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Green Synthesis, Characterization and Antimicrobial Activity of Zinc Oxide Nanoparticles from Leave Extracts of *Ziziphus spina-christi* Plant

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Abstract

The escalating incidence of antimicrobial resistance presents a global health challenge, underscoring the need to explore alternative antimicrobial agents. Medicinal plants with their rich history of therapeutic use, provide a source of bioactive compounds which may be effective against resistant microorganisms. Concurrently, nanoparticles derived from metals exhibits remarkable antimicrobial efficacy due to their unique physical and chemical properties. This study aimed at green synthesis, characterization and antimicrobial activity of Zinc nanoparticles from Leave extracts of *Ziziphus spina-christi* plant. The phytochemical screening was carried out using standard method, antimicrobial activity was done using agar diffusion method. ZnO NPs were successfully synthesized through bio-reduction (using the leaves extract) and characterized using UV-Vis spectroscopy, FTIR, XRD, and SEM techniques. Phytochemical screening revealed the presence of terpenoids, saponins, flavonoids, and tannins. The antimicrobial activity result indicates that extract and ZnO NPs exhibit moderate antimicrobial activity against the selected bacteria and fungi. Notable findings include a zone of inhibition of 22.00 mm against *Escherichia coli* (*E. coli*) for *Ziziphus spina-christi* extract and zone of inhibition of 20.33 mm on *Staphylococcus aureus (S. aureus)* for ZnO NPs. These findings underscore the potential of *Ziziphus spina-christi* extracts and green-synthesized ZnO NPs as alternative antimicrobial agents. The characterization of the synthesized ZnO NPs showed a maximum wavelength of absorption at 281 nm with UV-Vis spectroscopy, indicating their successful synthesis. FTIR analysis identified specific functional groups indicated through major peaks at 2918.5 cm^{-1} and 2851.4 cm^{-1} resulting from methylene C-H asymmetric or symmetric stretch of alkane, 1729.5 cm^{-1} resulting from a carbonyl C=O stretch, 1364.2 cm^{-} alkene –C-H bending, 1315.8 cm^{-1} resulting from Amine C-N stretch, 1237.5 cm^{-1} resulting from acid C-O stretch and alcohol C-O stretch 1147.0 cm^{-} which are involved in the bio-reduction and stabilization of the NPs. XRD pattern analysis revealed a crystallite size of 45.73 nm.

Keywords: Bio-Reduction, Nanoparticles, Phytochemicals, Terpenoids, *Ziziphus spina-christi*.

Introduction

The increasing prevalence of antimicrobial resistance poses a global health challenge, necessitating the exploration of alternative antimicrobial agents. In recent years, the field of mainstream medicine has demonstrated a growing openness to utilizing antimicrobials and pharmaceuticals derived from botanical and alternative sources. This shift is due to the diminishing effectiveness of traditional antibiotics, which are either products of microorganisms or their synthetically engineered derivatives (such as penicillin and cephalosporin). These antibiotics, which have been in use for several decades to combat microbial pathogens, are now showing reduced efficacy against newly emerging diseases, particularly those caused by viruses. This is primarily because these conventional antibiotics are no longer effective in addressing these new and increasingly resistant pathogens. This phenomenon is referred to as Antimicrobial Resistance (Holubar, 2019).

The World Health Organization (WHO) has classified Antimicrobial Resistance as a global health emergency due to the proliferation of these resistant pathogens and their ability to share their resistance genes with other microorganisms (World Health Organization, 2021). Medicinal plants represent a valuable source from which antimicrobial agents can be derived. Throughout history, the plant kingdom has provided a rich repository of drugs and potential pharmaceuticals for treating various diseases. There are over 300,000 extant species of seed plants worldwide, and roughly 60% of these have a historical medicinal use. Only a small fraction, typically between 1% and 10%, are used as sources of food for both humans and other animal species (Jiao *et al.*, 2011).

Nanotechnology has also gained increased attention as a promising avenue for exploring potential antimicrobial agents to combat the growing challenge of antimicrobial resistance. Nanotechnology involves the design, characterization, production, and application of materials and devices at the nanometer scale, which typically falls within the range of 1-100 nanometers (Weir *et al.*, 2008). The small size of nanoparticles results in unique properties, including increased interactions with cells due to a higher surface area-to-mass ratio and versatile and controllable applications.

The distinct advantages of using nanoparticles as antibacterial agents, as opposed to traditional antibiotics, can be summarized as follows: they overcome existing antibiotic resistance mechanisms, engage multiple mechanisms simultaneously in combating microbes, and serve as effective carriers of antibiotics (Wang and Hu, 2017).

Moreover, an emerging aspect of the significance of plant extracts is their ability to facilitate the reduction of metal salts to their corresponding nanoparticles, a process now referred to as "Green Synthesis." This method provides an environmentally friendly and sustainable approach to nanoparticles synthesis, utilizing plant-derived compounds in the reduction process.Green synthesis is a widely recognized eco-friendly technique for producing nanoparticles that are not only relatively stable but also environmentally friendly and non-toxic, while maintaining a high level of effectiveness Chaunhan *et al.*, 2012 and Inagale *et al.*, 2013. Among the various nanoparticles, (ZnO NPs) stand out due to their

diverse applications. Multiple studies have indicated the potential of ZnO NPs as therapeutic agents for treating various types of cancer. Additionally, they exhibit notable antimicrobial activity against a wide range of bacteria, including both gram-positive and gram-negative types, as well as a diverse array of fungi (Jinhua *et al*., 2018).

Ziziphus spina-christi, commonly referred to as thorn Jujube, belongs to the Rhamnaceae family. It is a shrub that sometimes grows into a tall tree, reaching heights of up to 20 meters and a diameter of 60 centimeters. The bark is light grey, significantly cracked and scaly, with a twisted trunk, a much-branched structure, and a thick crown. The shoots are whitish, flexible, and drooping, and the plant is characterized by thorns occurring in pairs, one straight and the other curved. The leaves are glabrous on the upper surface and finely pubescent below. They are ovate-lanceolate or ellipsoid in shape, with an acute or obtuse apex and almost entire margins. Lateral veins are conspicuous (Asgarpanah and Haghighat, 2012). Ziziphus spina-christi grows wild in tropical regions of Nigeria and is traditionally used in indigenous medicine for the treatment of pain and various inflammatory conditions across the regions in which it is found.

Statement of the Problem

The discovery of antibiotics has had an incredible impact on the general health of humans by reducing the morbidity and mortality rates associated with microbial infections. Consequently, due to the abuse and misuse of these antibiotics have led to an increase in antibiotic resistance among many Microorganisms. The lack of new antibacterial and the rise of antibiotic resistance has led scientist to research and develop new antimicrobial agents from plants and other substance which would serve as potential drugs for combating the antimicrobial resistance.

Objectives of the Research

The specific objectives of this research are:

- To carry out the phytochemical screening of the ethanolic extract of leaves *Ziziphus spina-christi.*
- To synthesize ZnO nanoparticles through the bio reduction of a zinc metal salt by the leave extracts of *Ziziphus spina-christi.*
- Characterization of the synthesized ZnO nanoparticles using UV-visible spectroscopy, Fourier Transformed Infra-Red spectroscopy, X-ray Diffraction and Scanning Electron Microscope.
- To Estimate the antimicrobial activity of the leaves extracts of*Ziziphus spina-christi* on some selected pathogenic microbial strains.
- To Estimate the antimicrobial activity of synthesized Zinc oxide nanoparticles on some selected pathogenic microbial strains.

Methodology

Sample Collection and Preparation

Fresh leaves of *Ziziphus spina-christi*were collected from the Kanawa forest in Kwadom, Yamaltu Deba local government area of Gombe state, Nigeria. Taxonomic confirmation of the leaves as those of *Ziziphus spina-christi* was conducted at the Department of Biological Sciences, Gombe State University.The freshly collected leaves were carefully washed with distilled water and left to air-dry at room temperature under shade. Once dried, the leaves were ground into fine particles using a mortar and pestle. The resulting dried leaf particles were then sealed in polythene bags and stored at a temperature of 4°C within a refrigerator.

Extraction of the Leaf Extract

Exactly 10 grams of fine particles were soaked in 250 ml of ethanol. The mixture was left to sit at room temperature, with periodic stirring, for 48 hours. After this extraction period, the extract was filtered using Whatman No. 1 filter paper. The resulting filtrate was collected in a beaker and subsequently concentrated. This concentration process was carried out using a rotary evaporator. The concentrated filtrate (crude extract) was then collected in clean sterile containers and stored for future use.

Qualitative Phytochemical Screening

The qualitative phytochemical screening of the leaf extracts was performed using the methods described by A.O.A.C. (2005).

Test for Tannins

The solution of the extract was shaken with a small quantity of ferric chloride. The formation of a blue-green precipitate indicates the presence of tannins.

Test for Flavonoids

The solution of the extract was mixed with two drops of ammonia, resulting in a yellow-brown color, signifying the presence of flavonoids.

Test for Saponins

The solution of the extract was shaken with about 5 ml of distilled water and then heated to a boil. The formation of frothing indicates the presence of saponins.

Test for Alkaloids

The solution of the extract was warmed with 1% HCl for two minutes, filtered, and a few drops of Dragendorff's reagent were added. A reddish-brown color and turbidity with the reagent indicate the presence of alkaloids.

Test for Terpenoids

A mixture of 3 ml of various solvent extract, 1 ml of chloroform, and 1.5 ml of concentrated H_2SO_4 was created. The formation of a reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for Glycosides

A 0.1g of plant powder was soaked in 1 ml of glacial acetic acid with one drop of ferric chloride solution, followed by the addition of 1 ml of sulfuric acid. The presence of a brown ring indicates the presence of glycosides.

Green Synthesis of Zinc Oxide Nanoparticles

To synthesize ZnO NPs, the methods of Selim *et al*., (2020) was adopted and modified. 10 grams of dried leaves were placed in a 250 ml flask filled with distilled water and boiled for 20 minutes. The mixture was then allowed to cool at room temperature and filtered using Whatman No. 1 filter paper to obtain the filtrate. Subsequently, 50 ml of the filtrate was heated on a magnetic stirrer at temperatures ranging from 60°C to 80°C.

When the temperature reached 60°C, 5 grams of zinc nitrate hexahydrate was added, and the mixture was left for approximately one hour until a whitish precipitate was observed. This mixture was then left undisturbed for 12 hours in an oven until a creamy paste was formed. The paste was collected and washed several times with a solution of distilled water and ethanol. Finally, the collected paste was heated in a furnace at 400°C for 2 hours to obtain a white powder. This white powder was collected into a container and stored for further studies.

Characterization of the Synthesized Zinc Oxide Nanoparticles

To confirm the synthesis of ZnO nanoparticles, several analyses were conducted. Ultraviolet-Visible Spectrometer (Cary Series, Agilent Technology) was used for scanning the sample with a wavelength range of 200nm – 800nm. X-ray diffractometer (Bruker D8, Advance diffractometer, Coventry, UK) was employed for analyzing the crystalline material with Cu k-alpha radiation of wavelength 1.5402. Fourier-transform infrared (FT-IR) spectrometer (Perkin Elmer FT-IR) was utilized to detect the functional groups of the green-synthesized nanoparticles. The scanning was conducted within a wavenumber range of 500–4000 cm–1. Scanning electron microscopy (SEM) with a Nova NanoSEM 450 analyzer was used to produce high-resolution images of the synthesized nanoparticles. The SEM images were captured with an acceleration voltage of 10 KV.

Microbial Strains Selection and Growth Conditions

Various microbial strains, including both gram-positive and gram-negative bacteria, as well as fungi, were selected to assess the antimicrobial efficacy of the plant extracts and the synthesized ZnO nanoparticles. These microorganisms include *Staphylococcus aureus*,

Escherichia coli, *Salmonella typhi*, *Shigella*, *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, and *Aspergillus flavus*. Muller Hinton (MH) medium was used for the bacterial strains' growth, while Potato dextrose (PD) medium was employed for fungal strains.

Evaluation of Antimicrobial Susceptibility Testing Using Disc Diffusion Methods

Antibacterial and antifungal assays were performed using the agar well disc diffusion method in Mueller Hinton Agar (MHA) plates for bacteria and Mueller Hinton agar medium with added 2% glucose and 0.5µg/ml methylene blue dye for fungi. Pure colonies of the test microorganisms were suspended in sterile saline to match McFarland tube number 0.5 (1.5 × 106 CFU/ml). These adjusted organisms were swabbed onto the appropriate agar plates. Sterile paper discs (6 mm in diameter) were impregnated with specific concentrations of the plant extracts or nanoparticles, dried at 100°C for two hours, and placed on the inoculated agar plates. The plates were then incubated based on the growth requirements of each microorganism. The zones of inhibition were measured and recorded in millimeters (including the 6 mm disk).

This testing was conducted in triplicates, and the results provided insights into the antimicrobial activity of the plant extracts and ZnO nanoparticles (CLSI, 2009).

Results and Discussion

Phytochemicals

The qualitative phytochemical analysis of the plant extracts revealed the presence of terpenoids, saponins, flavonoids and tanins while alkaloids and glycosides were not detected. These findings correspond to the findings of Abalaka *et al.*, (2010). The presence of these phytochemical compounds in the *Ziziphus spina-christi* leaf extracts is of particular interest. Alkaloids and flavonoids, for example, are known for their potential antimicrobial properties. Furthermore, these phytochemicals can serve as bio-reducing and stabilizing agents in the green synthesis of nanoparticles, as has been observed in studies by Agarwal *et al.*, (2017) and Ramesh *et al.*, (2014).

Table 1: Phytochemical screening of crude extract of *Ziziphus spina-Christi* leaf

Phytochemicals	Results
Alkaloids	_
Terpenoids	+
Saponins	+
Flavonoids	+
Tannins	+
Glycosides	_

Keys: + = present, _ = absent

Characterization

ZnO nanoparticles biosynthesized utilizing the aqueous extracts of *Ziziphus spina-christi* due to its rich phytochemical contents. The UV-Vis spectrum analysis revealed that the synthesized nanoparticles have a maximum wavelength of absorption at 281 nm which is within the range for the specific absorption of ZnO nanoparticles due to surface plasmon resonance. This finding is also similar to results obtained by Shekhawat *et al*., (2014) who synthesized zinc oxide nanoparticles from *H. enneaspermus* and observed absorption peak at 300 nm from leaf extract, stem extract at 290 nm and the root extract at 288 nm. These differences in the maximum absorption wavelengths could be attributed to the difference in the synthesis approach and the phytochemical constituents of the parts of the plants utilized.

FTIR spectroscopic analysis of the synthesized ZnO nanoparticles aids in identifying the specific biomolecule functional groups that are directly involved in the bio reduction process of the zinc metal salts to form the nanoparticles and also the functional groups that are responsible for the stabilization and capping of the synthesized nanoparticles. The major signals and their corresponding functional groups identified from the figures that could be associated to the reduction of the Zinc metal salt are 2918.5cm^{-1} and 2851.4cm^{-1} resulting from methylene C-H asymmetric or symmetric stretch of alkane, 1729.5cm^{-1} resulting from a carbonyl C=O stretch, 1364.2cm^{-1} alkene –C-H bending, 1315.8cm^{-1} resulting from Amine C-N stretch, 1237.5cm^{-1} resulting from acid C-O stretch and alcohol C-O stretch 1147.0 cm^{-1}. This results are also similar to FTIR results obtained by Chaudhary *et al*., (2018) in their synthesis of ZnO nanoparticles from *Aloe vera* peel extracts and reported FTIR peaks at 3369.34 cm^{-1}due to O–H stretching vibration, at 2136.52 cm^{-1} (CC stretching vibration), 1626.42 cm^{-1} (C= stretching vibration), 1416.48 cm^{-1} (C–H deformation vibration), 1155.47 cm^{-1} (CH_3 deformation vibration), 1077.46 cm^{-1} (C–C *stretching vibration), 1044.45 cm^{-1}, 701.50cm^{-1} (CH_2 deformation vibration) and 472.52 cm^{-1} (C–C skeleton vibration).

XRD pattern analysis of the synthesized ZnO nanoparticles displayed a major diffraction band at2Θ value of38.036 corresponding to a height of 118.27 and several other minor bands. To obtain the size of the nanoparticles,these major band parameters were incorporated into the Scherrer formula which states that:

$$p = \frac{k\lambda}{\beta\cos\theta}$$

Where:

P – Crystallite size

K – Constant (0.9)

λ – Wavelength (1.542A°)

β – Full maxima half width

θ- Diffraction angle

The particle size of the synthesized ZnO nanoparticle was found to be 45.73 nm in size.

SEM image of the synthesized nanoparticles was produced to obtain the morphology of the synthesized nanoparticles. The obtained SEM image displayed an irregular grained topography, indicating that the surface of the nanoparticles is not uniform but rather exhibits variations in grain size and shape. Additionally, the image depicted the formation of aggregate nanocrystals, suggesting that the ZnO nanoparticles tend to cluster together to form larger structures.

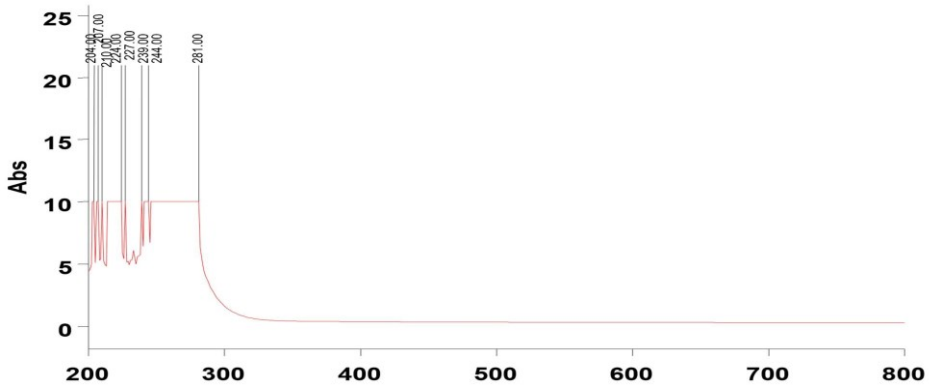

Fig. 1: UV-visible spectroscopy pattern of ZnO Nps of *Ziziphus spina-Christi* leaf

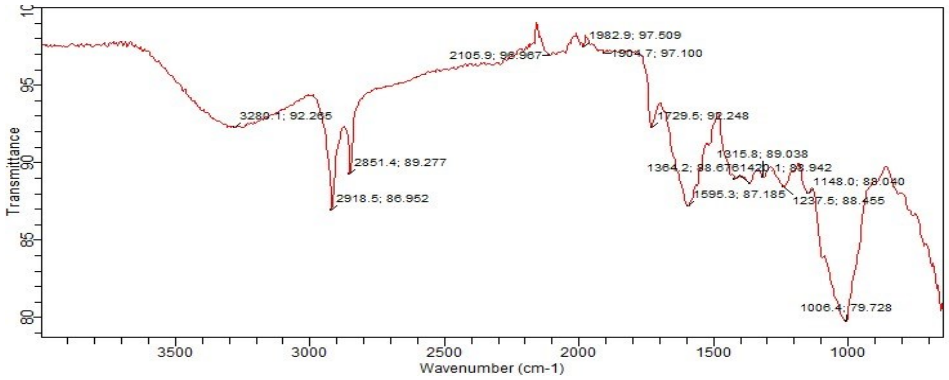

Fig. 2: FT-IR Spectra of leaves extract of *Ziziphus spina-christi* plant

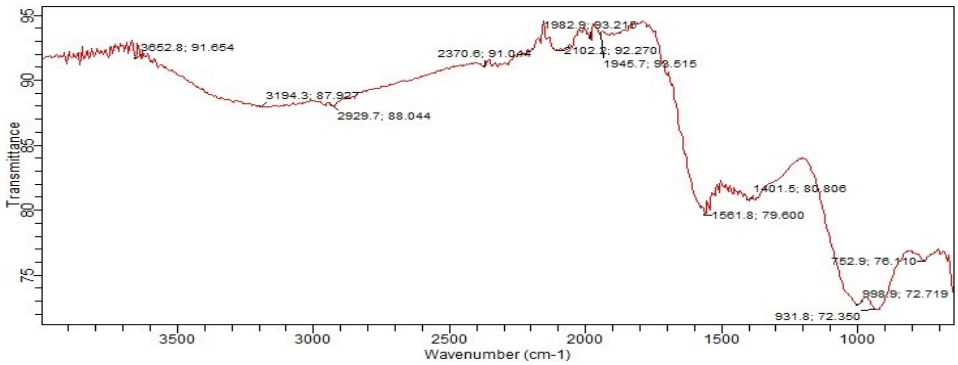

Fig. 3: FT-IR Spectra of ZnO Nps – of *Ziziphus spina-christi* leaf

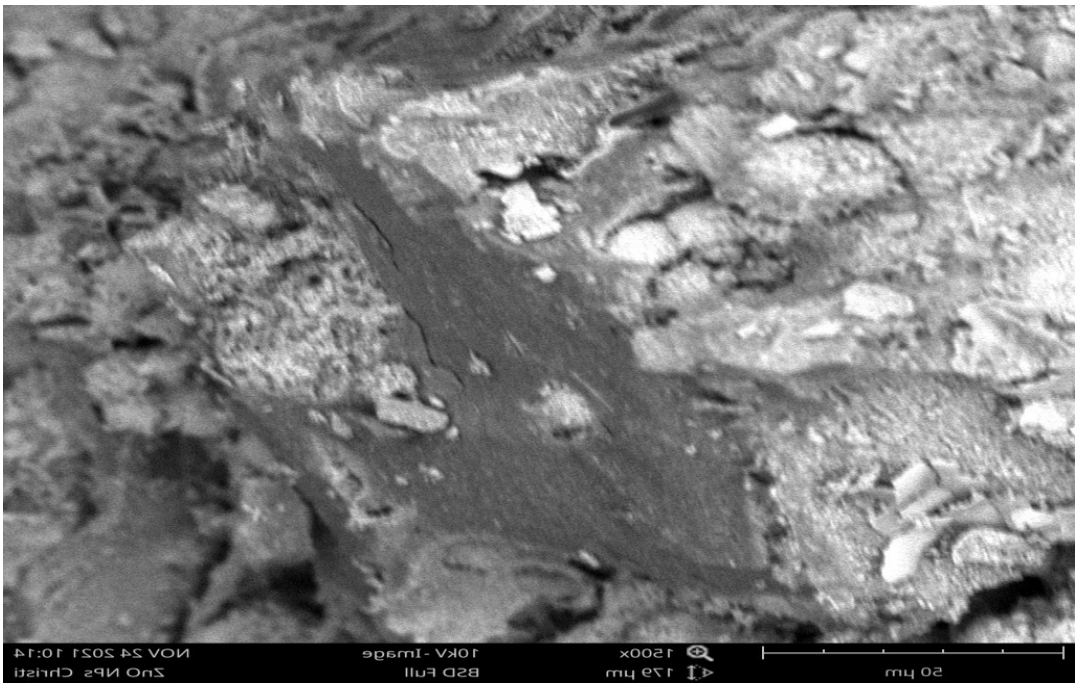

Fig. 4: SEM image of ZnO Nps of *Ziziphus spina-christi* plant

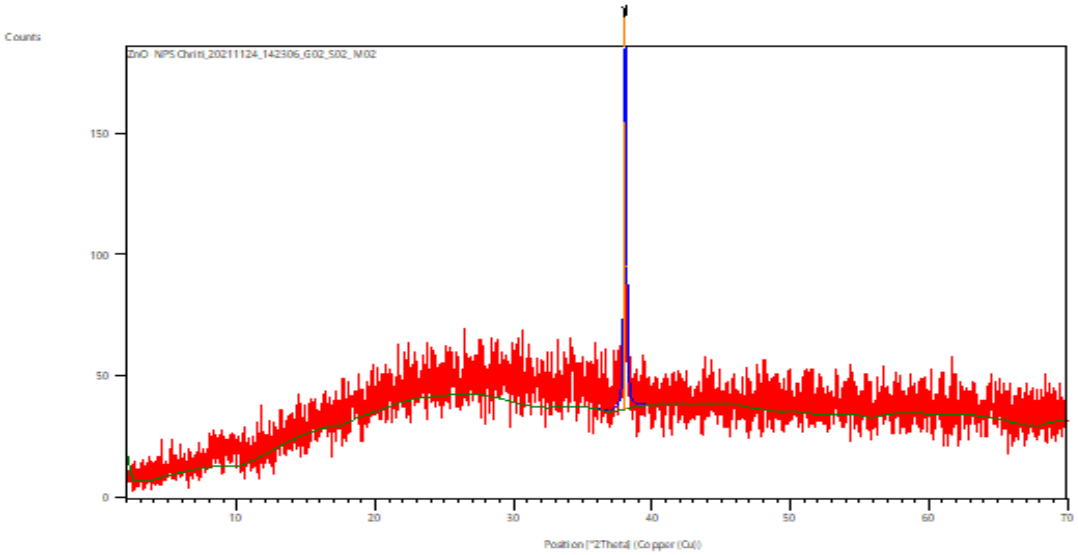

Fig. 5: XRD pattern of ZnO Nps of *Ziziphus spina-christi* leaf

Antimicrobial Analysis

In the antimicrobial analysis, the antibacterial and antifungal activities of both the *Ziziphus spina- christi* leaf extracts and the synthesized ZnO nanoparticles were evaluated against a range of microorganisms, including both gram-positive and gram-negative bacteria as well as fungi. The experiments were conducted using the disc diffusion method, and the results were observed by measuring the zones of inhibition in millimeters. To provide a basis for

comparison, positive controls were employed: Augmentin for antibacterial activity and ketoconazole for antifungal activity.

The antibacterial activity was assessed against a panel of bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Shigella*. These microorganisms were grown on Muller Hinton Agar medium, and the disc diffusion method was employed to evaluate the inhibitory effects of the leaf extracts and ZnO nanoparticles. The zones of inhibition, indicating the regions where bacterial growth was suppressed, were measured in millimeters. The experiments were conducted in triplicate to ensure the reliability of the results.

Similarly, the antifungal activity of the plant extracts and nanoparticles was tested against *Candida albicans*, *Candida tropicalis*, *Aspergillus niger* and *Aspergillus flavus*. These fungi were cultured on Potato Dextrose (PD) medium, and the disc diffusion method was applied to assess the inhibitory effects of the compounds. Zones of inhibition, representing areas where fungal growth was hindered, were measured in millimeters. Similar to the antibacterial experiments, the antifungal tests were also conducted in triplicate to enhance the accuracy and consistency of the results.

Table 2: Antibacterial susceptibility of leaves extract of *Ziziphus spina-christi* plant

	Zones of Inhibition (mm)			
Organisms	**500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**Aug. 30 mg µg/ml**
S. aureus	16.00±0.58	13.00±0.58	6.00±0.00	30.33±3.84
S. typhi	16.67±0.67	13.33±0.88	6.67±0.33	31.33±2.03
E.coli	22.00±0.58	15.00±2.08	8.67±0.67	25.67±1.76
Shigella	21.00±0.58	14.67±0.67	10.67±0.33	21.33±1.76

All measurements were carried out in millimeter (mm), the results were recorded as mean ±standard error of mean and Aug. 30 mg (Augmentin) is the positive control for the bacteria. The results of the antibacterial activity of the *Ziziphus spina-christi* leaf extracts, as shown in Table 2., reveal that the plant extracts exert a moderate effect on the tested bacterial strains, which include *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli* and *Shigella*. The inhibition areas observed varied with the concentration of the plant extract used, with the highest inhibition area of 22.00±0.58 mm recorded for *Escherichia coli* at a concentration of 500 µg/ml.

It is worth noting that *Staphylococcus aureus* and *Salmonellatyphi* exhibited a slower response to the plant extracts, with no significant inhibition area recorded at a concentration of 125 µg/ml. These findings are in accordance with results obtained by

Abalaka *et al*. in their 2010 study, which evaluated the antimicrobial activities of two *Ziziphus* species, *Ziziphus mauritiana* and *Ziziphus spina-christi*. In Abalaka's study, the zone of inhibition recorded for *Staphylococcus aureus* was 15.00±1.00 mm, and for *Escherichia coli*, it was 17.00±3.00 mm. This similarity in results suggests that *Ziziphus spina-christi* leaf extract may exhibit consistent antibacterial effects across different studies.

Interestingly, in a more recent study by Kamil *et al*. in 2021, ethanolic extracts of *Ziziphus spina-christi* leaves showed a higher inhibition area of 24±0.1 mm at a concentration of 100 µg/ml on *S. aureus*. This value is significantly greater than the inhibition area of 16.00±0.58 mm observed in the current research at a concentration of 500 µg/ml. The substantial difference in results may be attributed to variations in the phytochemical constituent concentrations of the plant extracts, which could arise due to differences in the geographic locations of the plants used by the researchers.

Table 3: Antifungal susceptibility of leaves extract of*Ziziphus spina-christi*plant

	↓ Zones of Inhibition (mm) ↓			
Organisms	**→ 500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**kt50 mg µg/ml**
C. albicans	16.67±1.33	12.00±0.58	7.67±0.67	24.67±1.86
C. tropicalis	17.33±1.67	11.67±0.88	6.00±0.00	26.00±2.08
A. niger	17.33±0.67	11.33±0.88	6.00±0.00	24.33±2.03
A. *flavus*	20.00±0.58	10.33±0.33	8.67±0.67	23.00±1.15

Key: Kt.50mg (Ketoconazole) is the positive control for the fungi

All measurements were carried out in millimeter (mm), the results were recorded as mean ±standard error of mean.The results of the antifungal activity of *Ziziphus spina-christi* leaf extracts, as presented in Table 3, demonstrate that the plant extracts exhibit moderate activity against the tested fungal organisms, which include *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, and *Aspergillus flavus*. The inhibitory effects varied with the concentration of the plant extract used, with the highest inhibition area of 20.00±0.58 mm observed on *Aspergillus niger* at a concentration of 500 µg/ml.

Notably, no inhibition zones were observed on *Candida tropicalis* and *Aspergillus niger* at a concentration of 125 µg/ml. These findings suggest that the plant extract may be more effective against certain fungal species at higher concentrations.Interestingly, these results differ from those obtained by Abalaka *et al*. in their 2010 study, in which their extracts did not demonstrate any activity against the test fungi. This discrepancy in results may be attributed to differences in the phytochemical composition of the plant extracts, variations in the fungal strains used, or methodological differences between the studies.

Table 4: Antibacterial susceptibility of ZnO Nps*Ziziphus spina-christi*leaf

	↓ Zones of Inhibition (mm) ↓			
Organisms	**→ 500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**Aug. 30 mg µg/ml**
S. aureus	20.33±1.20	14.67±2.33	13.33±1.45	29.00±2.08
S. typhi	9.33±2.03	8.67±1.45	6.00±0.00	26.67±2.19
E.coli	15.33±2.33	9.33±0.88	10.33±1.45	25.33±1.86
Shigella	9.67±1.20	7.67±0.88	6.33±0.33	20.00±2.52

Key: Aug.30 mg (Augmentin) is the positive control for the bacteria

All measurements were carried out in millimeter (mm), the results were recorded as mean ±standard error of mean. The results for the antibacterial susceptibility of the synthesized ZnO nanoparticles (ZnO NPs), as shown in Table 4, indicate that the ZnO NPs possess moderate antibacterial activity against the test bacterial organisms. The highest inhibition area of 20.33±1.20 mm was recorded for *Staph. aureus* at a nanoparticle concentration of 500 µg/ml.

These findings are consistent with those of Naseer *et al*. (2020), who conducted a study on the antimicrobial activity of Zinc oxide nanoparticles against several bacterial strains, including both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli*, *Klebsiella Pneumoniae*, and *Pseudomonas aeruginosa*). Naseer and colleagues also observed significant antibacterial activity on all the tested bacterial strains, supporting the effectiveness of ZnO nanoparticles in inhibiting bacterial growth.

Table 5: Antifungal susceptibility of ZnO Nps *Ziziphus spina-christi*leaf

	↓ Zones of Inhibition (mm) ↓			
Organisms	**→ 500 µg/ml**	**250 µg/ml**	**125 µg/ml**	**kt50 mg µg/ml**
C. albicans	12.67±2.03	7.67±0.88	6.00±0.88	28.00±3.00
C. tropicalis	15.67±2.40	14.67±2.33	10.00±0.58	23.00±1.00
A. niger	7.67±1.67	6.00±0.00	6.00±0.00	28.67±0.50
A. flavus	20.33±1.20	9.33±0.88	7.67±0.88	24.33±2.50

Key: Kt.50mg (Ketoconazole) is the positive control for the fungi

All measurements were carried out in millimeter (mm), the results were recorded as mean ±standard error of mean, similarly, the results for the antifungal susceptibility of the synthesized ZnO nanoparticles, presented in Table 5, indicate that ZnO NPs have a moderate ameliorating effect on all the tested fungal organisms. The highest inhibition

area of 20.33±1.20 mm was recorded for *Aspergillus niger* at a nanoparticle concentration of 500 µg/ml.

These results align with those of George *et al*. (2021), who investigated the antimicrobial activity of Zinc oxide nanoparticles (ZnO NPs) against various microorganisms, including both gram-positive (*Staphylococcus aureus* and Bacillus subtilis) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), as well as fungi (*Aspergillus niger* and *Candida albicans*). George and colleagues also observed notable antifungal activity against the tested fungal strains, supporting the potential of ZnO NPs in inhibiting fungal growth.

Conclusion

This study justifies the potential of *Ziziphus spina-christi* plant extracts and green-synthesized Zinc oxide nanoparticles as alternative antimicrobial agents. The moderate antimicrobial activity observed against a variety of microorganisms presents opportunities for further research and applications in the fight against microbial pathogens.

This study also emphasizes the importance of harnessing the antimicrobial potential of natural resources and green nanotechnology to combat the challenges posed by drug-resistant microorganisms. It opens the door to further exploration and innovation in the field of antimicrobial research and applications.

Recommendations

Further studies should be carried out to determine the minimum inhibition concentration (MIC) and the minimum bactericidal concentrations (MBC) values of the leaf extracts of *Ziziphus spina Christi* plant and its corresponding zinc oxide nanoparticles.

Conflicts of Interest

The authors declare no conflicts of interest.

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