International Journal of
Industrial Biotechnology
and Biomaterials

ISSN: 2455-7323
Volume 10, Issue 1, 2024
DOI (Journal): 10.37628/IJIBB

http://biotech.journalspub.info/?journal=JIBB&page=index

Research IJIBB

Development of Azadirachta Indica Zinc Oxide Nanoparticles and their Prospective Role in Wastewater Treatment

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Abstract

An advanced and eco-friendly method for development of nanoparticles is the fundamental role of Nanotechnology. In recent times, the domain of nanotechnology and nanoscience research has witnessed remarkable advancement, resulting in its colloquial name "tiny science." The convergence of nanotechnology and biology leads to the emergence of a highly sophisticated domain known as nanobiotechnology. Nanoparticles are considered the primary structural units in nanotechnology. These particles, referred to as NPs, are typically sized between 1 and 100 nm and are composed of carbon, metal, metal oxides, or organic compounds. The biogenic synthesis of nanoparticles (NPs) exhibits a range of favourable attributes, including cost-effectiveness, minimal environmental hazards, and a biological reduction process. As a result, it has emerged as an appealing alternative to chemical methods. Utilization of bacteria, fungi, algae, yeast, and plants characterizes the green approach. These green methods have been found to be safe as compared to the physical and chemical methods. The biosynthesized metallic nanoparticles have an unlimited range of biomedical applications. Their use has been increasing day by day in a variety of processes such as drug delivery, biosensors, gene delivery, wastewater treatment and many more. Access to clean and safe water is essential for the holistic development of society and a prosperous economy. Rapid population growth, increased industrialization, urbanization, and intensive agricultural activities have led to the production of wastewater, which has not only contaminated water sources but also made them hazardous. Countless individuals lose their lives annually as a result of illnesses transmitted by consuming water tainted with harmful pathogens. Nanoparticles exhibit a substantial surface to volume ratio, exceptional sensitivity and reactivity, a remarkable adsorption capacity, and ease of functionalization, making them well-suited for utilization in wastewater treatment. This paper presents a comprehensive study on the green synthesis of Zinc oxide nanoparticles utilizing leaf extracts obtained from Azadirachta indica. The NPs' structural and optical properties were thoroughly investigated using UV-Visible spectroscopy [UV-Vis] and Fourier Transform-Infrared Spectroscopy [FT-IR]. The antibacterial efficacy of ZnO NPs was evaluated using the paper disc diffusion method against coli formic bacteria. The results revealed that ZnO NPs synthesized with the aid of Azadirachta indica exhibited potent antimicrobial activity against pathogens. These findings suggest that the plant-based synthesis of NPs can serve as an excellent strategy for the development of versatile and eco-friendly bio medicinal products.

Keywords: Azadirachta indica, Nanoparticles, Zinc nanoparticles, Wastewater treatment, green synthesis

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Received Date: March 30, 2024
Accepted Date: April 05, 2024
Published Date: April 17, 2024

Citation: Yashfeen Ansari. Development of Azadirachta Indica Zinc Oxide Nanoparticles and Their Prospective Role in Wastewater Treatment. International Journal of Industrial Biotechnology and Biomaterials. 2024; 10(1): 32–51p.

INTRODUCTION

A relatively new field of study called nanoscience studies materials at extremely small sizes, usually in the nanometer range, which is defined as 1 to 100 nanometers. A key area of study under nanoscience is nanotechnology, which deals with the production, modification, and management of nanoparticles, which are minuscule particles with special characteristics due to their small size.

A classic quote from Nobel winner Richard P. Feynman sums up nanotechnology perfectly: "There is plenty of room at the bottom." This claim emphasizes the enormous potential that comes with working with matter at such small scales. One of the distinctive attributes of nanoparticles is their significantly high surface area to volume ratio. This property allows them to interact with a wide range of molecules, improving their thermal conductivity, catalytic reactivity, chemical stability, and nonlinear optical performance.

Environmental concerns are associated with the physical and chemical processes used in the typical synthesis of nanoparticles. As a result, biological processes are becoming more popular due to their environmentally beneficial nature. An alternate production strategy is provided by the defensive systems of organisms in metal-rich environments, which frequently result in the synthesis of nanoparticles. Plants, fungi, and bacteria can work together to create environmentally benign metallic nanoparticles, which have been studied in great detail for their variety of qualities. Plant extracts, which are abundant in biomolecules that are active, help to stabilize and reduce nanoparticles [1,2].

Because of its unique qualities and wide range of uses, zinc oxide (ZnO) has garnered a lot of interest from scientists [3-6]. Energy storage, nanosensors, personal care products, nano-optics, and nanoelectronics are just a few of the industries that use ZnO-based nanomaterials [7–12]. The biodegradability and low toxicity of ZnO nanoparticles are noteworthy features. Concerns about their possible toxicity in biomedical applications, however, are starting to surface [13–15]. It is still unclear exactly what causes them to be toxic, especially in terms of intracellular zinc ion release and nanoparticle dissolution [15].

Based on the previous literature reports, ZnO nanoparticles have been synthesized from various plant extracts such as *Azadirachta indica*[16,17],*Passiflora caerulea* [18], *Aloe vera* [19,20], *Vitex trifolia* [21], *Trifolium pratense* [22], *Bauhinia tomentosa* [23], *Cinnamomum verum* [24], *Camelliasinensis* [25],*Artocarpus gomezianus* [26], *Durantaerecta* [27], *Moringa oleifera* [28], *Matricaria chamomilla*, *Olea europaea*, and *Lycopersicon esculentum*[29], and their antimicrobial activities were also reported.

The tree *Azadirachta indica* belongs to the Meliaceae family. The majority of the African nations as well as the Indian subcontinent are home to it. Numerous phytochemicals can be found in the fruit, seeds, leaves, stems, and bark of neem. Neem, known for its antimicrobial properties, has been widely utilized in the pharmaceutical industry for a long time. In addition to its medicinal applications, the flowers of the neem tree play a significant role in various Indian celebrations, such as Ugadi. In India, the tender shoots and flowers of the neem tree are consumed as vegetables. The bark of the neem tree is effective in treating malaria, stomach and intestinal ulcers, skin conditions, pain, and fever. Utilized for various purposes, the neem leaf is known for its effectiveness in treating conditions such as leprosy, bloody noses, intestinal worms, stomach upset, appetite loss, skin ulcers, cardiovascular disease, fever, diabetes, gingivitis, and liver issues. Additionally, it is used for inducing abortions and controlling fertility. The antimicrobial activity profile of these plants was then examined in relation to coliform bacteria. In addition to its many traditional uses, this study explored the use of *Azadirachta indica* leaf extract as a capping and reducing agent for the synthesis of ZnO NPs.

METAL NANOPARTICLES

Metal nanoparticles, usually ranging in size from 1 to 100 nm, exhibit special optical and electrical properties called localized surface plasmon resonance (LSPR). This effect, which allows noble metals to absorb visible light, is particularly noticeable in copper, silver, and gold. As a result, these nanoparticles are highly useful in many different fields, such as electronics, conductivity, catalysis, and quantum technologies. Furthermore, critical metal nanoparticles—like zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), and their oxides—are particularly versatile for a wide range of applications due to their benign nature and significance to plant metabolism.

Zinc has strong reduction properties, which contribute to its high elemental activity. One simple byproduct of zinc's oxidation process is zinc oxide, which is acceptable as an inorganic material. This substance is white in color and exhibits a variety of nanostructures. ZnO serves as a representation of its chemical makeup.

The unit cell in the crystalline structure of zinc oxide is hexagonal. In this configuration, four cations at a tetrahedron's corners encircle each anion, demonstrating sp3 covalent bonding and tetrahedral coordination. The non-centrosymmetric structure of ZnO is produced by its tetrahedral form.

Synthesis of Nanoparticles

There are 2 approaches in the synthesis of nanoparticles as given shown in Figure 1:

a. Top-Down Approach.
b. Bottom-Up Approach.

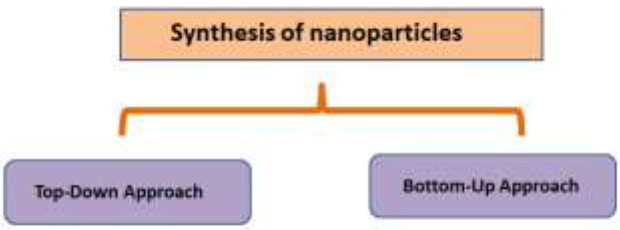

Figure 1. Two different approaches for nanoparticle synthesis.

Green Synthesis of Nanoparticles using Plant Extract

Biological nanoparticle synthesis is the method of creating nanoparticles with the help of biological agents such as plants or bacteria. Green synthesis is the process of making nanoparticles using plant extracts. Historically, plants have paid little attention to the creation of nanoparticles. However, because they are non-pathogenic and the NPs they create are discovered to be more stable, plants have emerged as the main contenders for synthesis rather than bacteria in recent years.

In order to achieve the necessary nanoparticle synthesis, a prepared salt solution is added after carefully chosen plant extract. Agitation is the process that forms nanoparticles. By using different components of medicinal plants-leaves, roots, and stems, for example- green synthesis is an environmentally benign method that reduces pollution at its source. Figure 2 given below shows antimicrobial activity of ZnO nanoparticles synthesised using *A.* indica.

Beyond its positive effects on the environment, green synthesis also has the advantage of requiring less time, producing non-toxic byproducts, and facilitating large-scale synthesis. Researchers are favouring green approaches more and more because they don't require high pressure, harmful chemicals, excessive energy, or high temperatures. Notably, green synthesis produces more stable nanoparticles with less contamination and makes scaling up easier.

Consequently, the creation of nanoparticles using components originating from plants appears to be a better option. The research interest in this technique has been significantly piqued due to its remarkable scalability and effectiveness in the production of nanoparticles. The table that follows shows the distinct ZnO nanoparticles that were made from different plant resources.

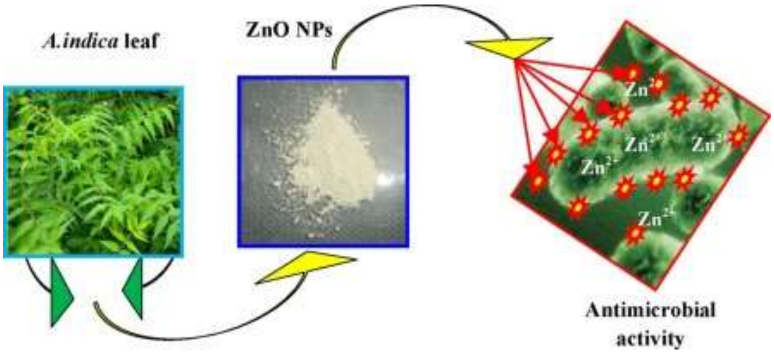

Figure 2. Antimicrobial action of A. indica mediated ZnO nanoparticles.

Table 1. Nanoparticles synthesized from different plant parts.

Plant	Plant part	Nanoparticles	References
Lippiaadoensis	Leaf	ZnO	[30]
Euphorbia hirta	Leaf	ZnO	[31]
Cassia fistula and Melia azadarachta	Leaf	ZnO	[32]
Raphanus sativus	Root	ZnO	[33]
Ricinus communis	Root	AgNPs	[34]
Clitoria ternatea	Flower	AuNPs	[35]
Rosa canina	Seed	AgNPs	[36]
Grewia lasiocarpa	Stem bark	AgNPs	[37]
Nephelium lappaceum L.	Fruit peel	TiO2	[38]

METHODOLOGY

Collection of Plant Leaves

Fresh leaves of *Azadirachta indica* were collected from outside the department of Biotechnology, C.C.S. University, Meerut, UP, India. The leaves were separated, washed with gentle tap water and dried in hot air oven at 80ºC for 2 hours. Then, using mortar and pestle, the leaves were grinded to fine powder.

Preparation of Leaf Extract

20 gm of the powder was mixed in 200ml distilled water and kept on a magnetic stirrer at 70ºC for 1 hour. After some time, the colour starts changing, then the extract was filtered using Whatman filter paper. Figure 3 shows extract of *A.indica.*

Biosynthesis of ZnO NPs

The biosynthesis of ZnO nanoparticles was done following the already done procedures with slight changes. 0.5 M Zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) was prepared in distilled water. For this 35.94 gm Zinc sulphate heptahydrate was mixed in 250 ml distilled water. It was then stirred for 15 minutes for proper mixing. The leaf extracts of *M. koenigii* was added to the solution drop-by-drop with continuous stirring. Colour starts to change and the synthesized nanoparticles are sent further for characterization.

We also prepared different concentrations of NPs viz.

1. 5ml plant extract+95ml salt solute
2. 15ml plant extract+85ml salt solution
3. 30ml plant extract+70ml salt solution

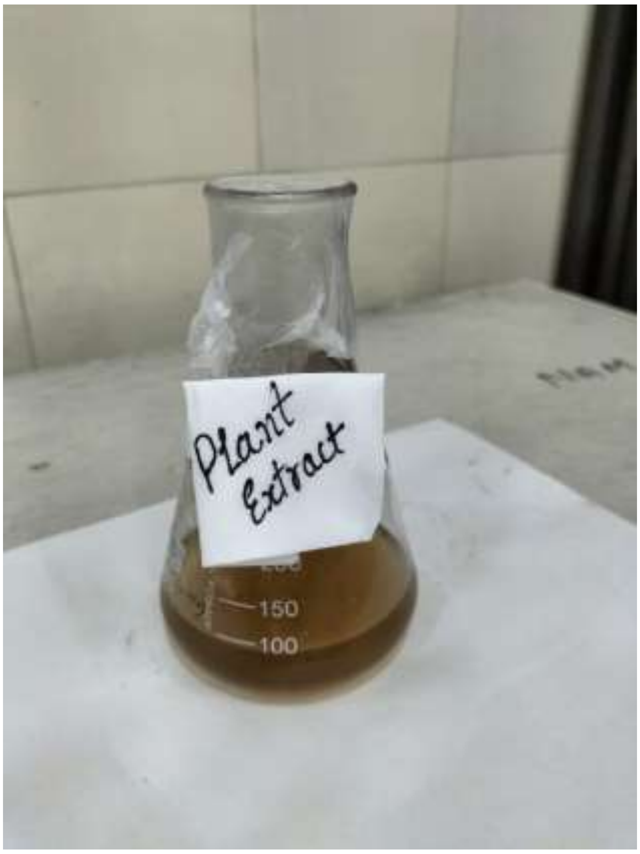

Figure 3. *A. indica* leaf extract.

Figure 4. Nanoparticle formation indicated by change in colour [5:95].

Figure 5. Nanoparticle formation indicated by change in colour [15:85].

Figure 6. Nanoparticle Formation indicated by change in colour (30:70).

Wastewater Collection

Samples of wastewater were collected from different sites: 1-Kali River in Meerut, UP, India; 2-Stagnant water from a random place in Meerut, UP, India; 3-Krishna River, Ranchhar, Baghpat, UP, India.

Growing Coliformic Bacteria from Wastewater

For this, NAM Media was prepared:

- NaCl – 1.25gm
- Agar – 5gm
- Peptone – 0.75gm
- Beef Extract – 0.75gm
- Distilled Water – 250ml

All the components were mixed and then autoclaved.

5 ml of wastewater was taken in petri and 30ml NAM media was poured over it. Few such replicas are prepared. The Petri plates are incubated for 24 hours. It was seen that bacteria appeared in Petri plates.

Preparation of NAM Broth

NAM broth was prepared as:

- NaCl – 0.5gm
- Peptone – 0.3gm
- Beef Extract – 0.3gm
- Distilled Water – 100ml

This NAM Broth was taken in 12 test tubes and then autoclaved. These test tubes were inoculated with isolated bacteria. The samples were subsequently placed in an incubator at room temperature for a duration of 24 hours to facilitate bacterial growth. Following this incubation period, the test tubes were subjected to agitation on a shaker set at 150 revolutions per minute for a period of 2 hours.

Checking of Anti-microbial activity of Nanoparticles

250 ml of NAM Media was prepared. 2ml of grown bacteria were pipetted in each of the 5 Petri plates. Then, 20ml of NAM media was poured over it. The Petri plates were incubated for 24 hours in LAF. Wells were made in the Petri plates and the prepared NPs were poured in the wells as depicted in Figure 7.

Chemical Synthesis of ZnO NPs

2gm of Zinc Sulphate was mixed in 15ml distilled water to obtain a Zinc Sulphate solution (Figure 8) and it was stirred on a magnetic stirrer for 5 minutes. In another beaker, 8gm Sodium hydroxide was mixed in 10 ml distilled water and the solution (Figure 9) was stirred for 5 minutes. Zinc Sulphate solution was mixed in sodium hydroxide solution and the mixture (Figure 10) for allowed to stir for 5 minutes. Then, 100ml ethanol was added to the above mixture. This results in the formation of ZnO NPs (Figure 11).

Figure 7. Bacteria growing on NAM.

Figure 8. ZnSO4 Solution.

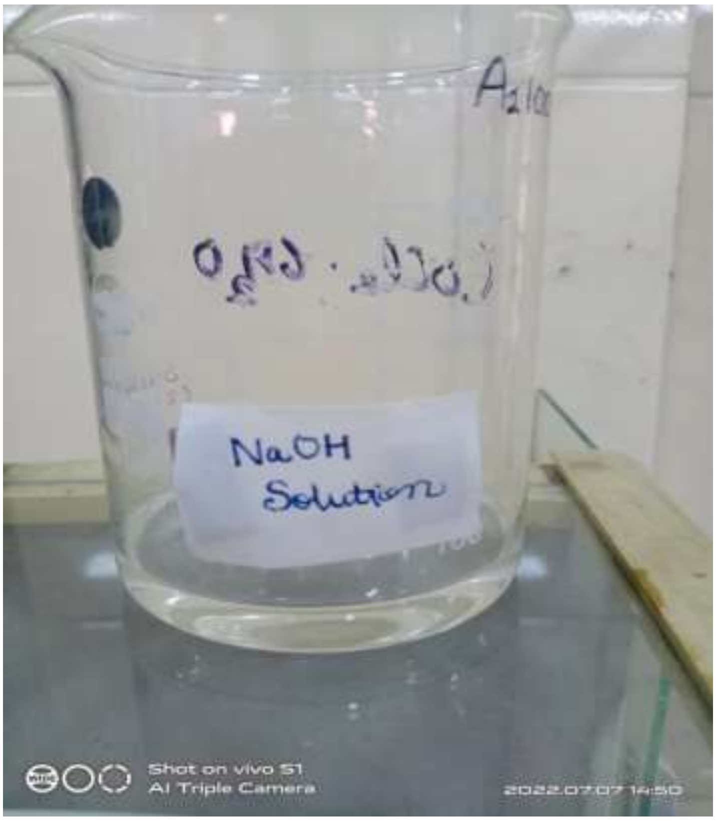

Figure 9. NaOH Solution.

Figure 10. ZnSO4 + NaOH mixture.

Figure 11. ZnO NPs.

Then, wastewater taken in a boiling tube was treated with this nano-solution. After some time, it was observed that the solid waste particles settled down. It was then filtered and an almost clear solution result (Figure 12).


Figure 12. Wastewater, nano solution and treatment [showing accumulation of solid waste particles].

RESULTS

Zinc oxide nanoparticles were successfully synthesis using leaf extracts in a green synthesis process. The transformation of zinc ions into Zinc Oxide Nanoparticles leads to a noticeable alteration in color, transitioning from a light yellow hue to a deep brown shade. This modification occurs due to the occurrence of the Surface Plasmon Resonance phenomenon, which is brought about by the existence of unbound electrons within the metal nanoparticles.

Ultraviolet-Visible (UV-Vis) Spectral Analysis

UV-Vis spectra of A. indica I leaf extract (Figure 13) and Zinc Sulphate solution (Figure 14) was visualized. Later, Zinc oxide nanoparticles' UV-Vis absorption spectrum was observed (Figure 15) and these biosynthesized samples showed strong absorption bands in the 300–360 nm range, which is the characteristic band of ZnO nanoparticles. The absence of any additional peak spectra demonstrates the purity of the synthesised ZnO NPs. Additionally, it has been noted that the peak positions of UV-visible spectra are related to nanoparticle size and shift towards the blue the smaller the nanoparticles' crystal size [39].

Fourier Transform-Infrared Spectroscopy [FT-IR]

FT-IR spectroscopy is based on the principle of Fourier transformation, which allows for the conversion of raw data into a spectrum that provides information about the chemical composition and structure of a sample. The purity of the synthesized ZnO nanoparticles is a significant factor to consider. Furthermore, it has been observed that the peak positions in the UV-visible spectra are influenced by the size of the nanoparticles, with a shift towards the blue indicating smaller crystal sizes. FTIR spectra of the different concentrations of nanoparticles was visualized:

1. 5:95 (Figure 16)
2. 30:70 (Figure 17)
3. 15:85 (Figure 18)

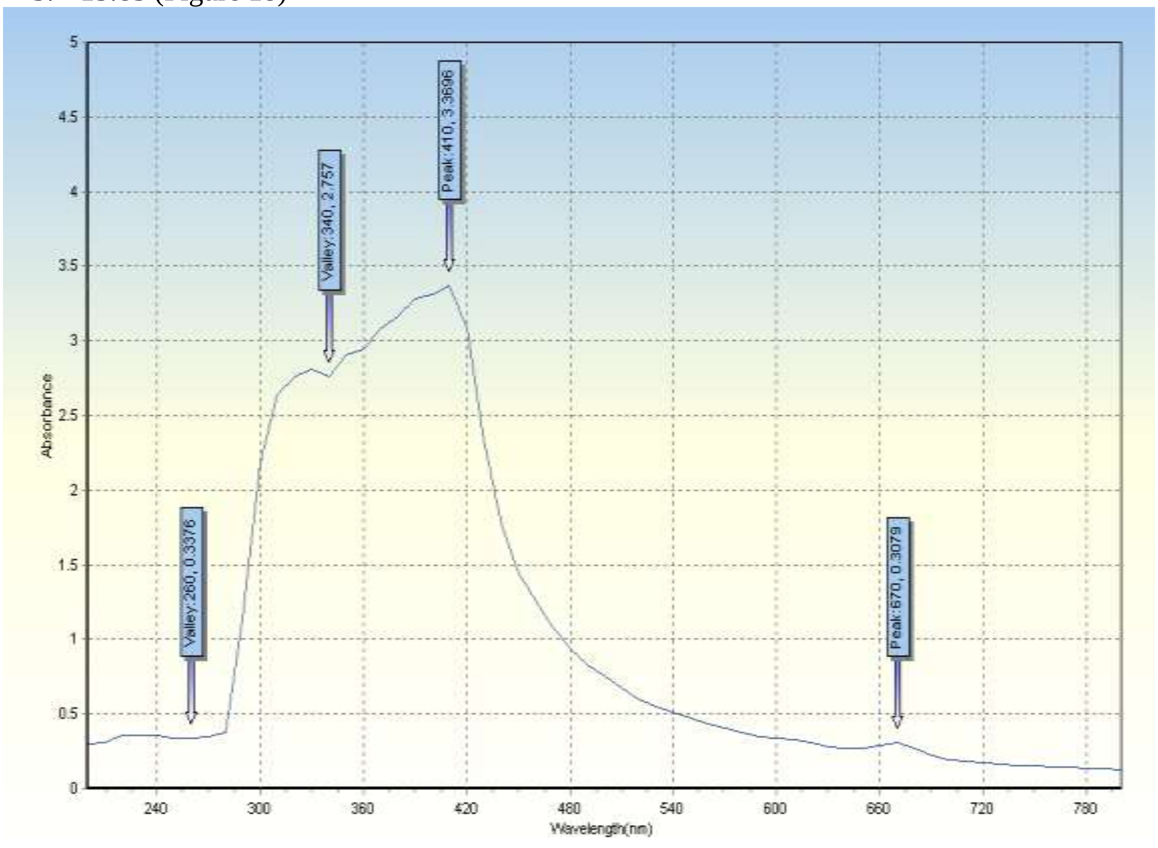

Figure 13. Absorption peaks of A. indica leaf extract.

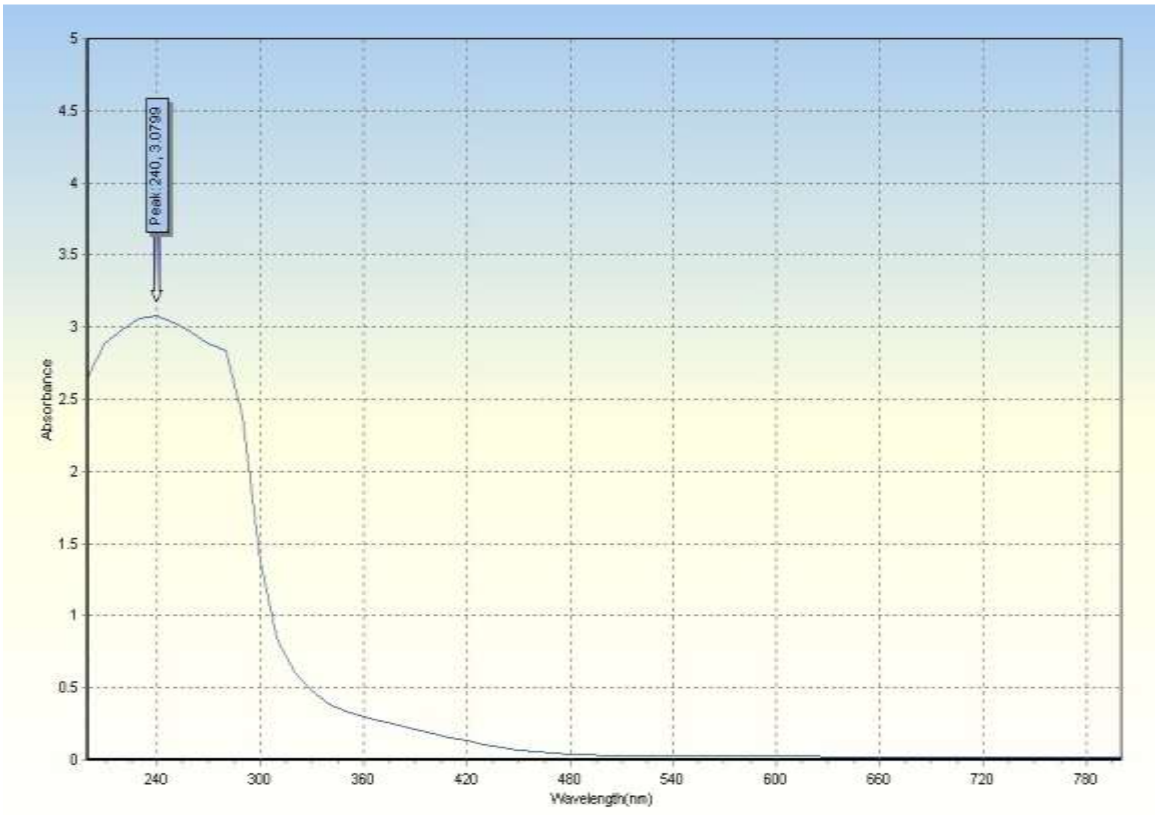

Figure 14. Absorption peaks of Zinc Sulphate salt solution.

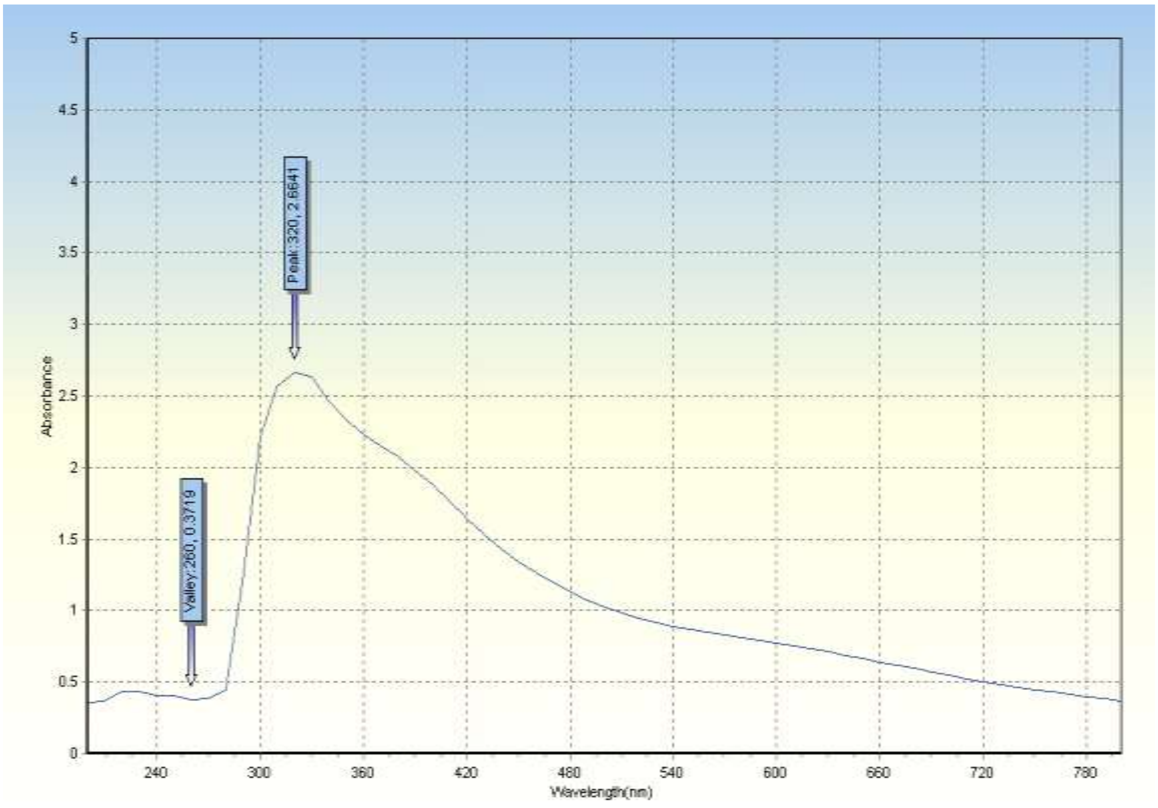

Figure 15. Absorption peaks of A. indica mediated ZnO nanoparticles.

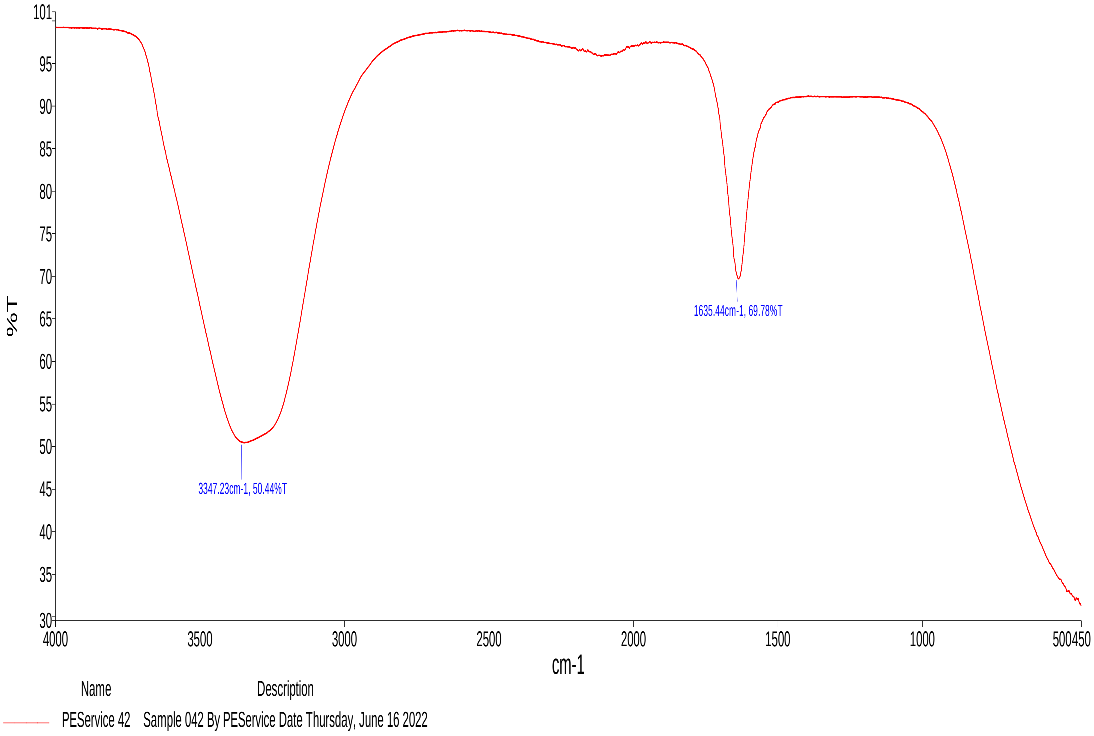

Figure 16. 5:95.

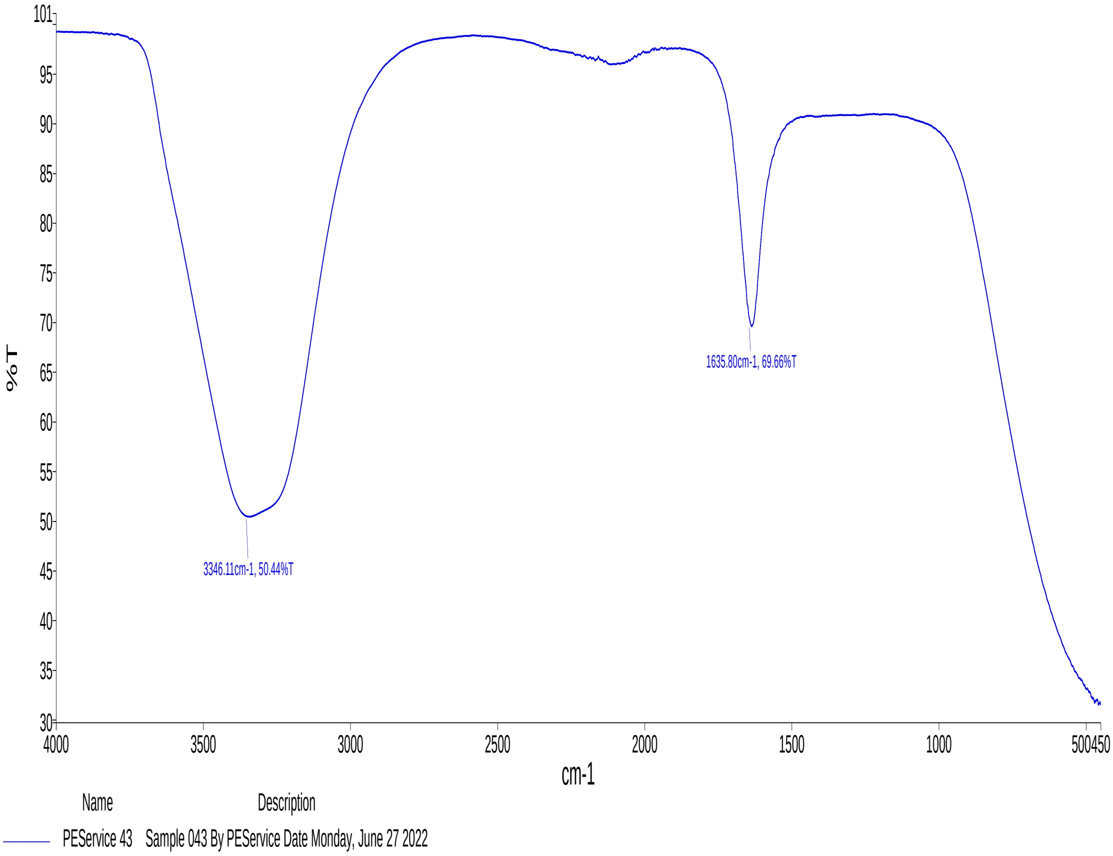

Figure 17. 30:70.

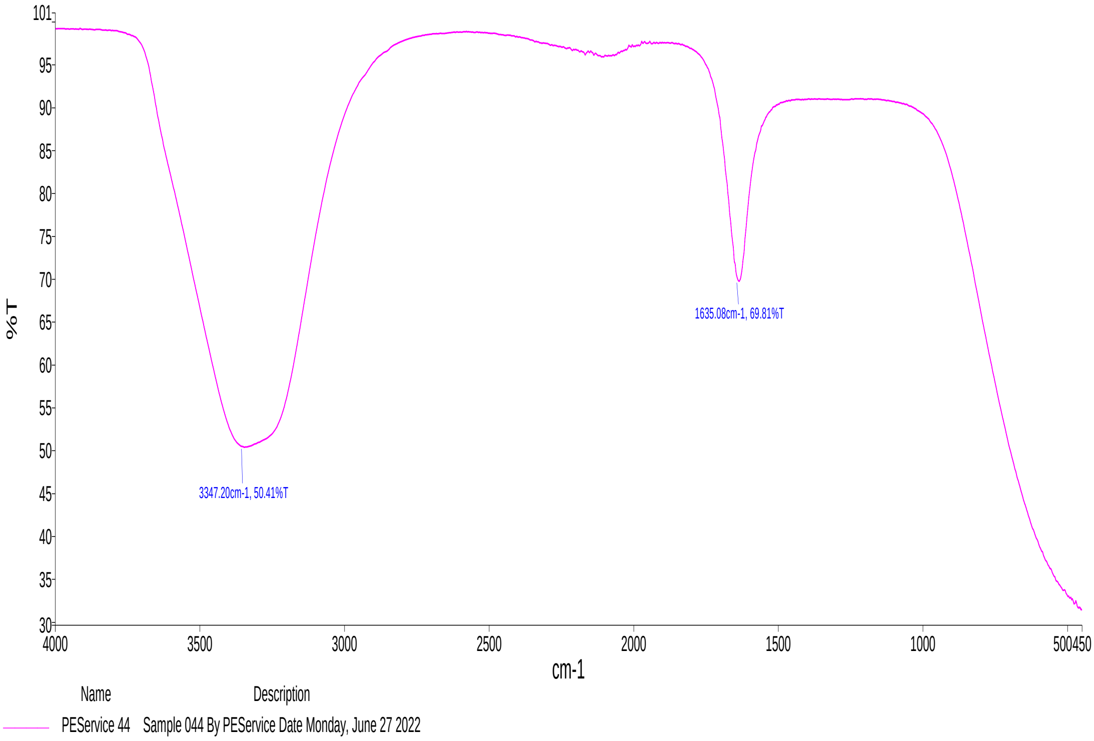

Figure 18. 15:85.

Antibacterial Activity Analysis of ZnO Nanoparticles

The antibacterial activity of ZnO NPs that were biosynthesized was evaluated against mature pathogens using the Disc Diffusion method. Overall, the leaf extract-based biosynthesized ZnO NPs showed potent antibacterial activity against bacterial strains at all concentrations as shown in Figure 19, 20, 21 respectively.

Figure 19. Effect of *A. indica* mediated ZnO NPs [5:95] on bacteria.

Figure 20. Effect of *A. indica* ZnO NPs [15:85] on bacteria.

Figure 21. Effect of A. indica mediated ZnO NPs [30:70] on bacteria.

A previous study found that ZnO nanoparticles had greater bactericidal activity against Gram-positive bacteria than Gram-negative bacteria [40]. The variation in the morphological composition of these microorganisms could potentially explain the contrasting sensitivity observed between Gram-positive and Gram-negative bacteria. Gram-negative bacteria possess an outer lipopolysaccharide membrane that acts as a hindrance for chemical antibacterial agents to penetrate the cell wall. Conversely, gram-positive bacteria are more susceptible due to their sole outer layer of peptidoglycan, which lacks effectiveness as a permeability barrier. Consequently, Gram-negative bacteria exhibit more intricate cell walls compared to Gram-positive bacteria, serving as a diffusion barrier and diminishing their vulnerability to antibacterial agents. There are a few potential bactericidal mechanisms that could explain how ZnO NPs interact with bacteria. Some claimed that smaller NPs released Zn2+ because of their easier cell penetration and higher surface reactivity. The fundamental concept driving antibacterial mechanisms involves the liberation of Zn2+ from ZnO NPs, which has been shown to impede various bacterial cell processes such as active transport, bacterial metabolism, and enzyme activity, leading to the eventual demise of bacterial cells [41]. The formation of reactive oxygen species (ROS), which results in oxidative stress and subsequent cell damage or death, is the source of the other proposed antibacterial activity. ZnO NPs frequently use the formation of ROS as an antibacterial strategy [42]. ZnO NPs may exhibit antimicrobial activity through the attachment to the bacterial cell membrane via electrostatic forces, presenting another potential mechanism for their effectiveness. This interaction may alter the structure of the membrane plasma and harm the integrity of the bacterial cell, leading to the leakage of intracellular materials and ultimately to cell death [43].

In one set of Petri plates, wastewater and NAM media were taken and they were observed for bacterial growth. It was seen that a large number of bacteria were growing on the Petri plates.

Figure 22. Bacteria growing in wastewater.

In other set of Petri plates, wastewater treated with nanosolution and NAM media were taken and observed for bacterial growth which is illustrated in Figure 23. It was seen that no bacteria were grown on the Petri plates.

International Journal of Industrial Biotechnology and Biomaterials
Volume 10, Issue 1
ISSN: 2455-7323

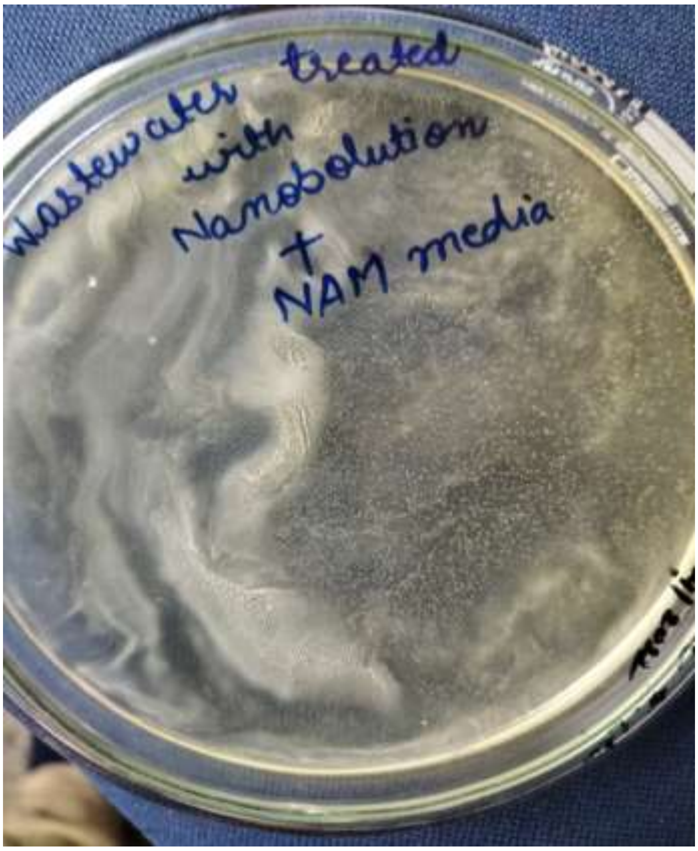

Figure 23. No bacteria seem to be growing in nanosolution.

Absorbance at Different Concentrations

Adding 10ml of nanosolution in varying concentrations of wastewater and recorded absorbance as shown in Figure 24.

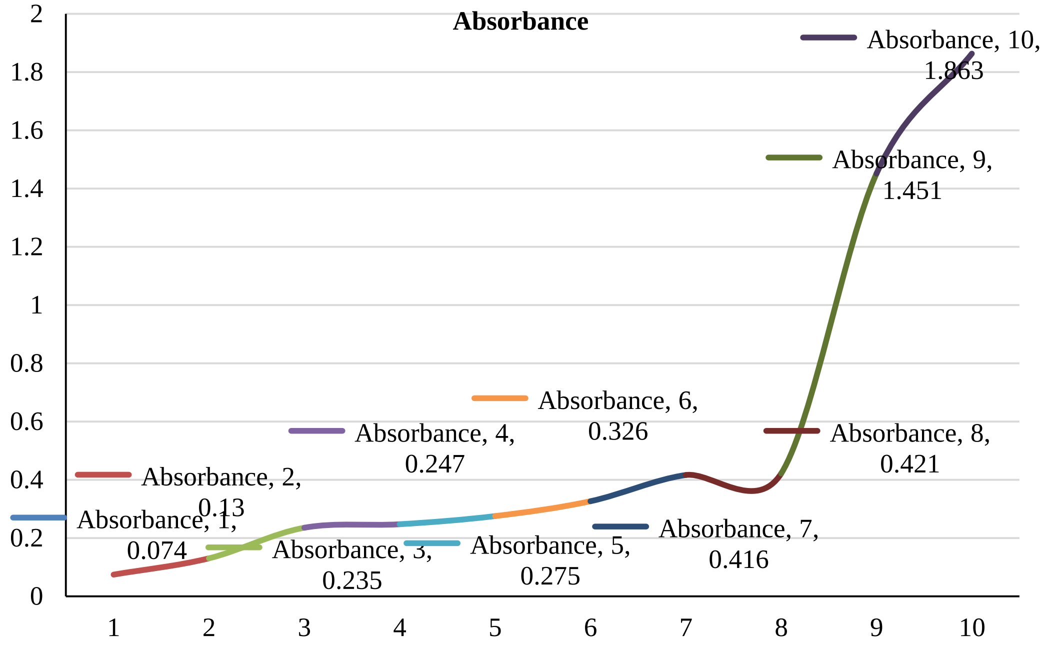

Figure 24. Graphical representation of Absorbance.

CONCLUSIONS

In this study, ZnO NPs were successfully produced from *Azadirachta indica* and *Murayyakoenigii* leaf extract using a straightforward, economical, environmentally friendly, and green method. This demonstrated that these plants might be used as efficient reducing and capping agents for ZnO NPs produced biologically.

FTIR and UV-V is techniques were used to characterise the biosynthesized ZnO NPs. UV-Vis was used to confirm that the NPs were stable. C-C stretching aromatic ring is visible in FT-IR. Some peaks exhibit ZnO stretching vibration, supporting the use of these plants as a reducing and capping agent in the synthesis of ZnO NPs.

Additionally, biosynthesized ZnO nanoparticles have demonstrated their efficacy against coliform bacteria, indicating a strong and promising effect of greenly synthesised ZnO nanoparticles against biological systems.

Chemically synthesized nanoparticles were tested on wastewater and the solid waste accumulated at the bottom and almost transparent solution results. Metal nanoparticles with antimicrobial properties are essential for the effective treatment of wastewater. Therefore, it is crucial to focus on the commercialization of these nanoparticles. By doing so, we can harness their potential to combat microbial contamination and improve the overall quality of wastewater treatment processes. By doing this, the amount of chemicals needed to treat wastewater is reduced.

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