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Green synthesis of zinc oxide nanoparticles (Zno-Nps) *Ailanthus excelsa* Roxb. stem bark extract and its antibacterial activity

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Abstract

Green nanoparticle synthesis from plant fruits aqueous extracts has been developed for a variety of applications. Zinc oxide nanoparticles by green synthesis method have recently piqued curiosity of researchers as well as scientists. Because of its in expensiveness, less harm to the ecosystem, non-toxicity, low cost and ability of act as acetrimide agent. Thus, the resent study reports synthesis of zinc oxide nanoparticles utilizing *Ailanthus excelsa* Roxb. stem bark methanolic extract. UV-visible absorption spectroscopy was used to evaluate a green chemistry produced zinc oxide nanoparticles. The antibacterial activity of synthesized ZnO-NPs against a bacterial strain of *Staphylococcus aureus* is demonstrated. It is observed that ZnO-NPs shows very potential antibacterial action by forming a zone of inhibition of 37 mm, it is a promising supplement which can replace hazardous chemical and physical antibacterial agents.

1. Introduction

Nanoparticles (NPs) because of their distinctive characteristics in size, shape, and surface area, have a variety of applications. The preparation of nanoparticles was done by using ecofriendly technique like green synthesis without using any harmful chemicals (Sudha *et al*., 2019). The use of NPs as catalytic agents provides the greatest advantage. NPs of noble metals including zinc oxide, gold, platinum are frequently use for consumer, medical goods in addition to catalyst. ZnO-NPs are one of the metal nanoparticles which is being tested in the search for more potent antibacterial agents.

Different morphologically structured zinc oxide nanoparticles (ZnO-NPs) having antibacterial action against bacteria, microfungi, and viruses were created employing a variety of synthetic methods, including: hydrothermal (Hush *et al*., 2010),wet chemical microemulsion (Yildirim, 2014), precipitation and co-precipitation route (An *et al*., 2012), solvothermal (Shamhari *et al*., 2018), surfactant assisted precipitation routes (Mahamuni *et al*., 2019), polyol chemistry, conventional methods using ecofriendly techniques like, sonochemical method (Khanna*et al.*, 2012), solgel method (Hasnidawani *et al*., 2016), microwave assisted combustion (Obopobjmt and Boe, 2013).

Because of the difficulties in scaling of the methods, the purification and separation of the nanoparticles out of the microemulsion and the energy needs, these approaches have significant drawbacks.

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The creation of simple, environmentally friendly processes for making zinc oxide nanoparticles is crucial and continues to be a difficult task for material scientists. According to literature review, plant extracted products have been recommended as a worth while alternative to synthetic techniques for producing zinc oxide nanoparticles (Awwad, 2020).

The size, shape and production processes of NPs hamper their antibacterial effectiveness. Due to the biomolecules that cap the NPs, those made from plant extract may have more potent antibacterial activity. Additionally, because of different plants produce a variety of different biomolecules, the antibacterial properties of NPs produced from various plants might vary. So, it is crucial to create nanoparticles from a fresh source, as doing so might improve their antibacterial effectiveness. Other benefits of employing plant extracts are their cost-effectiveness, lack of harmful chemicals during synthesis, and environmental friendliness (Iravani, 2011). It was observed that naturally synthesized ZnO-NPs have more potent antimicrobial and antifungal properties than chemically generated NPs (Bhabesh *et al*., 2022). Also, nowdays researchers have gain more interest over certain period of time in green synthesis of nano-particles, the reason is its physical characteristics (Tamanna and Madan, 2020).

From the ancient period of time, our ancestors used herbal plants for various antibacterial activity which is passed from generation-to-generation. That is the reason, pharmaceutical industries moving more towards the herbal medicinal product than chemical (Yamina *et al.*, 2019).

In this particular research work, the biological moiety was derived from the stem bark of the *A. excelsa* which is locally called as "the tree of heaven". It is big tree in size belonging to the family

Simaroubaceous and generally available in Indian and Shri Lankan region. This tree has many herbal and medicinal benefits like, in Indian region, it is used for cure of the fever. Its leaves and bark can be used as tonics after labor and the juice extracted from the leaves and bark is maximally consumed by the Konkani people as remedy to get rid any kind of pain. The methanolic extract was utilized to synthesize ZnO-NPs, which showed antibacterial properties that were further evaluated. For evaluation of synthesized green nanoparticles and to observe in their distinctive optical properties, UV- visible spectroscopy is generally used (Abubucker *et al*., 2021).

Figure 1: *Ailanthus excelsa* Roxb. stem.

2. Material and Methods

2.1 Chemicals and reagents

Methanol was used as a solvent for the Soxhlet extraction process, distilled water, zinc nitrate hexahydrate; *A. excelsa* stem bark (Hasegawa, 2001) was collected from the tree in the premises of Kamla Nehru College of Pharmacy, Butibori (Figure 1). The stem bark was ground to a coarse powder by a grinder, and ciprofloxacin as a standard drug for the comparison of the antibacterial study.

2.2 Preparation of *A. excelsa* stem bark extract

The *A. excelsa* plant's stem bark was crushed into a coarse powder before the Soxhlet extraction procedure was carried out. The thimble that was inserted into the Soxhlet device was filled with 50 g of coarse powder. Then, 80:20 solutions of methanol and purified water were added. It poured just enough to moisten the powder within the thimble before 100 ml of a solution of purified water and methanol was mixed. The solvent is then heated to reflux between 60 and 80ºC (Figure 2). For 48 h, the descent cycle (extraction) was completed. The methanolic extract was sun dried after being filtered through Whatman's filter paper and allowed to cool at ambient temperature (Figure 2).

Figure 2: Synthesis of zinc oxide nanoparticle.

2.3 Green synthesis of zinc oxide nanoparticles (ZnO-NPs)

In 100 ml of methanolic stem bark extract of *A. excelsa*, 2 g of zinc nitrate hex hydrate [Zn $(NO_3)2.6H_2O$] solubilized while being constantly stirred. After the mixture had completely dissolved, the mixture was vigorously agitated at 60°C for 2 h. After that it was allowed to cool at room temperature, and supernatant was discarded. The obtained NPs solutions underwent a 15 min centrifugation at 4500 rpm. After that, the raw pellets again suspended in purified water and kept at 4°C (Salem *et al*., 2015).

3. Characterization techniques

3.1 UV-Visible spectroscopic analysis

The obtained ZnO-NPs were characterized by UV-visible absorption spectroscopy. The mixture scanned at the wavelength (λ) range between 200 to 400 nm (Mohan and Renjanadevi, 2016). The biogenic production of ZnO-NPs was verified using UV-visible spectroscopy. The material was dissolved in deionized water for this common analysis, the UV-visible spectrum was ranged from 200 to 400 nm, as the λ_{max} observed was at 360 nm. The plant extract under went UV-vis analysis as well. The decrease in zinc metal salt for the generation of ZnO-NPs developed because of the presence of these molecules in the plant extract (Knapik *et al*., 1998).

3.2 Scanning electron microscopy and transmission electron microscopy

The ecological observation of green synthesized zinc oxide nanoparticles was done by the scanning electron microscopy in which the particles show different physical characteristics property like shape, size. The ecology (morphology) physical characteristics of ZnO nanoparticle, studied using transmission electron microscopy.

3.3 Energy-dispersive x-ray analysis

According to the scanning electron microscopy, the formation of zinc oxide nanoparticles was clearly visible .The other minor elements included in the ZnO-NPs were brought about by the occurrence of *A.excelsa* stem bark extract. Whereas the atomic weight of zinc was 37.16% and its weight present was 65.35%.

3.4 Potential

The zeta potential and size distribution of nanoparticle were analyzed by dynamic light scattering (DLS) technique. Its defines the typical measurement of surface charge on the particle and colloidal stability. 15 mv exhibiting suspensions are considered as stable collids. In studies, zeta potential of zinc oxide nanoparticle in purified water was measured as 22.1 mv, so it was considered as strongly anionic. The zeta potential verifies and supports the dispersion capacity of synthesized nanoparticles. The negative surface charge is due to the binding affinity of extract compounds with the NPs, conferring stability of the zinc oxide nanoparticles and alleviating the aggregation potential of the particles as shown in (Figure 3).

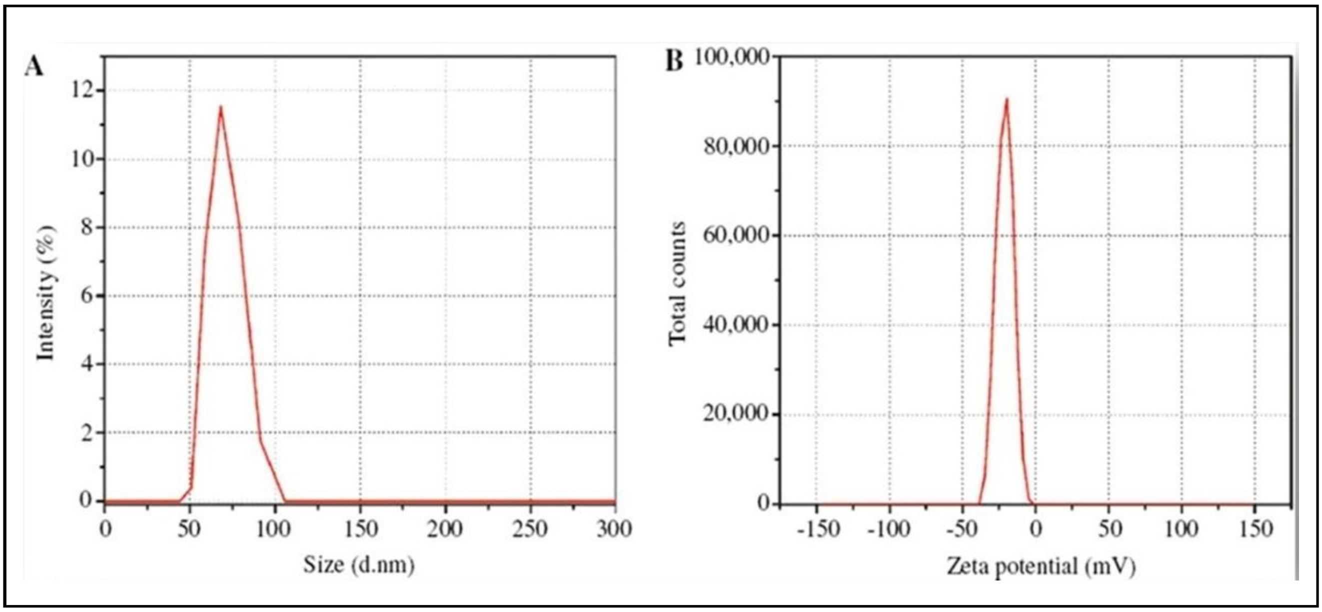


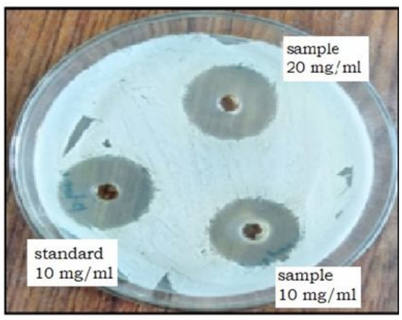
Figure 3: (A) Size distribution potential and (B) Potential distribution of synthesized ZnO-NPs.

3.5 Antibacterial activity

An assay that measures the bactericidal effectiveness of synthetic ZnO-NPs in agar wells was performed (Harathi *et al.,* 2017). Before use, all of the instruments and reagents were autoclaved. As a positive control, the standard antibiotic ciprofloxacin (Osonwa *et al.,* 2017), a pure medication, was employed. The way that this antibiotic works is by preventing bacterial growth. A fluorinated antibiotic called ciprofloxacin is used to treat bacterial infections. Inactivation of DNAgyrase and topoisomerase IV accounts for the majority of its antibacterial effect (Fournier *et al.*, 2000). Antimicrobial activity of green synthesized nanoparticles was performed by the well diffusion method (Swapna *et al.*, 2020).

The dormant *Staphylococcus aureus* bacterial isolates were sent from the Kamla Nehru College of Pharmacy's laboratory in Butibori. To assess the antibacterial properties of ZnO-NPs, they were stimulated inside the laboratory and used as test organisms. On an agar plate, three wells with a diameter of 6 mm were punctured using gel borer. With the use of sterile cotton swabs, the bacterial strain was uniformly wiped over the agar bed petri plate. Utilizing a micropipette, two wells on the plate were filled with 10 and 20 mg/ml of ZnO-NPs solution (Figure 4 A), respectively, with ciprofloxacin serving as the control in the other well. After being incubated at 37ºC for 24 h, the various zones of inhibition values were measured and evaluated (Manjunath *et al*., 2014).

(A) Dilutions made for spotting



(B): Zone of inhibition of standard drug and the sample.

Figure 4: ZnO-NPs against *S.aureus* bacteria.

4. Results and Discussion

4.1 Characterization of ZnO-NPs

Table 1: Particle size distribution

Run No.	Concentration of plant extract (ml)	Particle size distribution			
1	5	1	100	1	31
2	5	2	150	15	61
3	5	3	200	2	20
4	10	1	150	2	31
5	10	2	200	1	73
6	10	3	100	15	43
7	15	1	200	15	21
8	15	2	100	2	62
9	15	3	150	1	41

The findings revealed several peaks at various wavelengths between 200 and 520 nm. By measuring the spectrum, it was able possible to observed the optical characteristics of ZnO-NPs and observation was high absorption characterization peaks were in the range of 345 to 365 nm (Figure 5), which supported the development of ZnO-NPs.

Table 2: Absorbance of zinc oxide nanoparticles

Wavelength	Absorbance
203	1.016
352	2.939
396	0.794

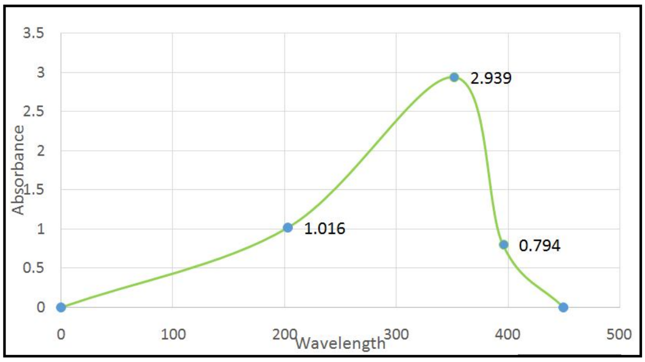


Figure 5: Spectrum of ZnO-NPs synthesized by green method.

4.2 Scanning electron microscopy

In this, it was observed that the particle shows cluster or lump form of structure. The transparency indicates that particles are available in equivalent that particles are available in equivalent or analogous nanoparticles. The size of the particle lies between 43.3-83.1 nm. The overlapping of particles occurs which leads to the rise in the size particle.

The occurrence of round shaped-hexadic particles, having grain size of 35.5 nm was found. The confirmation of zinc oxide nanoparticle was given by collating particle size acquired by TEM and X-ray diffraction process.

4.3 Energy-dispersive X-ray analysis (EDX)

The atomic weight of zinc was 37.16% and its weight present was 65.35%.

4.4 Antibacterial activity of ZnO-NPs

Table 3: Zone of inhibition

Bacterial strain	Concentration of zinc oxide nanoparticles (mg/ml)	Zone of inhibition
S. aureus	10	32
	20	35
	Concentration of standard ciprofloxacin (mg/m) 10	37

S. aureus was used in the disc diffusion technique test of the synthesized ZnO-NPs. The zone of inhibition against the bacterial strain expanded as ZnO-NP concentration zone.

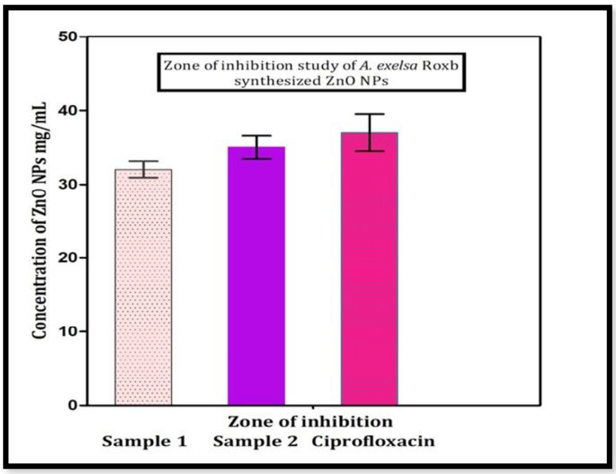


Figure 6: Antibacterial activity of the ZnO-NPs.

4. Conclusion

The current study looked at the green synthesis of ZnO-NPs utilizing a methanolic extract of the stem bark of *A. excelsa*. This synthesis's key benefits are its ease of use, economical method, and extensive production of ZnO-NPs. The examination of UV-visible absorption spectroscopy proved that ZnO-NPs had been formed. The produced nanoparticle shows promising antibacterial activity against the pathogenic bacterium, *S. aureus*. Based on the findings, it is concluded that green synthesis employing stem bark extract from *A. excelsa* is an effective nanoscale synthesis technique for ZnO-NPs, which may also be expanded for the production of other metal oxide nanoparticles.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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