Green Synthesis of Zinc Oxide Nanoparticles Using *Ziziphus mauritiana* Extract and Its Antibacterial Activity

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ABSTRACT

The zinc oxide nanoparticles (ZnONPs) used in this study were produced utilizing an easy-to-use, entirely safe method that used an aqueous plant extract from *Ziziphus mauritiana* leaves as an efficient stabilizing agent. The aqueous leaf extract underwent phytochemical screening, which revealed the presence of flavonoids, alkaloids, polyphenols, carbohydrates, proteins, and amino acids. UV, XRD, FT-IR, SEM and EDX were used to characterize the produced ZnONPs. This green synthesis suggests that this strategy is more effective than the chemical one. According to the study, green synthesized ZnONPs have strong antibacterial properties as well. *Salmonella typhi* and *Escherichia coli* are gram negative bacteria, while *Staphylococcus aureus* and *Micrococcus luteus* are gram positive bacteria. The medication chloramphenicol is used as the positive control.

KEYWORDS: *Ziziphus mauritiana*, Aqueous Extraction, Antibacterial Activity.

1. INTRODUCTION

For thousands of years, people in small communities all over the world have used medicinal plants as a source of healing. Even yet, they continue to be important in the modern day as a source of primary healthcare for over 85% of the global population and as a tool for drug discovery—80% of synthetic pharmaceuticals are derived from them. (1).Nearly every civilization uses medicinal plants as a resource (2). Alkaloids, flavonoids, terpenes, glucosinolates, and phenolic compounds are among the natural components and phytochemicals found in medicinal plants in abundance. Known by most as Indian jujube, (3) Ziziphus mauritiana (Rhamnaceae) is a pharmacologically varied medicinal plant. Numerous active phytochemical components of this plant have been identified thus far, including lanosterol, diosgenin, kaempferol, stigmasterol, quercetin, and sitosterol(4) . The leaves are used to treat lung problems, fever, diarrhea, stomach ailments, and liver damage (5). Ziziphus mauritiana is a tiny, prickly tree or shrub that can reach heights of three to fifteen meters. Leaves and fruits are used to manufacture dyes and medicines (6). The species evolved in the South East Asian area known as Indo-Malaysia(7). Because of its use in chemistry, medicine, and biotechnology, nanotechnology has grown enormously during the last ten years (8, 10). Zinc oxide (ZnO) has multifunctionality owing to its extraordinary Physico-chemical properties and functionality in a range of applications (11). New avenues in nano science have been made possible by advancements in this discipline, notably in medication delivery, gene delivery, nanomedicine, bio sensing, etc (12, 13). The high surface-to-volume ratio of nano-sized particles is one of their special characteristics (14). Because atoms on the surface of nanoparticles tend to be more active than those in the core, this property makes them more reactive than the bulk material. (15,16). It is possible to create these nanoparticles by chemical, physical, or biological means. Numerous physical and chemical techniques, such as hydrothermal, laser ablation, sol-gel synthesis, and lithography, call for specialized tools and trained personnel. Additionally, they have harmful impacts on health that are poisonous. It is discovered that the nanoparticles produced using the green synthesis process are non-toxic, reasonably priced, and naturally biodegradable.(17- 20). Many different uses require biocidal materials (materials that can kill living organisms), such as creating packaging that can actively protect food, developing medical equipment, and producing membranes that resist biological buildup (21). Because the process

makes use of natural resources like roots, leaves, and flower extracts as well as microorganisms like fungus, bacteria, algae, etc., (22-24). this environmentally friendly synthesis approach uses less dangerous substances .Current research demonstrates the value of environmentally friendly metal oxide nanoparticle production, where metal oxides such as zinc, silver, gold, nickel, copper, etc(25-27). are becoming more and more significant. Our goal is to create nanoparticles that may be used to realize optical biosensors; this requires materials with favourable optical characteristics, such as chemiluminescence, photoluminescence (28, 29). Optical emission, and so on. excellent electron mobility, a broad band gap, a large Exciton binding energy, and excellent optical transmittance are among (30, 31). The characteristics of ZnONPs, a class of metal oxides. The production of sensors makes use of zinc oxide's optical characteristics (32-34). Applications for the produced nanoparticles include drug discovery, lasers, sensors, and other areas. Depending on the use, the synergistic effects can be quantified. Shape and size are significant factors in applications that use the antibacterial(35), anticancer(36), antimicrobial(37), ant biofilm(38), and antityrosinase (39) ,properties of nanoparticles. The current work assessed the photo-catalytic activity of the Ziziphus mauritiana leaf and concentrated on the green synthesis of ZnONPs. Plant-based synthesis is used in green synthesis processes to create nanoparticles. Chemicals that are relatively free of pollutants are used in green synthesis procedures to create nanostructures. Yeast, fungus, and bacteria are among the biological systems that have been employed safely in biogenic syntheses of nanoparticles [40].

2. MATERIALS AND METHODS

2.1 COLLECTION OF SAMPLES

The plant's aerial portion was gathered from the Salem district's Seeragapadi village. The plant sample was immediately cleaned with regular water when it was collected, and then twice more with demineralized water to remove any remaining moisture.

EXTRACTABLE MATTER OF THE PLANT

A water bath device was used to perform the extraction. Aqueous solution was used to remove the coarse leaves. Following extraction, the extracts were dried at room temperature to produce a viscous mass before being concentrated using a rotary evaporator. Weighing and storing the extracted materials was done.

Section of a plant: Leaves

HARMONY OF THE ORGANIC EXTRACT

A 62-gram Ziziphus mauritiana leaf was taken together with 300 milliliters of water. After that, the plant material was removed using a water bath that was heated to 80° C for one hour and thirty minutes. Following extraction, what man filter paper in vacuum filter was used to filter the extract. After that, the extract was kept in sealed bottles for 12 hours at 4°C in a refrigerator.

2.2 ANALYSIS PHYTOCHEMICAL

Check for alkaloids

1. The Wagner Test

Two milliliters of the plant extract, five milliliters of hydrochloric acid, and a few drops of Mayer's reagent were added. The mixture was then tested for the development of a yellow or brown color.

2. The Haggerty Test

Two milliliters of the plant extract, five milliliters of hydrochloric acid, and a few

1. One percent lead acetate

Add a few drops of a 10% lead acetate solution to two milliliters of the plant extract, and watch for the creation of a white precipitate.

2. cellulose

Add a few drops of gelatin reagent to two milliliters of the plant extract, and watch for the creation of a white precipitate.

3. FeCl3 in neutral form

Add a few drops of neutral FeCl3 solution to two milliliters of plant extract, and watch for the creation of a white precipitate.

Check for carbs.

After adding two milliliters of plant extract, two milliliters of albumin bovide, a few drops of NaOH, and heating, the color changed from brown to yellow.

Check for proteins.

After adding 2 ml of the plant extract and 2 ml of NaOH, heat for a few minutes. Include five or six.

2.3 GREEN ZINC OXIDE NANOPARTICLES SYNTHESIS

The aqueous leaf extract of Ziziphus mauritiana, zinc nitrate (ZnNO3), and double-distilled water were utilized in the bioreduction synthesis of nanoparticles. Ziziphus mauritiana aqueous leaf extract (10 ml) was carefully added five times to 25 ml of 1 mm aqueous ZnNO3 solution. The 50ml of extract was then agitated for two hours using a magnetic stirrer.Next, take a crucible, set the heat mantle to 160°C, and gradually pour the extract in. After solidification, the temperature was raised by 20°C to 400°C. After the crucible reached 400°C steadily for 30 minutes, scrape the solid with a spatula and weigh it. that after four hours, the crucible was set at 550°C in a muffle furnace. Next, remove the crucible and discard the materials.

2.4 Ziziphus mauritiana's Anti-Bacterial Activity

Test Microorganisms: Bacteria and fungi

Staphylococcus aureus, Micrococcus luteus, Escherichia coli, and Salmonella typhi were the test microorganisms employed in the study. After being morphologically recognized, the microorganisms underwent a biochemical test to confirm their identity at the biochemical level.

INOCULUMS PREPARATION

The stock culture was kept on nutrient agar at 4°C. A loopful of cells from the stock cultures were transferred to a test tube containing Muller-Hinton broth for bacteria, and the experiment was then brought to life by incubating the bacteria for 24 hours at 37°C without any agitation.In order to attain the optical densities that equate to 2.0 X 106 colony forming units (CFU/ml) for bacteria, the culture was diluted with fresh Muller-Hinton broth.

ii. STERILE SWAB PREPARATION

Autoclaving or using dry heat (exclusively for wooden swabs) were the methods used to prepare and sanitize cotton wool swabs on wooden applicators or plastics. The swabs were packed in culture tubes, tins, papers, etc. to sterilize it.

iii. FORCEPS STERILIZATION

You can dip forceps to sanitize them.

THE MULLER-HINTON AGAR PREPARATION

In a conical flask with a flat bottom, one liter of distilled water and 38 milligrams of Muller-Hinton agar powder were combined. To fully dissolve the media, the mixture was heated while being stirred frequently and brought to a boil for one minute. After securing the flask firmly with cotton wool, aluminum foil was placed on top of it. The mixture was allowed to cool to room temperature after being autoclaved for 15 minutes at 121°C. A laminar flow of the media was used to fill the Petri dishes to a consistent depth of 3–4 millimeters. Before being used, the Petri plates with the media were then sealed in sterile plastic bags and kept between 2 and 8 °C.

Utilizing the Agar Well Diffusion Method for Anti-Bacterial Assay

Muller Hinton Agar (MHA), obtained from Himedia, was used to screen the system of in vitro antibacterial exertion. The sterilized petriplates were filled with 15 ml of molten medium to create the MHA plates. After 0.1 inoculums were slightly swabbed and allowed to firm for 5 twinkles, the inoculums were allowed to dry for 5 twinkles. Twenty microliters of the various test medication were added to wells that had been cut. Additionally, the plates are incubated for 24 hours at 37 °C. The perimeter of the inhibitory zone that developed around the well was measured in order to assess the antibacterial exertion. A slice of chloramphenicol was employed as a positive control.

3. RESULTS & DISCUSSION

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES

The various tests and analyzing methods are indicated the nanoparticles from the leaf extracts of *Ziziphus mauritiana*

Figure 1: Green synthesis of ZnONPs

3.1 PHYTOCHEMICAL ANALYSIS OF *Z.mauritiana*

Table 1: Phytochemical Present in *Z.mauritiana* leaf extract

TEST FOR ALKALOIDS	
Wagner reagent	++
Hager reagent	++
Dragendorff reagent	+

Mayer reagent	+
TEST FOR POLYPHENOLS	
1% Lead acetate	+
Gelatin	+
Neutral FeCl3	+
TEST FOR CARBOHYDRATES	
Molisch reagent	+
Benedict reagent	+
TEST FOR PROTEINS	
Biurette reagent	+
TEST FOR AMINO ACIDS	
Ninhydrin test	-
TEST FOR FLAVANOIDS	
Stock solution (10% NaOH)	+

Note: ++ = high positive; + = medium positive; - = negative.

3.2 CHARACTERIZATION OF ZnONPs

3.2.1 UV-VIS SPECTROSCOPY

The UV-Visible is effective tools to identify and characterize nanoparticles. Hence, UV-Visible spectrum of the product formed was recorded at wavelength ranges from 200-1200 nm

and the maximum absorption peak was recorded at 372.62 nm which confirmed the formation of ZnONPs.

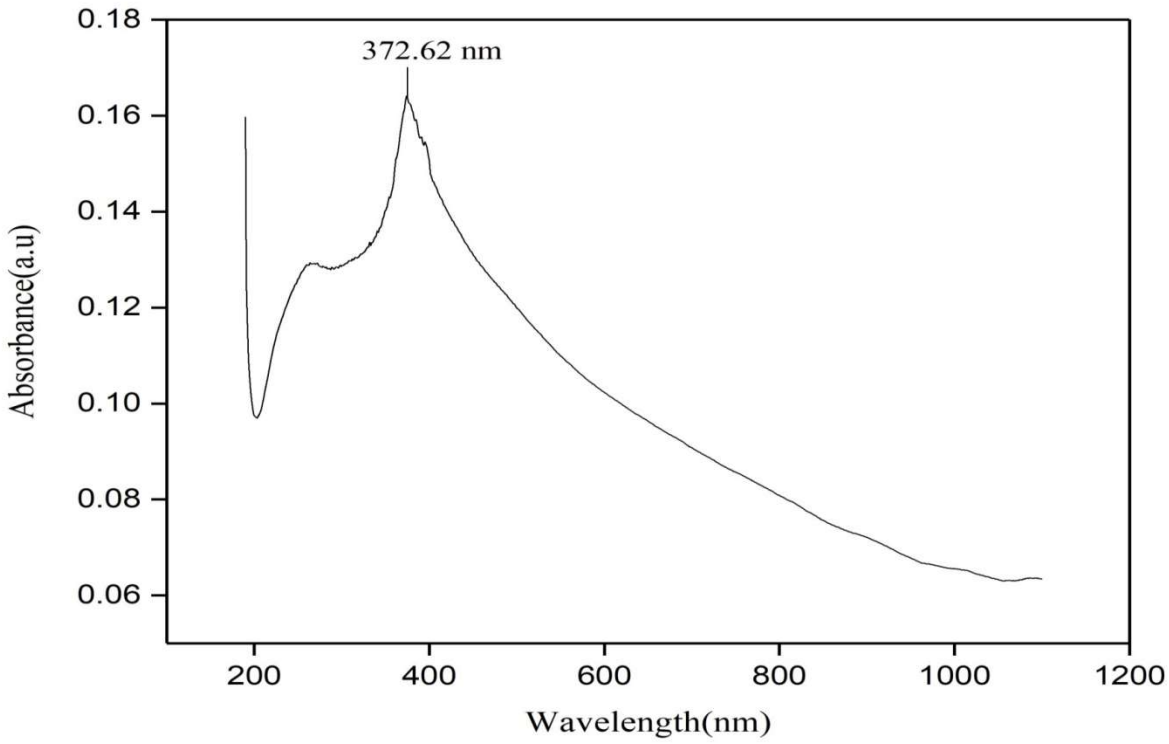


Figure 2: UV-Visible spectrum of the green synthesized ZnONPs

3.2.2 X-RAY DIFFRACTION (XRD)

The ZnONPs' XRD diffraction pattern was measured and analyzed to determine the purity and structure of the resulting compounds. The pattern was recorded within a 2θ range. The ZnONP XRD patterns that were created with Z. mauritiana leaf extract. The produced nanoparticles, their purity, crystallinity, and hexagonal form are all visible in the XRD pattern. Index numbers for the observed peaks are (100), (002), (101), (110), (102), (103), and (112). These peaks met the requirements of JCPDS standard no. (36-1451).

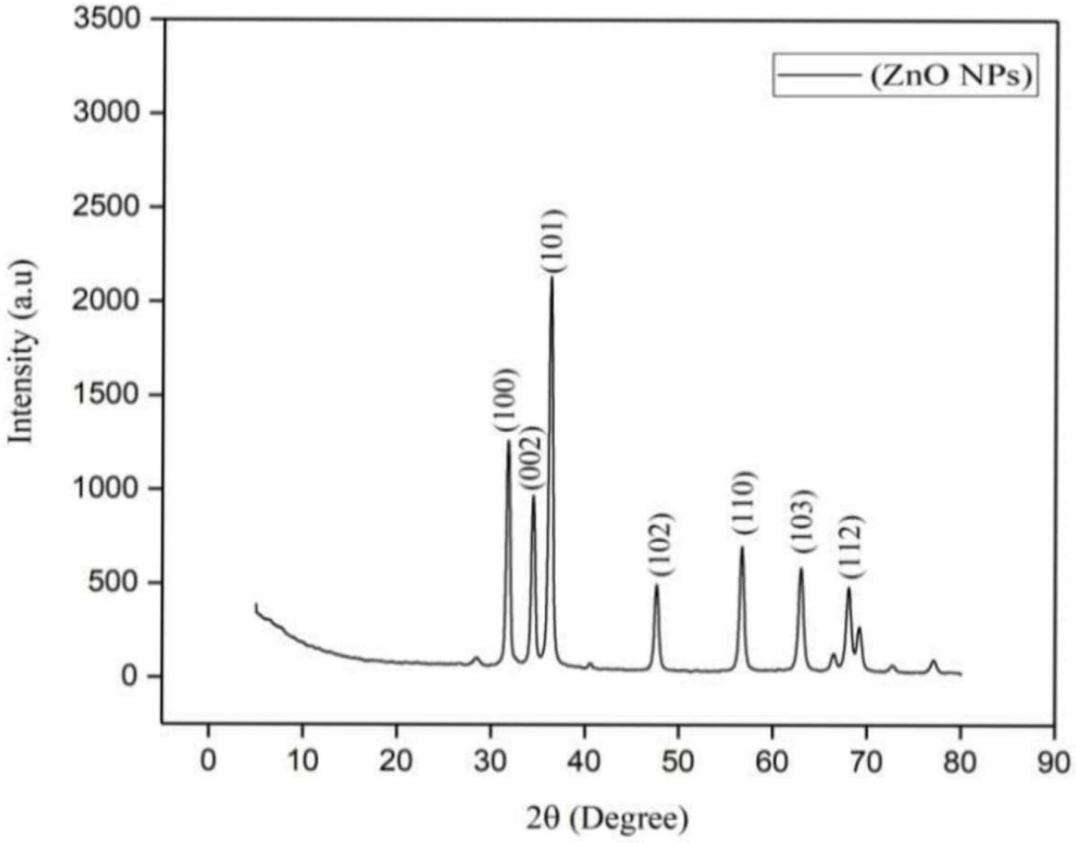


Figure 3: XRD pattern of the green synthesized ZnONPs

Table 2: XRD ANALYSIS

2θ	MILLER INDICES
31.80	100
34.57	002
36.41	101
47.65	102
56.49	110
62.94	103

68.10	112

3.2.3 Infrared Spectroscopy using Fourier Transform (FT-IR)

The OH group is represented by the peak that was seen at 3444.62 cm-1, which is the result of stretching and deformation related to water adsorption on the metal surface, respectively. Similar peaks can be found at 1639.54cm-1, 1385.27cm-1, and 1050.62cm-1, showing the presence of distinct functional groups in the synthesized particle and its potential for usage in a variety of applications. The frequencies of metal oxides were measured at 517.78 cm-1.

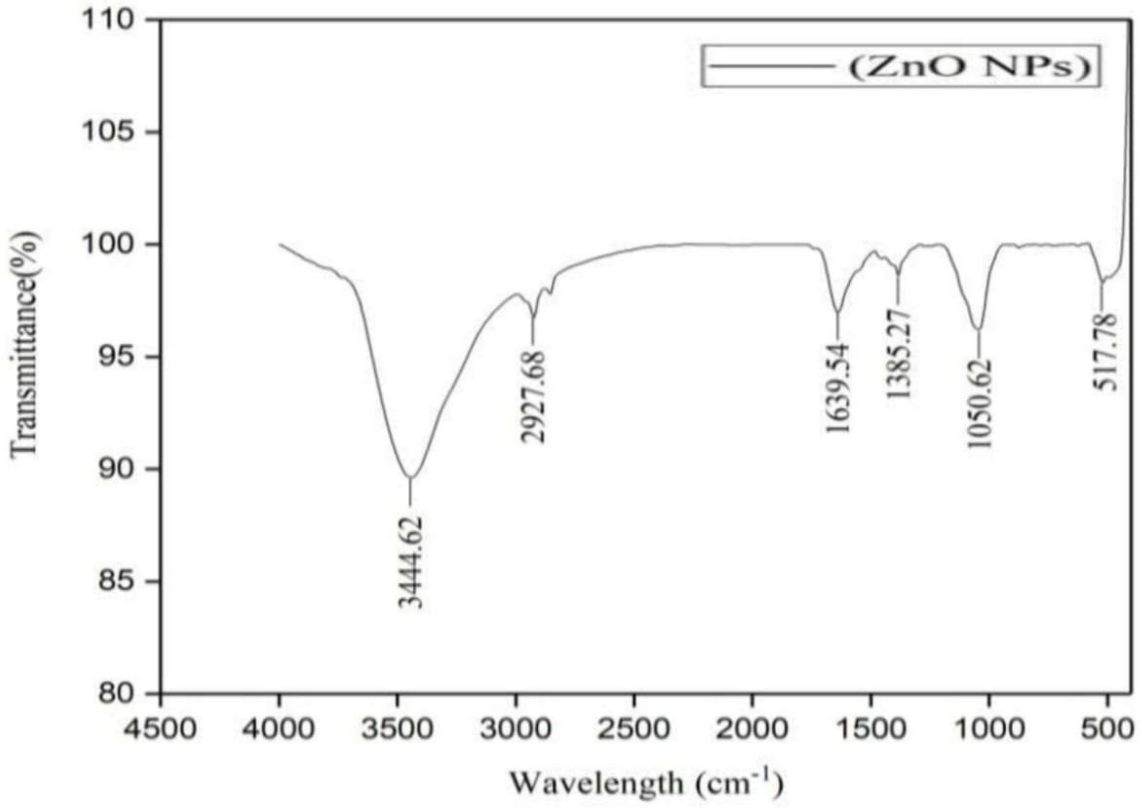


Figure 4: FT-IR spectrum of the green synthesized ZnONPs

Table 3: Functional group of the green synthesized ZnONPs

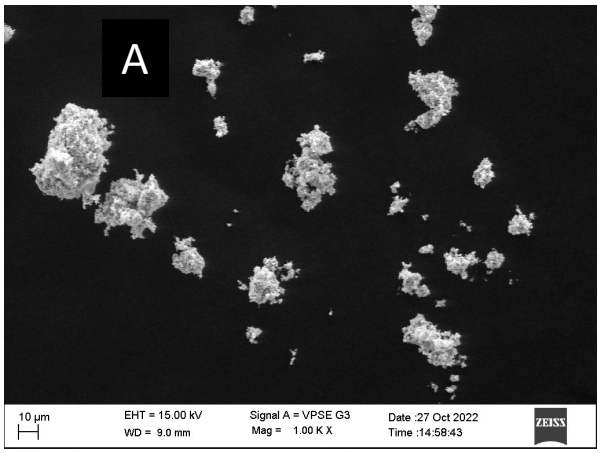
WAVENUMBER(cm^{-1})	BOND	FUNCTIONAL GROUP

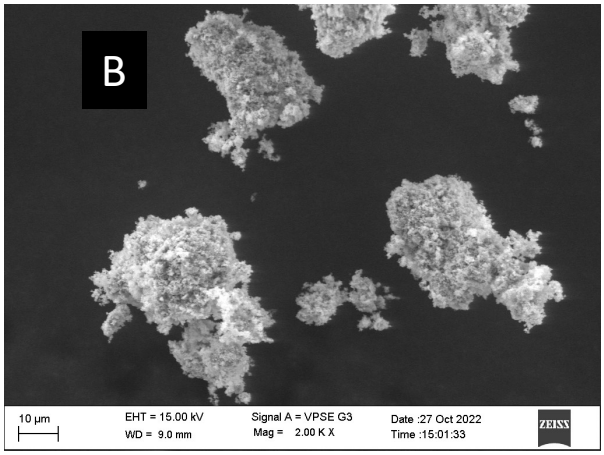
3444.62	O-H stretch bond	Alcohols
2927.68	C-H stretch bond	Alkane
1639.54	C=O	Ketone group of amide
1385.27	C-O	Amide
1050.62	C-N stretch	Aliphatic amines
517.78	C-Cl	Alkyl halides

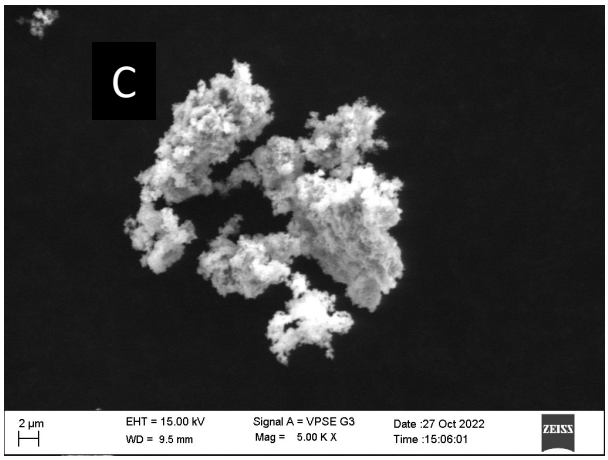
517.78	C-Cl	Alkyl halides

3.2.4 SCANNING ELECTRON MICROSCOPY (SEM)

In SEM image, the synthesized ZnONPs are present in uniform and structure is identified as sponge like morphology.






C
2 µm
EHT = 15.00 kV
WD = 9.5 mm
Signal A = VPSE G3
Mag = 5.00 K X
Date :27 Oct 2022
Time :15:06:01
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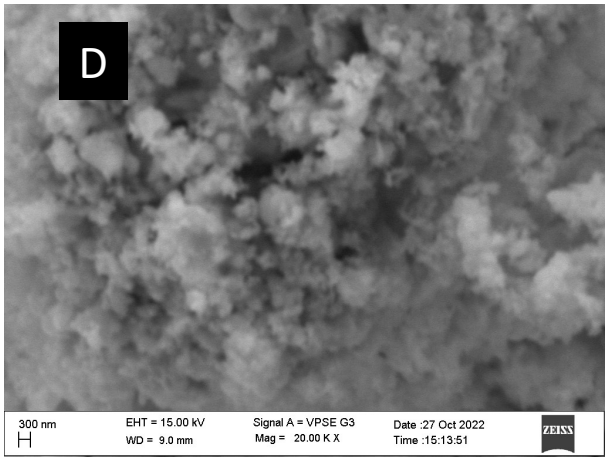
D
300 nm
EHT = 15.00 kV
WD = 9.0 mm
Signal A = VPSE G3
Mag = 20.00 K X
Date :27 Oct 2022
Time :15:13:51
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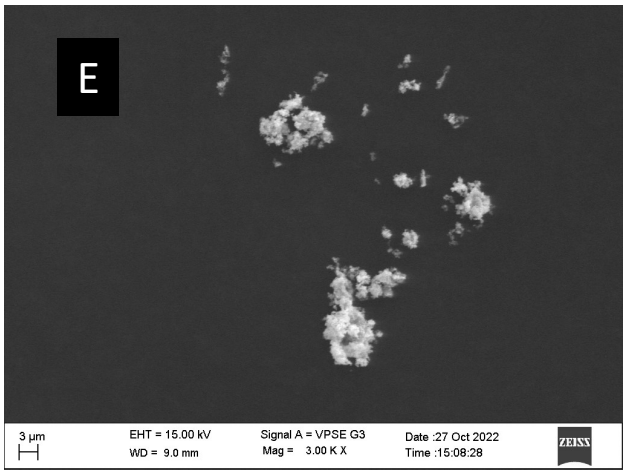
E
3 µm
EHT = 15.00 kV
WD = 9.0 mm
Signal A = VPSE G3
Mag = 3.00 K X
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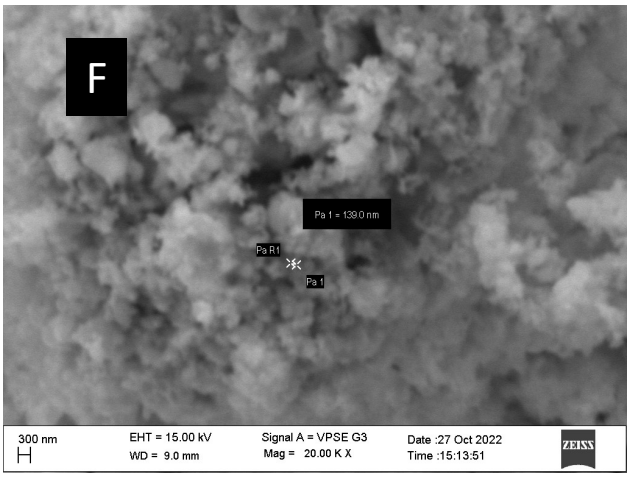
F
Pa 1 = 139.0 nm
Pa R1
Pa 1
300 nm
EHT = 15.00 kV
WD = 9.0 mm
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Mag = 20.00 K X
Date :27 Oct 2022
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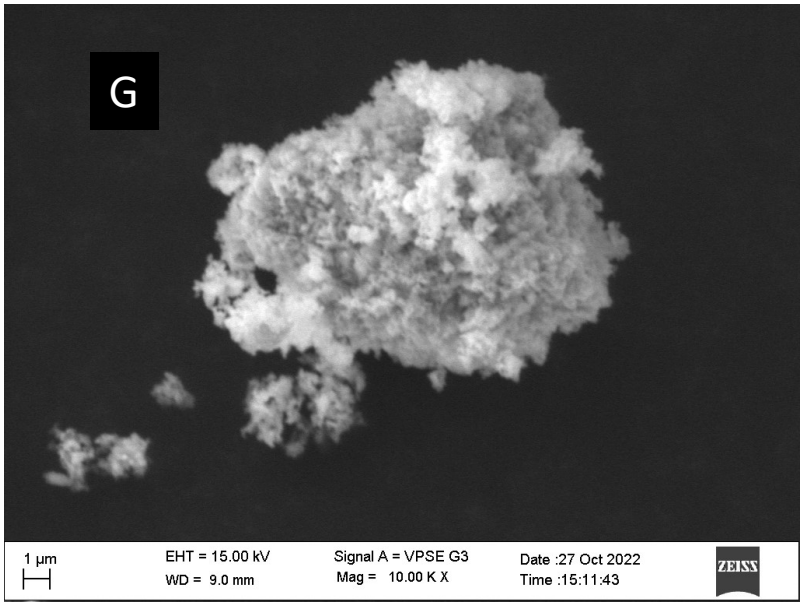

Figure 5: SEM of the green synthesis of ZnONPs

3.2.5 ENERGY-DISPERSIVE X-RAY ANALYSIS (EDX)

The Energy dispersive X- ray analysis (EDX) pattern confirms the presence of zinc and oxygen. Other signal including K and C are also recorded possibly due to the elements present in the green synthesis of ZnO nanoparticles and also % of atomic weight include in the table.

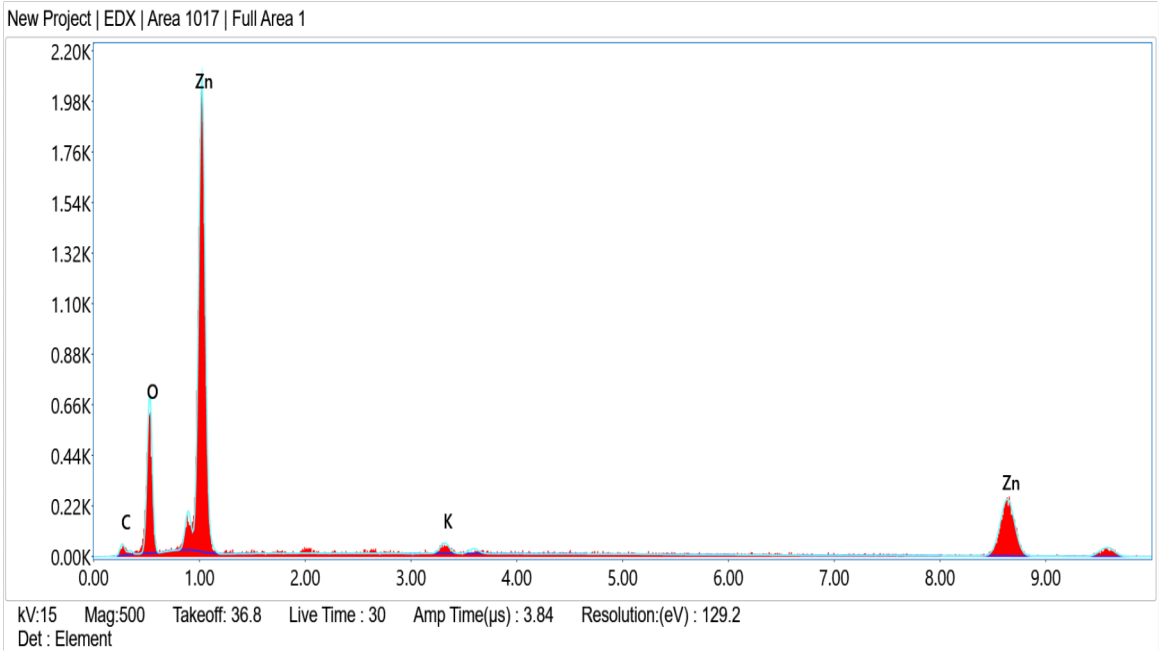

Figure 6: EDX pattern of the green synthesized ZnONPs

Table 4: Elements structural of ZnONPs

Structural Parameters of ZnO NPs			
Element	**Weight %**	**Atomic %**	**Error %**
C K	1.5	5.2	41.3
O K	14.2	38.4	10.5
K K	1.4	1.5	15.5
Zn K	82.9	54.8	5.7

C – Carbon

O – Oxygen

K – Potassium

Zn – Zinc

3.3 Ziziphus mauritiana leaf extract's antibacterial activity

Traditional in vivo bioassays are slower, more difficult, and more expensive than in vitro techniques. Despite remarkable progress in the detection of antibacterial drugs, infectious illnesses caused by pathogenic and opportunistic microorganisms continue to pose a serious threat to public health. Antibiotic-resistant bacteria that are moving toward the last line of antibiotic defense have emerged as a result of the indiscriminate use of antibiotics. Given that they can accomplish their intended function with fewer adverse effects than synthetic antibacterials, plant-based antibacterials offer a great deal of therapeutic potential. Plant-derived

biomolecules seem to be one of the key players in the regulation of these antibiotic-resistant human infections.

Table 5: Antibacterial Activity of ZnONPs of *Ziziphus mauritiana* leaves extract

S. NO	MICROORGANISMS NAME	ZONE OF INHIBITION (mm)				
		100	75	50	25	Positive control
1	*Staphylococcus aureus*	18	16	14	13	24
2	*Micrococcus luteus*	21	19	15	11	25
3	*Escherichi coli*	20	18	16	15	25
4	*Salmonella typhi*	18	14	13	11	23

ZONE INHIBITION OF GRAM POSITIVE BACTERIA

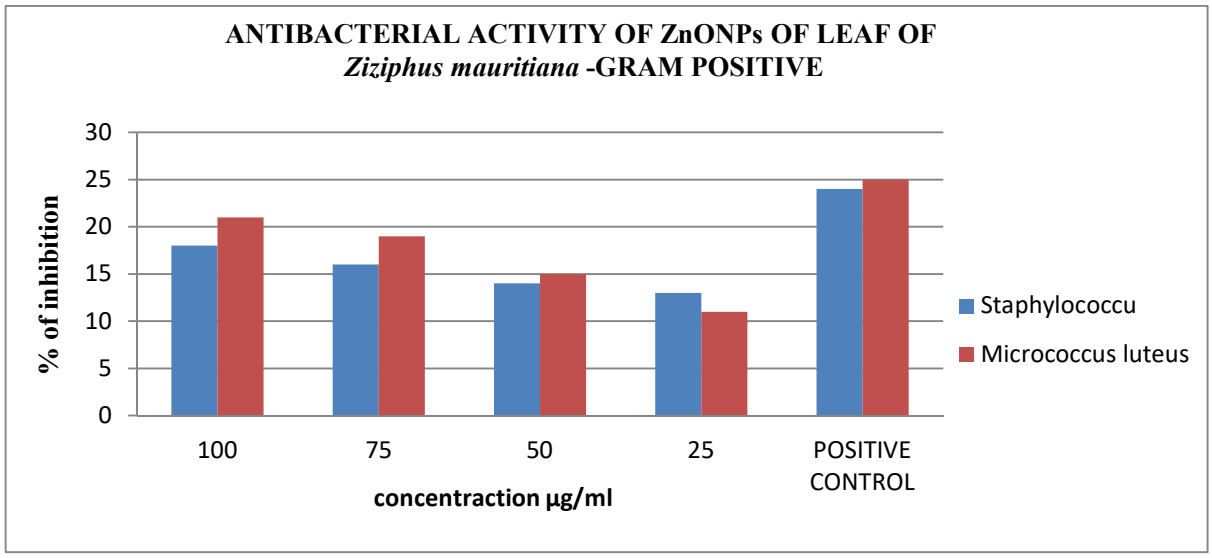

Figure 7: Zone Inhibition Of Gram Positive Bacteria

ZONE INHIBITION OF GRAM NEGATIVE BACTERIA

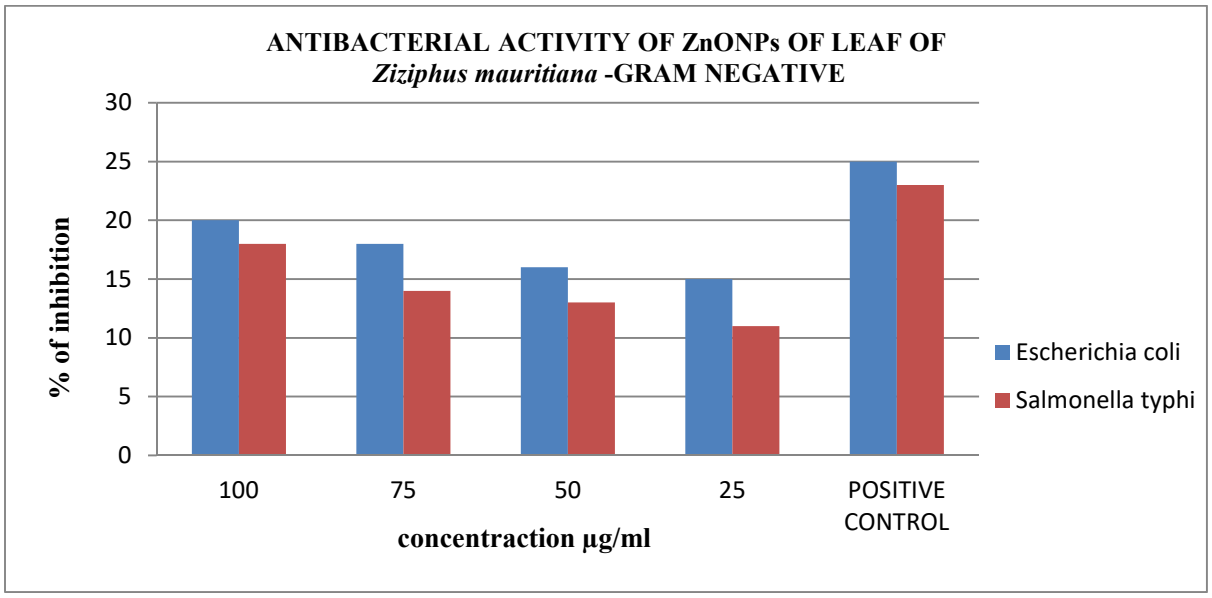

Figure 8 :Zone Inhibition Of Gram Negative Bacteria

ANTI-BACTERIAL ACTIVITY OF GRAM POSITIVE BACTERIA

(A) *Staphylococcus aureus* **(B) *Micrococcus luteus***

Figure 9: Anti-Bacterial Activity of ZnONPs against gram positive bacteria

ANTI-BACTERIAL ACTIVITY OF GRAM NEGATIVE BACTERIA

(C) *Escherichia coli* ***(D) Salmonella typhi***

Figure 10: Anti-Bacterial Activity of ZnONPs against gram negative bacteria

4. CONCLUSION

The synthesis of ZnONPs utilizing Ziziphus mauritiana leaf extract and its use for antibacterial activities are successfully reported in this study. The existence of proteins, carbohydrates, phenols, alkaloids, and flavonoids has been verified by qualitative analysis. The ZnONPs that were produced were examined using FT-IR, SEM, and XRD spectroscopy. The crystalline quality of the green produced zinc oxide nanoparticles is indicated by the strong and narrow XRD peaks. The SEM displays the agglomerates of porous nanoparticles that resemble sponges. According to the study, green synthesized ZnONps have strong antibacterial properties as well. Salmonella typhi and Escherichia coli are gram negative bacteria, while Staphylococcus aureus and Micrococcus luteus are gram positive bacteria. The medication chloramphenicol is used as the positive control. There are many benefits to this straightforward process, including cost

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