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Synthesis of Zinc Oxide Nanoparticles from Medicinal Herb *Bryophyllum pinnatum*.

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ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) have attracted considerable attention in several sectors, including medical and health technology, and environmental science, due to their unique properties consisting of a huge surface area, biocompatibility, and antimicrobial activity. This study aims to synthesize ZnO nanoparticles using a green, eco-friendly approach by employing *Bryophyllum pinnatum*, a widely known medicinal herb, as a naturally occurring stabilizing and reducing agent. *Bryophyllum pinnatum* is a prospective option for the synthesis of nanoparticles because of its many medicinal uses in traditional medicine, which include anti-inflammatory, antibacterial, and antioxidant activities. Under carefully monitored circumstances, an aqueous extract of *Bryophyllum pinnatum* was combined with a zinc salt precursor (zinc acetate) to create ZnO NPs. scanning electron microscopy (SEM), X-ray diffraction (XRD), and UV-visible spectrophotometry were used to track the reduction of zinc ions and the creation of ZnO nanoparticles. The ZnO NPs' hexagonal structure, as revealed by XRD analysis, is in line with ZnO's known crystal structure. With an average size range of 20–50 nm, the nanoparticles' spherical and evenly distributed shape was revealed by SEM analysis. These dimensions are in line with those of ZnO nanoparticles produced using environmentally friendly techniques. Significant antibacterial action against a range of bacterial strains was shown by the produced ZnO nanoparticles, indicating their potential for usage in medical applications such infection control and wound healing. The ZnO NPs' potential for therapeutic uses was further increased by antioxidant testing that revealed significant free radical scavenging activity. To sum up, this study demonstrates the ecologically friendly production of ZnO nanoparticles utilizing *Bryophyllum pinnatum*, offering a substitute for traditional chemical synthesis techniques. The biosynthesized ZnO NPs demonstrated advantageous traits for biomedical uses.

INTRODUCTION

Nanoparticles, ranging from 1 to 100 nanometer, are characterized as organic, inorganic, or carbon-based. Their small size enhances properties like reactivity, strength, surface area, sensitivity, and stability compared to bulk materials. Nanoparticles are synthesized using mechanical, chemical, and physical methods, with significant advancements over time for research and commercial applications (1). Nanoparticles, known for their antibacterial and antioxidant properties, have gained attention for use in a variety of sectors, such as food technology and medical. The study compares chemically synthesized ZnO nanoparticles (Chem-ZnO) with Biogenic ZnO nanoparticles (BPLE-ZnO) made from leaf extract from *Bryophyllum pinnatum*, analysing their properties and photocatalytic activity using multiple characterization techniques (2). Numerous studies support the potential of nanotechnology in addressing agricultural challenges, including disease control and plant health (3). Nanotechnology encompasses the design, production, and application of nanoscale structures (0.2–100 nm), while nanobiotechnology focuses on biological applications. Nanoparticles can be made via top-down or bottom-up methods, influenced by various factors. Biological methods, particularly using plant extracts, are preferred for their simplicity, reliability, eco-friendliness, and ability to produce high-quality, precisely-sized nanoparticles more rapidly than other methods.

Green synthesis using medicinal herbs for nanoparticle production avoids harmful chemicals from traditional methods, offering an eco-friendly alternative. Medicinal plants, used since ancient times, are utilized for their therapeutic properties (4). Nanoparticles are classified into inorganic (e.g., magnetic, noble metals like gold and silver, semiconductors like zinc oxide) and organic (e.g., carbon-based). Inorganic nanoparticles, particularly noble metals, are valued for their superior properties, versatility, and potential in medical imaging, disease treatment, and controlled drug delivery due to their compatibility and functionality (5). In history, nanoparticles were solely produced by physical and chemical means. Physical and chemical methods that are frequently used comprise ion spattering, solvothermal synthesis, reduction, and the sol gel approach. Nanoparticle biogenesis involves reduction and oxidation reactions. Green synthesis offers a cost-effective, safer alternative to chemical methods, avoiding harmful compounds often absorbed during chemical production, ensuring better suitability for medical applications. Scientists use microbial enzymes and plant extracts for cost-effective nanoparticle synthesis, leveraging their reducing properties for greener, safer alternatives to chemical and physical methods (5). *Bryophyllum pinnatum* (Crassulaceae)

exhibits various pharmacological activities, including antiviral, antimicrobial, anti-inflammatory, antidiabetic, and diuretic effects. It contains bioactive compounds like flavonoids (luteolin, quercetin), alkaloids, tannins, and anthocyanidins, contributing to its anti-inflammatory, antibacterial, and kidney stone-removal properties, as well as its antioxidant and diuretic effects (6). *Bryophyllum pinnatum*, Native to Madagascar and extensively cultivated in tropical and subtropical areas, it is popular for its traditional medicinal uses. for its numerous medicinal properties and the production of plantlets from phylloclade edges (7).

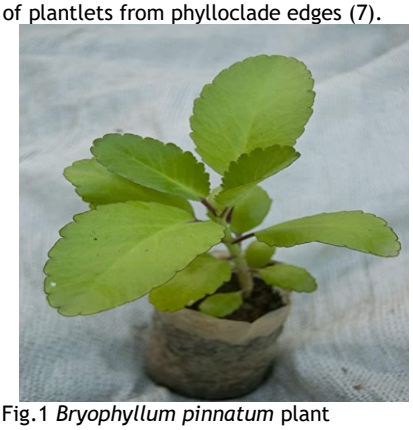

Fig.1 *Bryophyllum pinnatum* plant

Bryophyllum pinnatum, used globally for its anthelmintic, hepatoprotective, anti-inflammatory, and other medicinal properties, contains bioactive compounds like alkaloids, flavonoids, triterpenes, and glycosides with synergistic pharmacological effects (7). *Bryophyllum pinnatum*, a succulent annual, thrives in hot, humid climates like Bengal, reaching 1-1.5 meters. Its hollow, four-angled stem branches, and opposite, succulent leaves (10–20 cm) display distinctive dark green color with scalloped, scarlet-edged margins. The compound leaves have 3–5 leaflets, with latent vegetative buds. Terminal panicle inflorescences measure 10–40 cm. The pinnately compound leaves have 3-5 leaflets, 10–30 cm long, with crenate edges and latent vegetative buds. Inflorescences are 10–40 cm (8). The plant thrives in hot, humid climates, particularly Bengal, generating bell-shaped scarlet to purple flowers. Its fruit contains smooth, striate seeds and four septa. Known for homeostatic and wound-healing properties, it tastes sour, astringent, with a sweet aftertaste. The plant is valued for its therapeutic properties in both modern and traditional medicine (8). *Bryophyllum pinnatum*, used in traditional medicine for treating various ailments, contains secondary metabolites like steroids, flavonoids, and bufadienolides. Studies show its extracts exhibit antibacterial, antihypertensive, anticancer, anti-diabetic, anti-ulcer, and immunomodulatory effects (9,10). Green-produced nanoparticles are efficient agents in biomedicine, energy conversion, environment, and electronics, with notable catalytic and antibacterial applications (11). *Bryophyllum pinnatum* was intended for the green synthesis of ZnO nanoparticles (ZnONPs), which were characterized to assess their physio-chemical properties. Their antibacterial, antifungal were also evaluated for the first time (12).

4.2. Material and Methodology:

4.2.1. Sample Collection:

The *Bryophyllum pinnatum* Plant leaves were obtained from the Botanical Garden in Yashavantrao Chavan institute of science Satara & from household premises (13).

4.2.2. Preparation of Leaves extract:

Preparing *B. pinnatum* Leaf Extract and ZnO NPs: 30 grams of fresh B. pinnatum leaves were gathered, properly cleaned on both surfaces with lots of distilled water to get rid of any dust, and then dried to get rid of any remaining moisture. After that, leaves were outward sterilized with an unqualified alcohol. The leaves were chopped into small pieces. After transferring the cut leaves to a 250 ml conical flask with 100 ml of sterilized distilled water, they were heated for 15 minutes. Within 60°C. Following that, Whatman's filter paper no. 1 was used to filter the extract, and vacuum was used to further filter it. Filter with 0.2 µm pores. For future usage, the finished filtrate was kept in a dry, cool location (13, 16).

4.2.3. Synthesis of ZnO NP's using *B. pinnatum* leaf extract:

After heating 100 ml of the previously prepared leaf extract at 45°C for 10 minutes, 100 milliliter of a 1 mM zinc acetate solution was added, and the combination was agitated for five minutes using a magnetic stirrer. After that, 1000 µl of 1mM NaOH was added dropwise while being stirred. After that, the reaction mixture was left alone for 40 minutes to allow for full reduction and NP precipitate development. After stirring, the complex was collected and centrifuged for 10 minutes at 10,000 rpm. Following a two-hour transfer of the particles to a silica crucible cup and muffle furnace at 500°C, the result was a powder with a grey color that was properly collected and packaged for characterization. Using a mortar and pestle, the material was crushed to a finer consistency for characterization. (13,17).

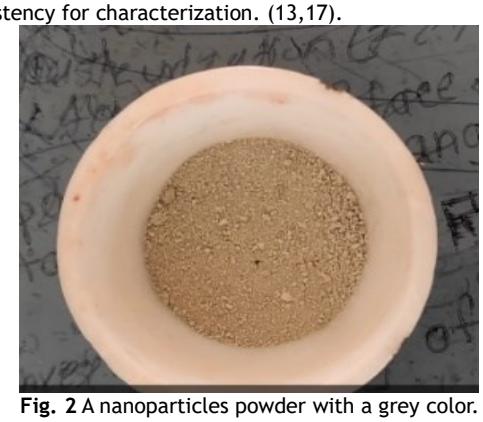

Fig. 2 A nanoparticles powder with a grey color.

4.2.4. Characterization of Synthesized ZnO Nanoparticles:

In order to confirm the synthesis of ZnO NPs and identify their size, shape, and morphology, a number of analytical techniques were employed, such as X-ray diffraction (XRD) and scanning electron microscopy (SEM). (14).

XRD spectroscopy:

Using an X-ray Diffractometer, the phase structure and material identification of ZNPs were investigated. In the wavelength range of 1.20 Ao, XRD scanning was carried out using the X-Ray Diffractometer. With an angle slit of 0.3 mm in 2θ/θ, the XRD scanned continuously within the range of 2θ of 10°-80° at 40 kV and 40 mA (14, 18).

SEM microscopy:

Scanning electron microscopy was used to analyze the generated nanoparticles' size, shape, and morphology. The produced nanoparticles were suspended in a diluted solution and sonicated for five minutes. After applying a drop of the prepared suspension to the gold grid, it was left for fifteen minutes to dry. The dried sample was then loaded into the pattern holder. The ZEISS EVO 18 SEM was used for the SEM examination (14,19).

4.2.5. Antimicrobial activity:

Zinc oxide nanoparticle solution was tested for antagonism using a plate assay after being infected with a bacterial strain that had been grown overnight. The inhibition zone was assessed to ascertain the antimicrobial activity (20). Numerous bacterial species, including Streptococcus, Proteus, Bacillus, Pseudomonas, and Salmonella, have been used to assess ZnO's in vitro antibacterial activity. In this study, the antibacterial properties of generated ZnO nanoparticles were investigated using a well-based diffusion technique and Gram-positive and Gram-negative bacteria. The nanoparticles' antibacterial activity was evaluated at different concentrations, ranging from 5 mg/mL to 10 mg/mL and 15 mg/mL. Growing material and lab equipment were autoclaved for 30 minutes at 115 °C and 15 pressure to disinfect them. The antibacterial activity was examined using the disc diffusion method. *Streptococcus, Proteus, Bacillus, Pseudomonas,* and *Salmonella* culture media were inoculated into 10 milliliters of nutritional agar solution to create a stock of bacteria that would multiply and regenerate the bacteria. A bacterial stock was created in order to inoculate culture media with bacteria in order to multiply and revitalize them. *Salmonella, Bacillus, Streptococcus, Proteus,* and *Pseudomonas* were placed in 10 milliliters of nutritional agar solution and incubated for 24 hours at 37 degrees Celsius. To test the bactericidal activity, the

inhibition approach was applied. After filling a petri dish with 150 mL of the nutrient agar mixture, the plate was disinfected for 20 minutes to solidify the solution in the nutrient agar. 0.1 milliliters of the microbial solution were then introduced into the growth solution. After 18 hours of incubation at 37 ˚C, the clear zone diameter was determined (13).

This feature plays a significant role in NPs' antibacterial activity. The antibacterial activity of ZnO NPs is also enhanced by increased concentrations; one of the main mechanisms explaining this activity is production (21).

4.2.6. Antifungal activity:

Aspergillus Niger and *Candida albicans*, two harmful fungi, were taken from the culture collection of the Ycis Institute of Science's Microbiology Laboratory. Satara *Aspergillus* and *Candida albicans* were cultivated on MH Agar plates at 25 ˚ C without light. With few modifications, the agar dilution method was used to conduct antifungal tests. ZnO NPs were added to autoclaved MH Agar media at doses of 5 mg/ml, 10 mg/ml, and 15 mg/ml. Following the solidification of the MH medium, the fungi were injected. Following that, the Petri dish containing the inoculums was incubated at 25 ˚C. The diameter of fungal colonies was used to gauge how well ZnO NP treatment worked. Every test was performed three times, and the result obtaining reported in centimeters (22).

In the studies, extracts of *Bryophyllum pinnatum* showed impressive efficacy against most of the test fungi (23).

4.2.7. Antioxidant Activity:

Antioxidants are a type of prevention against oxidants. Natural or artificial compounds known as antioxidants can stop or slow down oxidative cell damage (24). The DPPH assay, using a spectrophotometer or colorimeter, analyses antioxidants' capacity to reduce DPPH radicals. Variations in results arise from different methods, suggesting a standardized approach considering solubility, pH, and light sensitivity (25). Antioxidants inhibit oxidation, with primary antioxidants breaking chains and secondary antioxidants preventing oxidative damage. both are essential for protection (26). Oxidants cause oxidation; antioxidants inhibit it, with *Coriandrum sativum* tested by DPPH assay. Several concentrations of plant extracts were mixed with DPPH and incubated at 37℃ at dark conditions. The procedure involved dispersing 1 mL of ZnO NPs solution at 200, 400, 600, 800, and 1000 µg/mL concentrations

in methanol and then adding 3 ml of 100 µl DPPH produced in methanol. After being sonicated, the mixture was left in the dark chamber for half an hour. An additional experiment examined the absorbance trend of ZnO NPs scavenging activity using different doses (200, 400, 600, 800 and 1000 µg/mL). (33) DPPH scavenging a free radical. Following the incubation period, the absorbance of a specific tube was recorded at 530 nanometers. The percentage of free radical scavenging potential (FRS) of DPPH was calculated using the provided formula (Foudahet al., 2021).)

$$\text{Scavenging Activity (\%)} = \frac{(Ac - At)}{Ac} \times 100$$

Where Ac and as are absorbance of control and sample at 517 nm, respectively. (27, 28).

RESULTS AND DISCUSSION

5.1. Synthesis of Zinc oxide nanoparticles

The colour variations in the current investigation verified that ZnONPs were formed. After adding zinc acetate, The color of the solution shifted from dark yellow to pale apple greenish, indicating the ZnO NP synthesis, as showed in (Figure 3.)

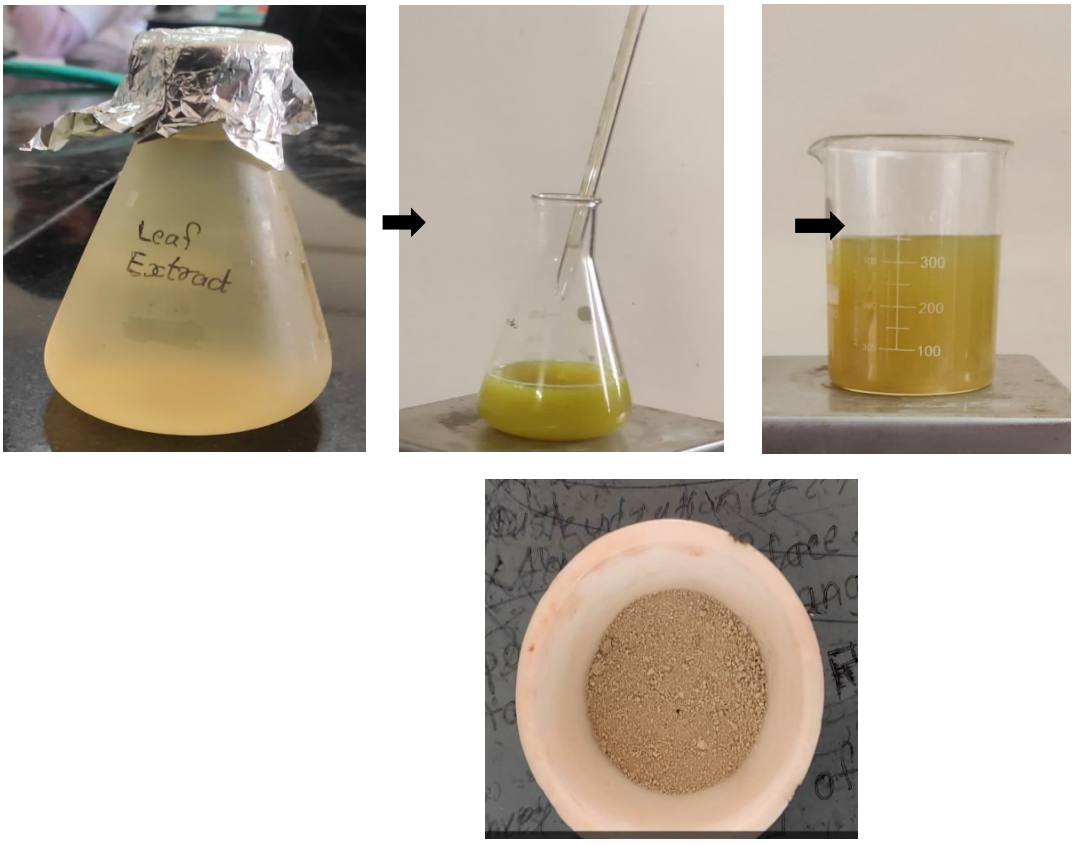

Fig .3. Color change indicates zinc-induced reaction or complex formation in solution.

5.2. Characterization of ZnO NPs

1.2.1. X-ray Diffraction Analysis

XRD was used in subsequent research to elucidate the nature of nanoparticles. (Figure 4) The peak location was in line with ZnO NPs that were metallic. A monochromatic X-ray beam was projected onto the material as the basis for this procedure. Out of the overall percentage of atomic mass of ZnO nanoparticles, we found that the masses of O = 24.72+2.32 and Zn = 75.28+7.76 in XRD. The material's crystalline structure, The X-ray diffraction (XRD) data provides information on phase purity and lattice characteristics., which show unique peaks that correspond to the material's crystallographic planes. These details shed light on the composition of the material and its possible uses.

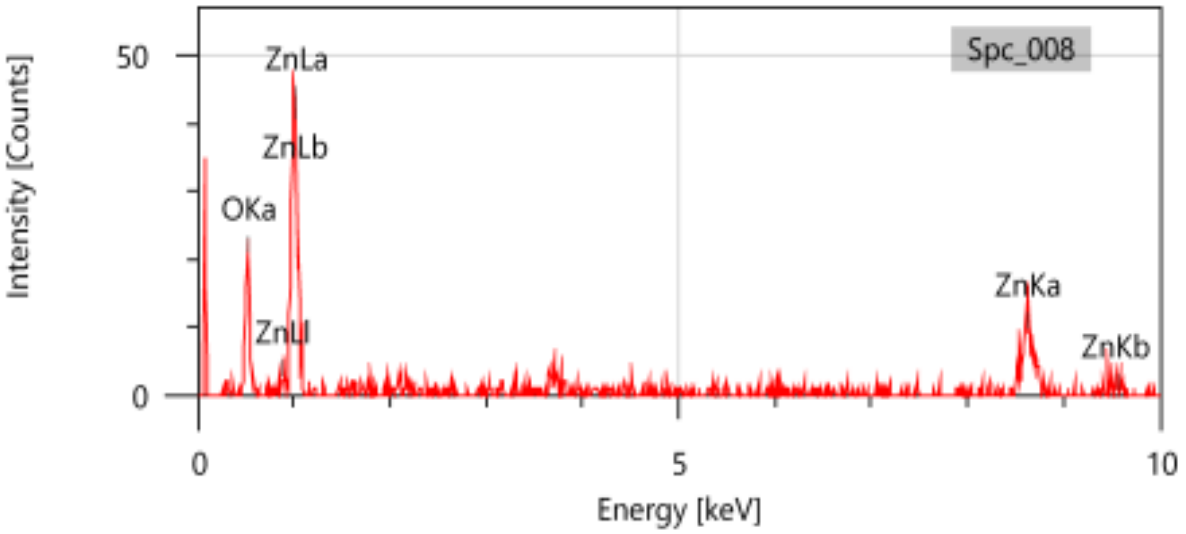

Fig 4. XRD graph showing the biogenic ZnO NPs' absorption wavelength

Table 1. EDAX showing the mass of ZnO nanoparticles in percentage.

Display name	Standard Data	Quantification method	Result Type
Spc_008	Standardless	ZAF	Metal

Element	Line	Mass%	Atom%
O	K	24.72+2.32	57.30+5.38
Zn	K	75.28+7.76	42.70+4.40
Total		100.00	100.00
Spc_008		Fitting ratio 0.5259	

1.2.2. SEM Analysis:

1.2.3. SEM, or Scanning Electron Microscopy, was utilized to describe the morphology of ZnONPs. This indicated that the appearance was, on average, between 50 and 70 nm in (Table no.2). The product is mainly made up of particles like ZnO NPs, as seen by the typical SEM pictures in Figures 0.5 and 6 (12).

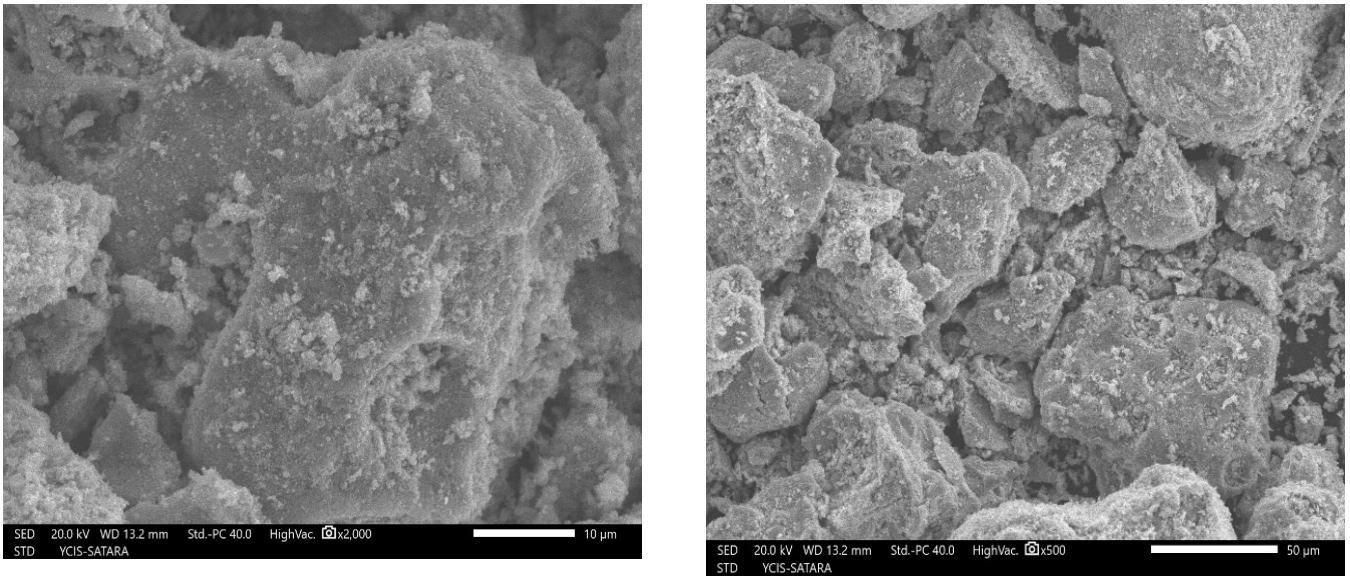

Fig 5. Scanning electron microscopy (SEM) image of *B. pinnatum* mediated ZnO NPs

Table 2. Size of nanoparticle.

No.	Value
1	53.76 nm
2	62.81 nm
3	67.13 nm
4	56.88 nm
5	56.61 nm

5.3. Antimicrobial activity:

ZnO nanoparticles' antimicrobial activity was examined against a number of bacterial species, including *Salmonella Typhi, Pseudomonas, Bacillus*, *Streptococcus,* and *Proteus*. When the antimicrobial assessment is conducted against Gram positive bacteria (*Bacillus and Streptococcus*), it is shown that the zone of inhibition changes for inhibitions of ZnO NPs with varying doses. *Salmonella typhi, Pseudomonas*, and *Proteus* are gram-negative. The zone of inhibition of prepared ZnO nanoparticles' antibacterial activity against the pathogen is depicted in Table .3 and Figure.7 along with the diameter of the zone of inhibition for the various ZnO NPs and the different concentrations used for each sample. These five, ten, and fifteen mg / ml are the concentrations.and in relation to other media, such as MH agar and NA agar.

The ZnO nanoparticle made by reducing zinc precursor using Pinnatum leaf extract has superior antibacterial properties against a greater number of microorganisms, according to the relative assessment of antimicrobial activity of synthesized ZnO NPs with respect to processing framework.

Table 3. Zone diameter reflects nanoparticle concentration effectiveness on bacterial growth.

Organisms	Zone of inhibition with different concentration (diameter in mm)		
	5mg/ml	10mg/ml	15mg/ml
Salmonella typhi	0	12	16
Pseudomonas	13	14	0
Bacillus	10	15	0
Streptococcus	14	17	13
Proteus	0	15	12

Antimicrobial activity by using *Streptomycin* Antibiotic:

Using streptomycin as a reference, ZnO NPs' antimicrobial properties mediated by *B. pinnatum* was evaluated against four bacterial strains. According to Table. 4 and Figure 9, the current investigation demonstrated the biosynthesized ZnO NPs from *B. pinnatum* to have strong antibacterial properties (12).

Table 4. Diameter of zone of inhibition of nanoparticles against the antibiotic and control.

Organisms	Streptomycin (diameter in mm)	Nanoparticle (diameter in mm)
Salmonella Typhi	13	9
Pseudomonas	12	6
Bacillus	11	2
Streptococcus	12	5

5.4. Antifungal activity:

Researchers looked into one of the many advantages of biosynthesized zinc oxide nanoparticles against harmful fungus species. To test the inhibitory impact on MH agar media, several concentrations of nanoparticles. The dosages were 5 mg/ml, 10 mg/ml, and 15 mg/ml. The antifungal activity result showed that the *Aspergillus* species' growth was significantly inhibited. According to Table 5 and Figure 10, our results also demonstrate that ZnO nanoparticles at lower concentrations are more successful in controlling disease. Additionally, the particles of nanoparticles play a major part in illness management in an environmentally benign manner. Scientists have successfully employed ZnO Nano material in recent years to inhibit the expansion of a variety of fungi, including *Aspergillus Niger, Fusarium graminearum,* and *Candida albicans* (33,34). The findings on ZnO nanoparticles' antifungal activity are consistent with earlier studies (35, 36).

Table 5. Nanoparticle inhibition zone diameter varies with concentration against fungal species.

Organisms	Zone of inhibition with different concentration (diameter in mm)		
	1mg/ml	3mg/ml	5mg/ml
Candida albicans	18	16	18
Aspergillus Niger	17	18	20

5.5. Antioxidant activity:

Utilizing *Bryophyllum pinnatum* tuber extract, The DPPH assay, which is commonly used to examine the radical scavenging

activity of green synthesized NPs, was utilized to evaluate the antioxidant capability of green produced ZnO single bond NPs. The deep violet color gradually turned pale yellow when ZnO NPs and ascorbic acid standard were added to the DPPH solution. This suggests that ZnO NPs have antioxidant properties, which are further corroborated by the colorimeter data. The absorbance at 520 nm decreases as ZnO NP concentrations increase from 100 µg/mL to 1000 µg/mL, demonstrating the radical scavenging activity of ZnO NPs. In all concentrations, the radical scavenging ability of ZnO NPs produced by *B. pinnatum* was marginally less than that of regular ascorbic acid. The drastic scavenging methods used by (33,37,38). By checking OD of the sample on colorimeter at 520 nm the antioxidant activity of ZnO nanoparticles was determined via the DPPH method and the results are obtained as follows (Table 6 and Figure 11)

Table 6. Antioxidant activity of ZnO nanoparticles.

Concentrations	Standard	Sample	% of inhibition (Standard)	% of inhibition (NPs)
200 µg/ml	0.2	0.21	21	22
400 µg/ml	0.3	0.36	31	37
600 µg/ml	0.5	0.4	51	41
800 µg/ml	0.6	0.32	61	33
1000 µg/ml	0.8	0.42	81	43

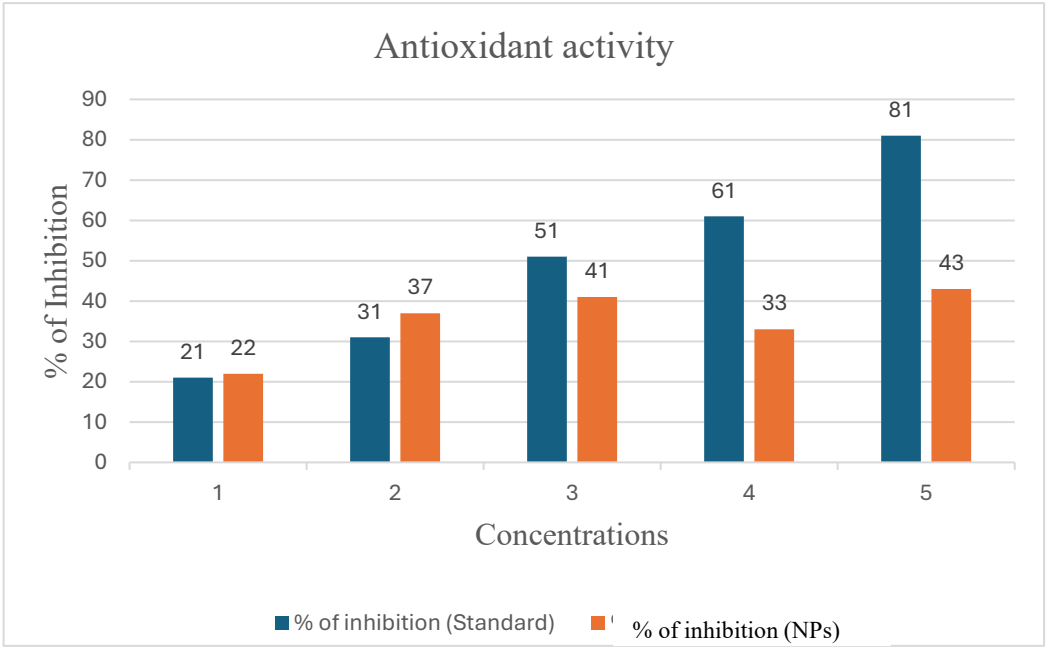

Fig11. ZnO NPs and ascorbic acid at varying concentrations exhibit radical scavenging action.

CONCLUSION

It is well known that, in comparison to chemical synthesis, green production of ZnO NPs is far safer and more environmentally friendly. The green production of nanoparticles is an economical and environmentally beneficial process. Numerous methods, including spectroscopic and microscopic investigation using XRD and SEM, were used to evaluate the produced ZnO NPs. It has been addressed how the bioactive substances in *B. pinnatum* leaves function as oxidizing agents. Antioxidant, antifungal, and antibacterial properties were noted. The findings of this study demonstrate how *B. Pinnatum* leaves can be used to create multifunctional ZnO NPs with antibacterial properties. It is anticipated that zinc oxide nanoparticles produced using the green synthesis process would find wider uses in biotechnology, sensing, medicine, catalysis, optical devices, coatings, and drug delivery.

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