LA-ZnONPs

The crystalline nature of the *L. angustifolia* mediated ZnONPs were analyzed by X-ray diffraction (XRD). Different peaks were observed at 2θ 31.722°, 34.370°, 36.224°, 47.471°, 56.54°, 62.790°, 66.361°, 67.893°, 68.989°, 72.471°, 76.881°,81.325°, 89.518°, 92.723°, 95.226° and 98. 522° corresponds to (100), (002), (101), (102), (110), (103), (112), (201), (004), (202), (104), (203), (210), (211) and (114) planes respectively. The XRD results were (figure 4) obtained is almost similar to the already published work in which *Cynara scolymus* leaves were used to synthesis of ZnONPs (Rajapriya et al.,2020), specifies the hexagonal wurtiz phase of LA-ZnONPs, in agreement with JCPSS file no.36-1451.

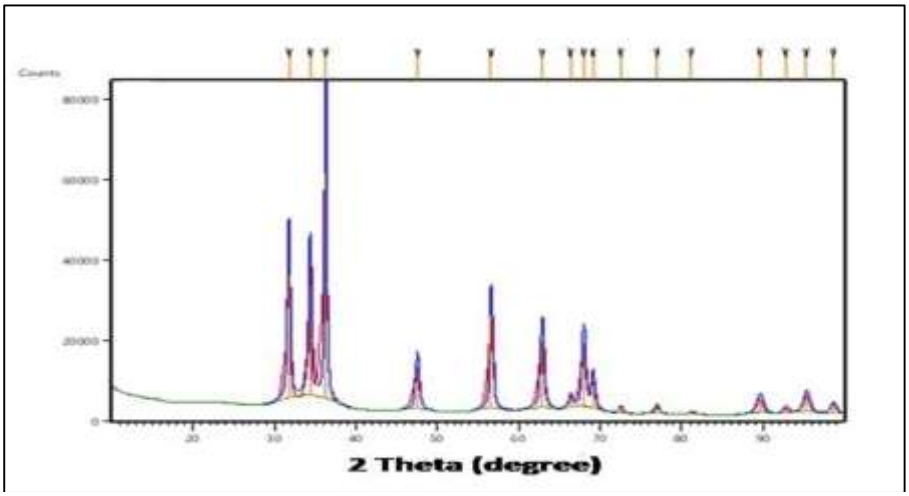

Figure. 4 XRD analysis of L-A LA-ZnONPs

Antibacterial activity of LA-LA-ZnONPs

The bactericidal activity of LA-ZnONPs were investigated against both gram-positive and gram-negative human pathogens. Both the strains of bacteria gram-positive *S. aureus (ATCC-25923)* and gram-negative *E. coli (ATCC -11229)* showed excellent antibacterial activity, on dose dependent manner as shown in (figure 5). The study is supported by previously reported work in which ZnONPs showed excellent bactericidal activity towards both gram-positive and gram-negative pathogens. The antibacterial activity is attributed by the presence of carboxyl groups and amines on their cell surface which offers higher affinity for Zinc ions to bind these groups resulted in higher antibacterial activity (Chikkana et al.,2019).

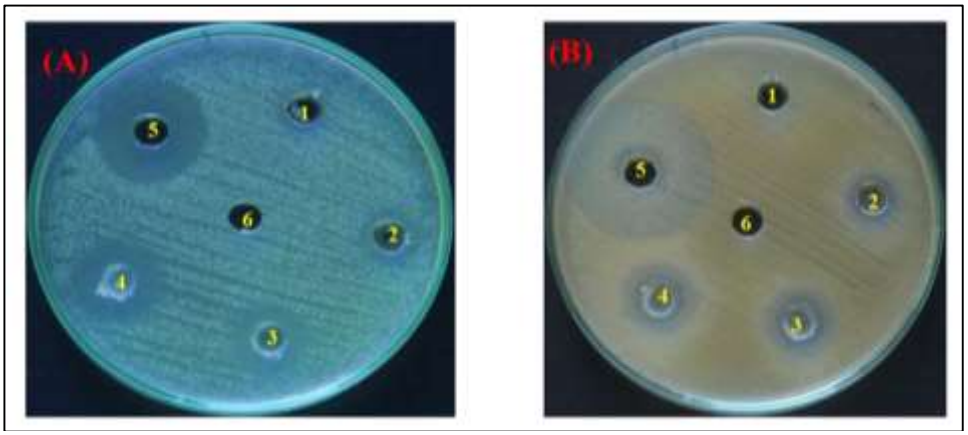

Figure 5. Antibacterial activity of LA-ZnONPs (A) S. aureus (B) E. coli; where 1:25µl ,2:50 µl,3:75 µl, 4: 100 µl, 5: Positive control and 6: Negative control

Table 1. Zone of inhibition (in mm) of green synthesized LA-ZnONPs

Sample Name	Pathogen Name	Concentration µg/mL				SD	NC
		25 µg	50 µg	75 µg	100 µg	10 µg	100 µg
LA-ZnONPs	*S. aureus (ATCC 25923)*	0 ± 0	12.66 ± 0.57	15.33 ± 0.57	20.33 ± 0.57	26 ± 1	0 ± 0
	E. coli (ATCC 11229)	0 ± 0	12.33 ± 0.57	14.66 ± 0.57	15 ± 0	33.66 ± 0.57	0 ± 0

Conflict of interest: No conflict of interest among the authors

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