Aswan University Journal of Science and Technology Volume 5, issue 1, March 2025

Green Synthesis of Zinc Oxide Nanoparticles using *Microtrichia Perotitii Dc* Plant Extract: Characterization and Antibacterial Activity

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Abstract

Green synthesis of nanoparticles (NPs) has attracted the researcher's attention, this is because of its rapid, cost-effective, sustainable and eco-friendly nature. The present study reports the successful green synthesis of Zinc oxide nanoparticles (ZnO NPs) using *Microtrichia perotitii* DC plant leaf extracts as a chelating and capping agent and Zinc Oxide (ZnO) as a precursor. The synthesized nanoparticles were thoroughly characterized using UV-visible spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy and thermogravimetric analysis. X-ray diffraction patterns revealed that ZnO NPs with an average crystallite size of 12.6 nm were synthesized. Scanning electron microscope images show a grain-like nature and with no well-defined morphology. Fourier transform infrared spectrophotometer analysis revealed that the strongest bond at 686 cm^{-1} corresponds to stretching vibration mode of Zn-O nanoparticles while TGA affirmed the thermal stability of the nanoparticles. The synthesized nanoparticles exhibited antimicrobial activity only against *B. subtilis* with 25.325 ± 3.51 mm zone of inhibition.

Keywords: Green synthesis; Nanoparticles; Crystallite size; Characterization; Antimicrobial

Introduction

Nanotechnology is widely recognized as a cutting-edge field with applications across diverse industries, including chemical, pharmaceutical, mechanical, food processing, power generation, optics, and environmental sciences **[1]**. It has revolutionized modern science and technology by addressing challenges in areas such as energy sufficiency, climate change, healthcare, and environmental sustainability. Furthermore, nanotechnology has raised living standards through advancements in beauty products, textiles, and the treatment of lethal diseases such as cancer and Alzheimer's disease **[2, 3]**.

Nanoparticles (NPs), the building blocks of nanotechnology, can be synthesized through various physical, chemical, and biological methods. While physical and chemical methods dominate, they are often associated with drawbacks such as long processing times, high costs, complex procedures, and the use of toxic chemicals that pose environmental and health risks **[4, 5]**. These limitations have driven research efforts toward green synthesis methods, which are eco-friendly, cost-effective, and efficient. Green nanoparticle synthesis, particularly using plant extracts, is gaining momentum as an emerging trend in green chemistry. This method not only reduces the need for hazardous reagents but also utilizes naturally derived biomolecules as reducing and stabilizing agents **[6–8].**

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Received July 29, 2024, received in revised form, December 05, 2024, accepted December 05, 2024.

(ASWJST 2021/ printed ISSN: 2735-3087 and on-line ISSN: 2735-3095) https://journals.aswu.edu.eg/stjournal

Metal oxide nanoparticles have garnered significant attention in the past decade due to their unique properties and wide-ranging applications in technological and biomedical fields **[9]**. Among these, zinc oxide nanoparticles (ZnO NPs) stand out as a promising material due to their exceptional physical and chemical properties. ZnO NPs exhibit a wide radiation absorption spectrum, a high electrochemical coupling coefficient, and excellent photostability, making them versatile for various applications **[10]**. They are also non-toxic, biocompatible, and possess remarkable medicinal properties, including anticancer **[11]**, anti-inflammatory **[12]**, and antibacterial activities. Other biomedical applications include targeted drug delivery **[13]**, wound healing **[14]**, and bioimaging **[15]**.

In recent years, the synthesis of ZnO NPs through green methods using plant extracts has become an area of intense focus due to its environmental and biomedical advantages. The use of non-toxic and eco-friendly materials in ZnO NP production not only reduces environmental hazards but also enhances the biocompatibility of the synthesized nanoparticles. **[16-18]**.

Herbal plants and extracts have a variety of medical qualities that have been exploited to produce novel medications **[19]**. Plant phytochemicals, such as alkaloids, polyphenols, flavonoids, and terpenoids, have emerged as effective reducing agents for metal ion reduction during nanoparticle synthesis **[20, 21]**. Thus, biogenic synthesis with plant leaf extract improves nanoparticle biocompatibility and accounts for the synergetic effect **[22]**.

In this regard, leaves extract of *Microtrichia perotitii* DC, a species of family Asteraceae (Compositeae), was used for bioconversion of manganese ions to NPs. *Microtrichia perotitii* DC is naturally available in several countries in west Africa, including Nigeria, Senegal, Mali, Port of Guinea, Sierra Leone, Ivory Coast, and Ghana **[23]**. Reports demonstrated that the whole leaves extract of *Microtrichia perotitii* DC is a rich source of several biogenic phytomolecules, including alkaloids, flavonoids, tannins, phenolic compounds, saponins, and triterpenoids with various biological applications **[23].** Which offers numerous advantages in the green synthesis of ZnO nanoparticles. It is an eco-friendly, cost-effective alternative to chemical reagents, providing a sustainable approach to nanoparticle synthesis. The extract is rich in bioactive compounds **[23]**, which serve as natural reducing and stabilizing agents, enhancing the biocompatibility, thermal stability, and antimicrobial activity of the nanoparticles **[16-18]**.

Zinc oxide nanoparticles (ZnO NPs) were prepared from aqueous fruit extracts of *Myristica fragrans* **[24]**. The ZnO NPs were characterized by different techniques such as X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, ultraviolet (UV) spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and thermogravimetric analysis (TGA). The synthesized NPs were evaluated for their possible antibacterial, antidiabetic, antioxidant, antiparasitic, and larvicidal properties. The results showed that the NPs were found to be highly active against bacterial strains both coated with antibiotics and alone. ZnO NPs displayed outstanding inhibitory potential against enzymes protein kinase, α-amylase, and α-glucosidase. Overall, the synthesized NPs have shown significant larvicidal activity. Similarly, tremendous leishmanicidal activity was also observed against both the promastigote and amastigote forms of the parasite.

The synthesis of ZnO nanoparticles (ZnO NPs) utilizing the potential of *Aloe barbadensis* Miller (*A. vera*) leaf extract (ALE). ALE-capped ZnO nanoparticles (ALE-ZnO NPs) were characterized using UV–Vis spectroscopy, Xray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), and transmission electron microscopy (TEM) analyses has been reported **[25]**. The synthesized zinc oxide nanoparticles were screened for antibacterial activities on gram-positive bacteria and gram-negative bacteria. Flow cytometry and atomic absorption spectrophotometry (AAS) data analyses revealed the surface binding and internalization of ZnO NPs in Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) cells, respectively. Significant antibacterial activity of ALE-ZnO NPs was observed against extended spectrum beta lactamases (ESBL) positive E. coli, Pseudomonas aeruginosa, and methicillin resistant S. aureus (MRSA) clinical isolates exhibiting the MIC and MBC values of 2200, 2400 lg/ml and 2300, 2700 lg/ml, respectively.

In this study, we report the green synthesis of ZnO NPs using *Microtrichia perotitii* DC plant leaf extract. This eco-friendly method takes advantage of the bioactive compounds in the plant extract to reduce zinc ions and stabilize the synthesized nanoparticles. The biogenic ZnO NPs were characterized using advanced techniques, including Fourier transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-vis) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). Additionally, the antibacterial activity of the synthesized nanoparticles was investigated, focusing on their potential inhibitory effects against bacterial pathogens.

Materials and Methods

Preparation of *Microtrichia perotitii* DC plant extract

The *Microtrichia perotitii* DC (a species of family Asteraceae (Compositeae) obtained from the northern part of Nigeria) utilized in the current study were rinsed with distilled water to eliminate any related dirt. The *Microtrichia perotitii* DC plant was air dried for one week. The *Microtrichia perotitii* DC leaves were ground using a pestle and mortar. The leaves (20 g) were mixed in 100 ml of distilled water and ethanol, then incubated at room temperature for 24 hours **[26]**. Following the incubation period, the resulting solution was properly filtered with Whatmann No.1 filter paper and used for antibacterial tests.

Preparation of Zinc oxide nanoparticles using *Microtrichia perotitii* DC plant extract

Zinc oxide (ZnO) was employed as a precursor. ZnO (80 mL, 0.1 M) is measured and carefully mixed with 10 ml of the plant extract, *Microtrichia perotitii* DC, drop wise with constant stirring. The mixture is then placed on the heating mantle with a magnetic stirrer for 2 hours at 80 degrees Celsius. After boiling for two hours, the mixture was allowed to cool and settle. The colloidal settling was then filtered using Whatmann no.1 filter paper and dried with a desiccator.

Antibacterial activities

Bacterial strains and Antibacterial Assay to assess the antibacterial activity of ZnO NPs against six bacterial strains. The disc diffusion method **[27-29]** was used with minor modifications for two Gram-positive strains (Staphylococcus aureus ATCC 33863, Bacillus subtilis ATCC 23857) and four Gram-negative strains (Pseudomonas aeruginosa ATCC 27853, Salmonella typhi ATCC 19430,

Escherichia coli ATCC 25922, and Clostridium botulinum ATCC 19397). The bacterial strains listed above were purchased from the University of Ilorin Teaching Hospital Laboratory. The surface of nutrient agar media plates was swabbed slowly in three separate directions with an aliquot of 100 µL of inoculum that had been pre-adjusted (10^8 cells/mL) for seeding density. The plates' surface was covered with sterile filter paper discs containing 5 µL (20 mg/mL DMSO) of ZnO NPs solution. The use of DMSO-infused discs as a negative control was contrasted with the use of ciprofloxacin (5 µg) loaded discs as a positive control. These was followed by an incubation period of 24 h at 37°C. After which the zone of inhibition (ZOI) was measured.

Characterization

The infrared spectrum of the solid ZnO NPs was recorded by FTIR spectrometer (ZN- FTIR 530) to identify the functional Groups present in the nanoparticles. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer (SPECORD-200). The particle shape of MnO NPs was determined by SEM. The model used is Phenom Prox model. XRD (Rigaku MiniFle 600 XRD Diffractometer) was used to determine the crystallographic structures of the nanoparticles and TG analyzer (TGA-Q500 series, TA instruments) was used to determine the thermal stability of the nanoparticles.

Powder XRD analysis has been carried out to examine the crystallinity and to check the purity of the synthesized Nps. The optical properties of the nanoparticle were analyzed using UV-Vis spectroscopy. The presence of functional group was analyzed by FTIR spectroscopy. The thermal stability of the synthesized nanoparticle was analyzed by TGA. Morphology of the synthesized manganese dioxide nanoparticles were analyzed using SEM.

Statistical Analysis

The data obtained from the experiment was utilized for statistical analysis. All trials were conducted in triplicate, and the findings are given as mean± standard deviation. To ascertain the statistical significance, we used SPSS with a predetermined significance level (at $p < 0.05$).

Results and discussion

Fourier transforms infrared spectroscopy (FTIR) studies

Figure 1 depicts the FTIR spectra of ZnO NPs produced using *Microtrichia perotitii* DC plant extracts. The FTIR analysis was performed utilizing the KBr pellet technique in the 4000 - 400 cm^{-1} range to evaluate the level of purity and identify the metal nanoparticles produced. Metal oxide absorption/transmission bands typically occur in the fingerprint region, which ranges from 400 cm^{-1} to 1500 cm^{-1} and are caused by inter-atomic vibrations **[30]**. The peaks at 3343 cm^{-1} could be attributed to O-H stretching. The observed peak at 1675 cm^{-1} correspond to C=O stretching vibration. The observed peak at 2888 cm^{-1} correspond to C-H stretching vibration. A peak at 1070.29 cm^{-1} is associated with C–O stretching vibrations. The signal at 686.53 cm^{-1} indicates Zn-O stretching. The metal-oxygen frequencies found for the various metal oxides are consistent with the literature reported **[30]** which found comparable FTIR spectra of zinc oxide nanoparticles in their investigation.

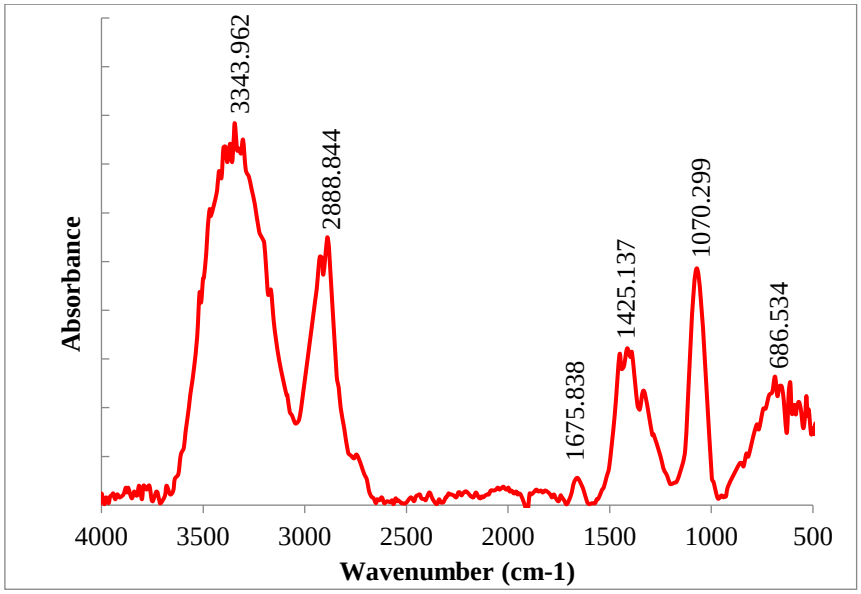

Figure 1. FTIR spectrum of *M. perotitii* synthesized ZnO NPs

Optical properties of ZnO NPs

The formation of ZnO NPs was followed by measuring the SPR peak using a UV-Vis spectrophotometer; the findings are shown in Figure 2. ZnO NPs exhibit a high absorption peak at 325 nm, attributed to their Surface Plasmon Resonance (SPR) band. The synthesis of ZnO NPs was confirmed by an SPR absorption band at 325 nm which is similar to the result obatained by **[31]**. As the wavelength increased, the absorbance intensity decreased, showing that formation did not occur at a long wavelength. The results are in agreement with prior investigations conducted by **[32]**.

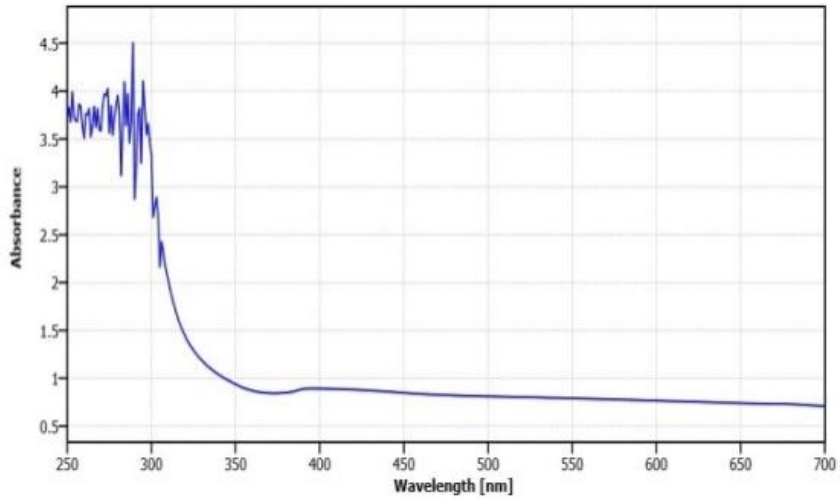

Figure 2. Absorption spectrum of *M. perotitii* synthesized ZnO NPs

3.3 X-ray diffractometry (XRD) analysis

The structural information and crystallinity of ZnO NPs generated using *Microtrichia perotitii* DC plant extract are investigated using an XRD pattern, as shown in Figure 3. The strong intensity and sharp peaks of ZnO NPs demonstrated that the resultant products are very crystalline. The presence of ZnO is responsible for the diffraction peaks at 2θ = 32.08°, 34.71°, 36.50°, 47.69°, 56.76°, 62.98°,

and 67.98° associated with the (111), (200), (200), (211), (221), (311) and (500) crystal planes (JCPDS file no. 04-013-6608). The crystallite size of ZnO nanocrystals was determined using Debye Scherer's equation:

$$D = \frac{0.89\lambda}{\beta \, x \cos\theta} \qquad (1)$$

Where D represents the average crystal size, λ is the X-ray wavelength, and β is the full width at half maximum (FWHM). The calculated average crystallite size was 12.6 nm. This result is in agreement with previous studies **[33]**. The lower crystallite size could be attributed to the approach used, as plant extract can act as both a fuel, chelating and capping agent, reducing the particle size **[26].**

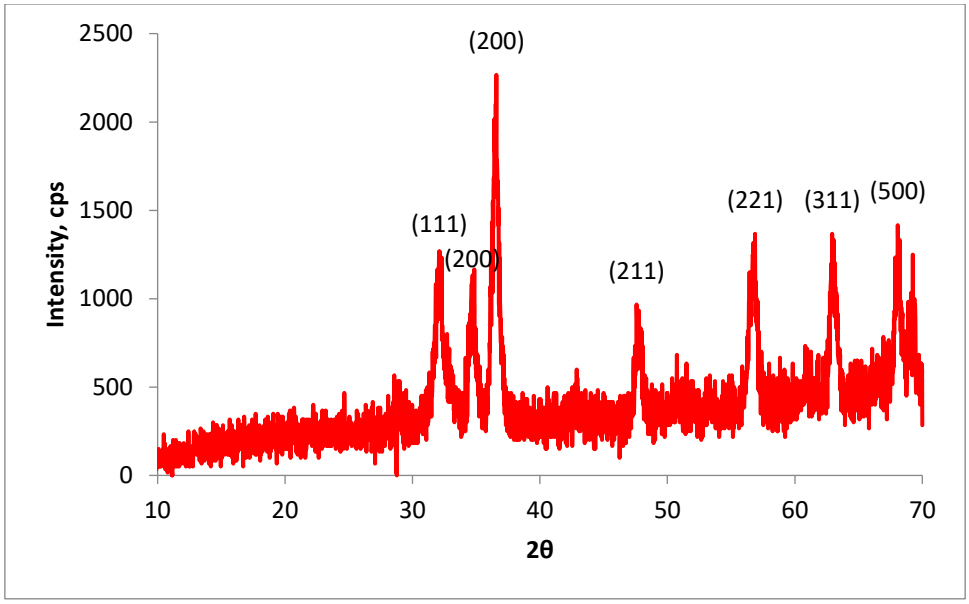

Figure 3. XRD pattern of *M. perotitii* synthesized ZnO NPs

3.4 Surface morphology characterization

The morphology of the ZnO NPs was examined using a scanning electron microscope (SEM). It demonstrates that it is completely covered with nanograined ZnO NPs with no well-defined morphology. The SEM micrograph shows that the particles are granular nanosized in nature with a rough surface which are aggregated which is similar with the result obtained by **[34]**. Figure 4 shows that these particles are uniformly dispersed. This could be due to low viscosity of the solution **[35]**.

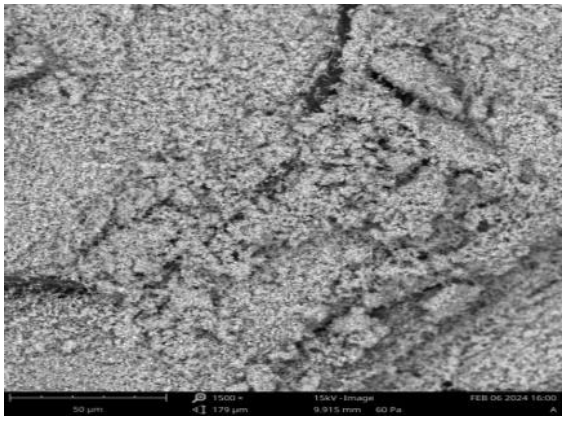

Figure 4. SEM micrograph *M. perotitii* synthesized ZnO NPs

3.5 Thermogravimetric Analysis (TGA).

Thermogravimetric analysis of *Microtrichia perotitii* DC plant extracts was carried out to determine the heat stability and degradation pattern of the produced nanoparticles. The TGA spectra of ZnO NPs show that the sample decomposes significantly with increasing temperature. First, the sample's initial loss between 25 and 100 °C was caused by the presence of ethanol and water **[36]**. As demonstrated in **Figure 5**, increasing the temperature to 450 °C results in a large 60-65% weight loss, which is most likely owing to the various volatile components contained in the plant sample. By increasing the temperature from roughly 450 °C to 900 °C, no additional loss in mass was recorded; the data thus confirm the stability of ZnO NPs within that temperature range. As a result, the thermogram clearly shows that ZnO NPs are thermally stable at temperatures up to 900 °C.

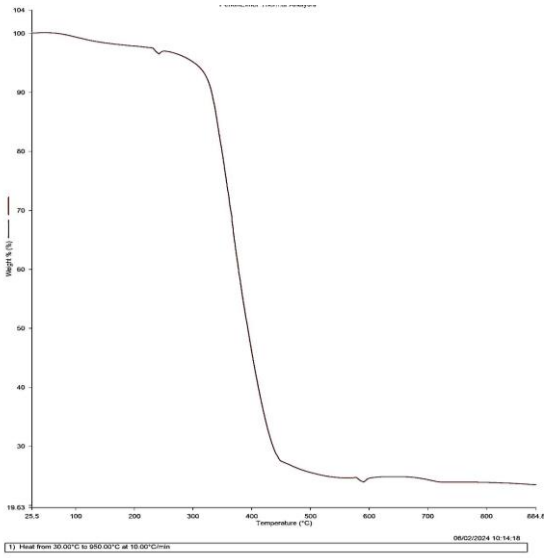

Figure 5. TGA curve of *M. perotitii* synthesized ZnO NPs.

3.6 Evaluation of antibacterial activity using ZnO NPs prepared using *Microtrichia perotitii* DC plant extract

The relative antibacterial activity of ZnO NPs prepared from *Microtrichia perotitii* DC plant extract was assessed against bacterial pathogens such as *Bacillus subtilis* (+), *Pseudomonas aeruginosa* (-), and *Escherichia coli* (-). The produced ZnO NPs was efficient against Gram positive Bacillus subtilis (25.325 ±3.51 mm), with a larger zone of inhibition compared to Gram negative bacterial pathogens, which showed no zone of inhibition as shown in **Table 1** and **Figure 6**. This observation demonstrates that Gram-negative pathogens have more resistance or tolerance than Gram-positive bacterial strains. The results are consistent with literature **[37]**. The reactivity of nanoparticles with bacterial cells is determined not only by the metal oxide used, but also by the bacterial species investigated **[38]**. The antibacterial activity of nanoparticles is determined by their size, stability, and concentration in the growing media. The nanoparticle has a higher reactivity with pathogen (*B. subtilis)* because the outer cellular membrane of bacterial strain contains nanoscale holes. The *Microtrichia perotitii* DC plant extract was capable of synthesizing ZnO nanoparticles with a particle size of 12.6 nm, as confirmed by the acquired XRD results.

Table 1. Antimicrobial activity of *M. perotitii* synthesized ZnO NPs

Test organisms	Activity of ZnO NPs (mm)	Ciprofloxacin (5 µg disc)
P. aeruginosa	0.000±0.00[a]	20.55±1.50[c]
E. coli	0.000±0.00[a]	19.70±3.57[c]
B. subtilis	25.325±3.51[b]	21.38±2.10[d]

The superscript lettered (a-f) indicate significant difference (at $p < 0.05$) when subject to SPSS test. The findings are given as mean ± standard deviation.

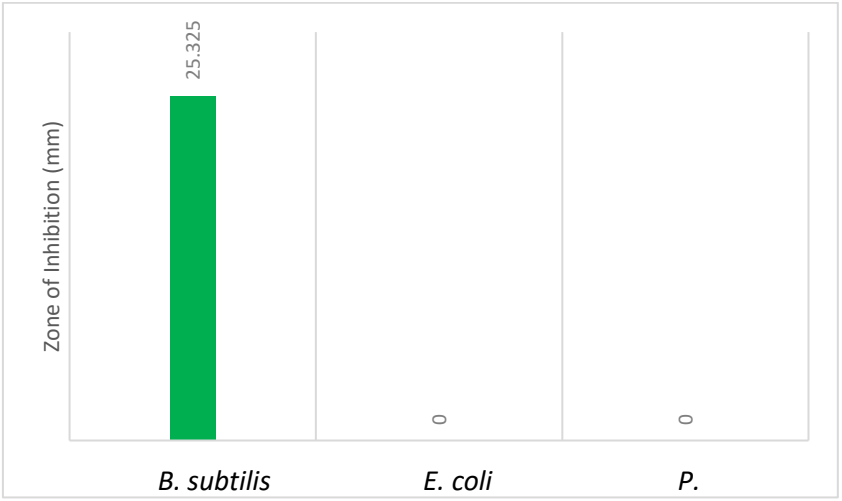

Figure 6. Antimicrobial activity of *M. perotitii* synthesized ZnO NPs

Conclusion

The current study describes a green synthesis of ZnO NPs that is both cost-effective and environmentally benign, with *Microtrichia perotitii* DC plant extracts serving as reducing, chelating and capping agents. UV-Vis spectroscopy, FTIR, SEM, XRD, and TGA were used to analyze the ZnO NPs. The UV-Vis spectrum showed an absorption band at 325 nm from ZnO NPs. The FTIR measurement revealed that metal oxide nanoparticles were reduced from zinc ion solutions to ZnO NPs with a stretching vibration at 686 cm^{-1}. SEM micrographs confirmed the nano-grained form of ZnO NPs. XRD examination confirmed the crystallinity of ZnO NPs, with a predicted crystallite size of 12.6 nm. The zone of inhibition data shows that the manufactured nanoparticle was more efficient against Gram-positive bacterial strains than Gram-negative ones.

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