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SHORT COMMUNICATION article

Bio-reducing properties of *bryophyllum pinnatum* aqueous leaves extract for the mediated synthesis of nickel oxide nanoparticles and its antimicrobial activities

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Abstract: *Bryophyllum pinnatum* is a medicinal plant from the *Crassulaceae* family. It's traditionally used in Ayurvedic and folk medicine to cure various ailments, and its phytochemicals are used in the reduction of metal ions to produce nanoparticles. This study is aimed at the biosynthesis of nickel oxide nanoparticles using *Bryophyllum pinnatum* as a reducing, capping, and stabilizing agent, characterizing the synthesized nanoparticles, and studying the anti-microbial activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis,* and *Pseudomonas aeruginosa* as well as fungi*; Candida albicans,* and *Aspergillus niger*. The synthesized nanoparticle was confirmed by UV-VIS, FT-IR, SEM-EDX, and XRD. The UV-VIS indicated the presence of chromophore and a surface plasmon resonance occurring in the sample with a band gap of 4.38 eV. The FT-IR result revealed the stretching vibration of the Ni-O bonds at 715 cm^{-1}. The SEM analysis showed the morphology of the nanoparticles, and the EDX revealed that NiO-NPs are composed of oxygen and nickel elements. The XRD confirmed a face-centered cubic structure with an average size of 16.377 nm. The antimicrobial analysis exhibited the tested minimum concentration against all the bacterial strains. The synthesized Nickel oxide nanoparticles are useful in inhibiting the growth of different types of bacteria.

Introduction

Inorganic nanoscale particles have gained significant attention as pivotal components driving technological advancements owing to their adaptable characteristics and superior performance compared to bulk materials. They have versatile applications in magnetic devices, electronics, sensors, photocatalysis, and biomedicines [1, 2]. Nickel oxide (NiO) nanoparticles (NPs) are one of the recently sorted NPs as regards their significant antibacterial, anti-parasitic, antifungal, antiviral, antioxidant, wound dressing, drug delivery system, and medical devices as well as coating [2, 3]. NiO is one of the NPs with distinct characteristics, non-toxic, p-type metal oxide with a wide band gap of 3.6-4.3 eV [4] that is highly biocompatible. NPs have emerged as a potent tool in the battle against microorganisms, offering innovative strategies that enhance traditional antimicrobial techniques.

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Their distinct properties and the ability to be modified with various antimicrobial agents, make them particularly effective in targeting and neutralizing pathogenic microorganisms. NP is one of the effective antimicrobial coatings and delivery systems [5]. NPs can combat microorganisms through several mechanisms. Metallic oxide NPs, such as titanium dioxide, zinc oxide, magnesium oxide, iron oxide, and copper oxide NPs, are known for their broad-spectrum antimicrobial activities [6-10]. These NPs can attach to microbial cell walls and disrupt their structure, leading to cell death [3]. Aside from this, NPs can target multiple cellular pathways of microorganisms. The development of new antimicrobial agents is crucial in the fight against antibiotic-resistant bacterial strains. Antimicrobial resistance is a major global health threat associated with 1.27 million deaths around the world in 2019 and has been identified by the World Health Organization (WHO) as one of the top 10 global public health threats facing humanity. Microorganisms are an integral part of our environment, playing critical roles in maintaining ecological balance and supporting life processes. However, they can also be vectors for disease, posing significant challenges to public health. Pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and various strains of influenza virus are capable of causing severe illness and even death. The transmission of these pathogens can occur through various means, including air, water, contaminated surfaces, and person-to-person contact. The impact of these pathogens can be seen in the morbidity and mortality rates associated with infectious diseases worldwide. Traditional methods for controlling microbial threats include chemical disinfectants, such as bleach and alcohol-based solutions, and physical methods, like heat sterilisation and ultraviolet light exposure. However, the emergence of antibiotic-resistant bacteria [11] and the environmental impact of chemical disinfectants [12] have led to the exploration of alternative strategies. Innovations in molecular biology and genetics have propelled forward our understanding of microorganisms and our need for more effective ways of fighting these harmful microorganisms.

Bryophyllum pinnatum is a medicinal plant known for its antimicrobial properties. *B. pinnatum* is rich in phytochemicals, including saponins, phenolic acids, triterpenoids, long-chain hydrocarbons, flavonoids, sterols, fenantrenic derivatives, ketones, bufadienolides, minerals, vitamins, sugars, amino acids, aromatic and acyclic organic acids, and fatty acids [13, 14]. The plant extract is an effective reducing, capping, and stabilizing agent for the synthesis of various NPs such as silver, selenium, copper, gold, zinc oxide, and iron oxide inorganic nanoscale particles [13-17]. However, there is limited research on the synthesis of NiO NPs using this plant extract. The green synthesis of NiO NPs has the potential to provide a new approach to the production of antimicrobial agents. We hereby report the synthesis of NiO NPs using *Bryophytum pinnatum* extract and evaluation of the antimicrobial activities of the synthesized NPs against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus niger*.

Materials and methods

Plant material: The leaves were collected from Okhuoromi, situated within the Oredo Local Government area of Edo State, Nigeria. (6.21916 ˚N and 5.58565˚E). The leaves were identified and authenticated as *B. pinnatum* (Lam.) Oken, voucher number: UBH-B593. The authentication of the sample was done by Dr. Akinnibosum Henry Adewale from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria.

Preparation of fresh leaf sample (Bryophyllum pinnatum) extract: The fresh leaves of *Bryophyllum pinnatum* were properly washed with distilled water three times, then crushed with a pestle and mortar and blended with an electric blender (**Figure 1**). The resulting extract was filtered through a funnel with a white degummed handkerchief and cotton wool. The filtrate was used immediately for the synthesis of NPs.

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Figure 1: Scheme of synthesis of NiO Nanoparticles

a: fresh miracle leaf, b: nickel sulphate heptahydrate precursor on magnetic stirrer, c: adding NaOH to sample and precursor, d: synthesised nanoparticles ready for centrifugation, and e: calcinated nanoparticles

Synthesis of nickel oxide nanoparticles: In a 500 mL, 250 mL of plant extract was mixed with 14.1 g of nickel sulphate heptahydrate. The mixture was stirred on a magnetic stirrer at 60°C, and 0.1M sodium hydroxide was added dropwise using a pipette and stirred for one hour at 24°C. The addition of sodium hydroxide changes the color from light green to dark green, indicating the formation of NiO NPs and increasing the pH from 4.2 to 7.5. The synthesized NiO NPs solution was centrifuged for 20 min at 4000 rpm, washed with distilled water to remove contaminants, dried in an oven at 100°C for one hour, and calcined in a furnace at 500°C for two hours (**Figure 1**). The biosynthesized NiO NPs were subjected to UV-VIS, FT-IR, SEM-EDX, XRD, and microbial analysis.

UV analysis: The UV analysis was done with the aid of a spectrophotometer (GENESYS 10S v1.2002L7J355002) to determine whether the plant sample contains aromatic rings and chromophoric groups. This is because the electronic transition of lone pair, σ- bond, and also **π**-bond electrons was detected.

FT-IR analysis: The FT-IR spectrum of *Bryophyllum pinnatum* was obtained using the CARY 630 Agilent Technologist spectrometer to identify the sample's bioactive compounds and detect their functional group.

SEM-EDX: The sample SEM-EDX was obtained using SEM-EDX (PHENOM PRO X S/N: MVE0224651193 Model no: 800-07334).

XRD analysis: The XRD analysis was performed using a Bruker D8 Advance X-ray diffractometer to determine the crystal structure and phase purity of the NiO NPs over a 2θ range of 20-80ºC.

Antimicrobial analysis: The antimicrobial activity of green synthesized NiO NPs was done using the agar well diffusion method [10]. This was carried out against various bacterial strains, namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Aspergillus niger* in the Pharmaceutical Microbiology Laboratory, University of Benin.

Preparation of antimicrobial agent: 0.05 g of *Bryophyllum pinnatum* NiO NPs was dissolved in 2 mL of 30.0% Tween 80 a diluent to give 25 mg/mL.

Agar plate preparation and antimicrobial process: 7.5 g of Mueller-Hinton agar was dissolved in 150 mL of distilled water in a sterile container. The agar solution was autoclaved at 12ºC for 15 min to sterilize it. The sterilized agar was cooled and allowed to be set on four different disc plates labelled with the name of each bacterium and fungi. Using a sterile corn borer, wells were bored in the solidified agar plates, into which the antimicrobial solutions were later added. The various bacteria and fungi were streaked on the surface of the prepared agar plates, and each of the plates bore its name. The plates were then incubated at a constant temperature of 37ºC for 24 hours. This was done to allow the organisms to grow and interact with the antimicrobial solution. A minimum inhibitory concentration (MIC) test was then conducted to determine the MIC of the *Bryophyllum pinnatum* NPs using a series of dilutions to create stock having concentrations of 12.5, 25, and 50 µL.

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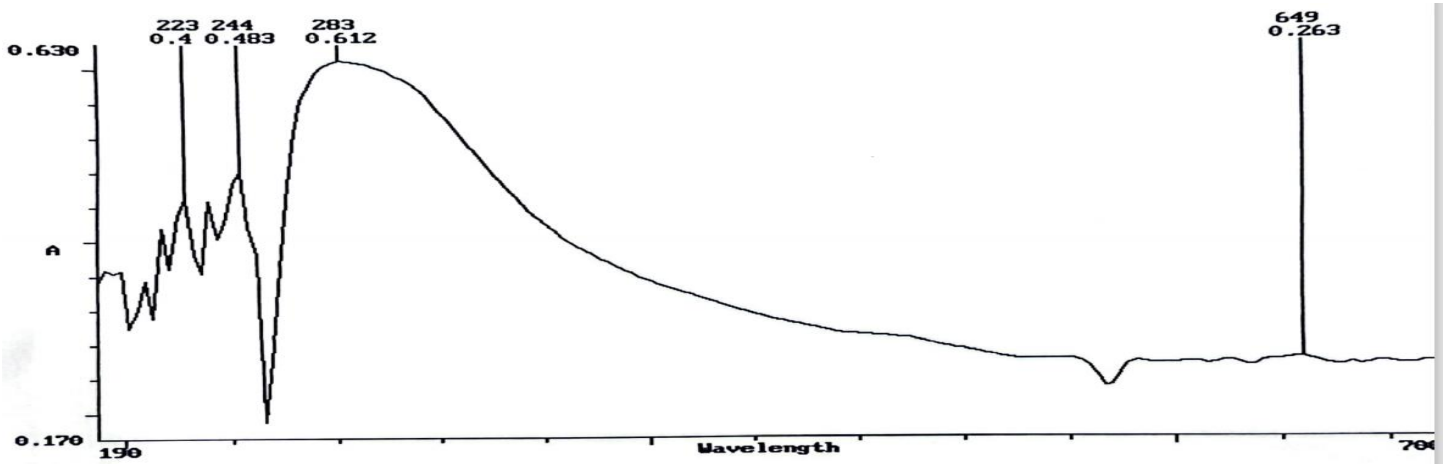
Results and discussion

In this study, the biogenic synthesis of NiO-NPs from nickel sulphate heptahydrate by incorporating the aqueous extract of *Bryophyllum pinnatum*, which serves as a capping, reducing, and stabilizing agent. The successful biosynthesis was visually confirmed by the observed colour changes from light green to dark green and later dark brown on calcinated. These color changes were in agreement with the previous reports of NiO NPs [3, 18].

UV-visible analysis: To ascertain the successful synthesis of NiO NPs UV-vis spectroscopy was employed. The peak at 283 nm, 244 nm, and 233 nm with absorbances of 0.612, 0.483, and 0.451, respectively, (**Figure 2**), suggested the presence of a chromophore that strongly absorbs in the UV region. These peaks may be attributed to electronic transitions within the NiO NPs. The peak at 649 nm with an absorbance of 0.263 suggests that the sample also absorbs light in the visible region. This peak may be due to a transition between energy levels within the NPs that corresponds to the absorption of light in the red part of the visible spectrum. There is a surface plasmon resonance (SPR) occurring in the sample and collective oscillation of electrons on the surface of the NPs, which is one of the characteristic features of SPR [19].

Figure 2: The UV-VIS spectrum of NiO NPS



Band gap: UV analytical technique is essential for assessing the band gap energy of NPs, which is a critical parameter in determining their suitability for various applications. The band gap (*Eg*) can be estimated using the formula *Eg* = 1240/wavelength. Where wavelength is the peak wavelength in nm. This formula gives an estimate of electron volts (eV). For the peak at 283 nm: Eg = $x = \frac{1240}{283}$ = 4.38 eV

This calculated band gap value of 4.38 eV is consistent with literature values for NiO, which generally range around 3.6 eV to 4.3 eV [4] and are estimated at 4.47 eV [20], depending on the particle size and synthesis method. The slightly higher value suggests smaller particle sizes or different crystalline forms due to the synthesis method (i.e., green synthesis). The application of these UV-Vis absorption characteristics indicates that these NiO NPs might be suitable for photocatalytic applications, particularly in absorbing UV light [21].

FT-IR analysis: The FT-IR spectroscopy revealed the functional group of the biosynthesized NiO NPs (**Figure 3**). The peak of 3153 cm^{-1} (alcohols or carboxylic acids O-H stretching). The peak of 2344 cm^{-1} (C≡N vibration stretching). The peak of 1628 cm^{-1} (C=O vibration stretching of ketone, carboxylic acid derivatives). The peaks at 1077 cm^{-1} (C-O stretching). The peaks at 715 cm^{-1} (Ni-O of NiO NPs) [16]. The FT-IR analyses show that the surface of synthesized NPs revealed the presence of proteins, carboxyl, and hydroxyl groups [22].

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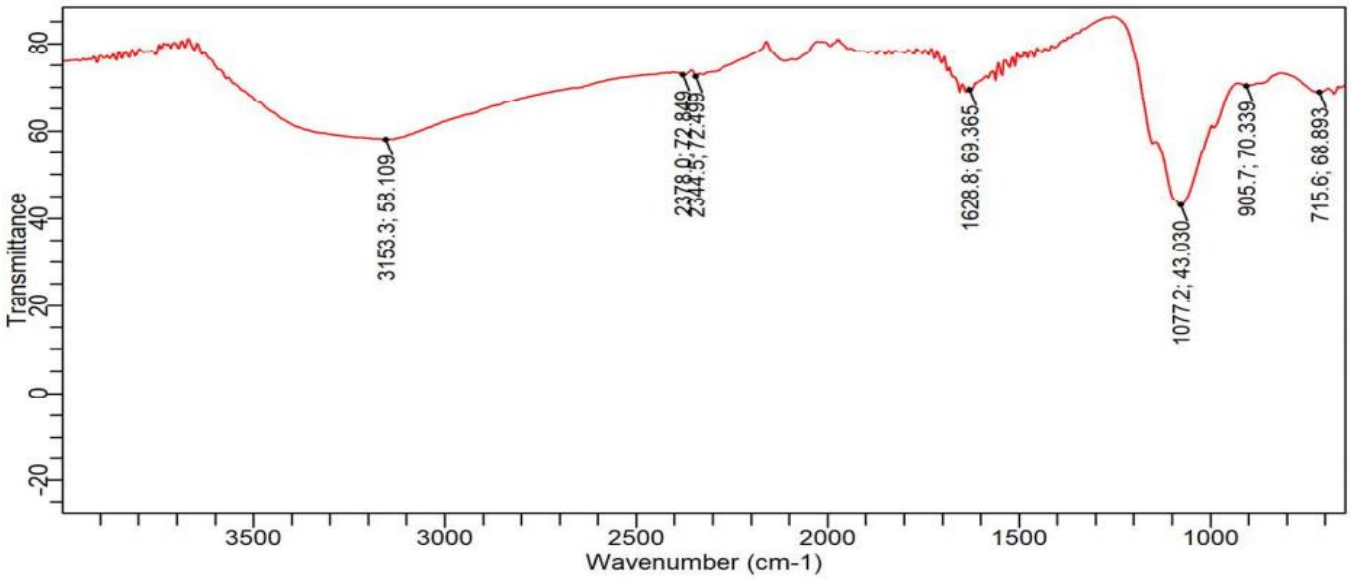
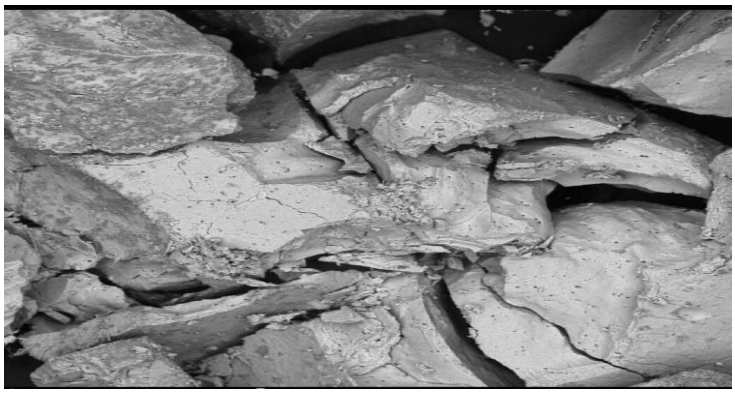
Figure 3: FT-IR Spectrum of NiO NPs



SEM-EDX analysis: The EDX is an analytical technique used to identify the elemental composition of a sample. SEM (scanning electron microscopy) revealed the morphology of NPs at a magnification of 500X (**Figure 4**). EDX revealed that the synthesized nanoscale particles are composed of oxygen and nickel elements (**Table 1**). The NPs are arranged in clusters and are highly agglomerated [16], as this is common in NP research.

Figure 4: SEM image of NiO NPs



XRD analysis: The X-ray diffraction (XRD) provides information on the average grain size, crystallinity, shape, strain, and crystal defects. XRD data also assist in correlating microscopic findings with bulk samples. The XRD pattern confirmed NiO NP formation, displaying intense reflection peaks at 2θ=37.31˚, 43.32˚, and 62.86˚ (**Figure 5** and **Table 2**). The detected peaks matched with the phases of 1 1 1, 2 0 0, and 2 2 0 of standard JCPDS card numbers 47-1049, indicating a face-centered cubic structure. The crystallite size (D) is estimated using the intense reflection peak corresponding to the 2 0 0 plane by Debye Scherrer's equation [23].

$D = \frac{K\lambda}{\beta \cos\theta}$: Where D is the average crystalline size of the NPs, K is the shape factor (usually equal to 0.9), λ is the wavelength of the X-ray radiation used (0.15418), β is the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg diffraction angle. The average crystalline size of 16.377 nm of NiO NPs computed using Scherrer's equation was in agreement with the 13.0-31.0 nm documented at different annealing temperatures [1, 3, 24].

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Table 1: EDX table

Element Number	Element Symbol	Element Name	Atomic Conc.	Weight Conc.
28	Ni	Nickel	42.13	68.73
8	O	Oxygen	53.00	23.57
82	Pb	Lead	0.52	3.01
16	S	Sulfur	1.77	1.58
29	Cu	Copper	0.29	0.51
50	Sn	Tin	0.15	0.49
15	P	Phosphorus	0.51	0.44
20	Ca	Calcium	0.35	0.39
26	Fe	Iron	0.25	0.38
11	Na	Sodium	0.38	0.24
25	Mn	Manganese	0.15	0.23
13	Al	Aluminium	0.30	0.22
19	K	Potassium	0.20	0.22
14	Si	Silicon	0.00	0.00
17	Cl	Chlorine	0.00	0.00
30	Zn	Zinc	0.00	0.00
22	Ti	Titanium	0.00	0.00
12	Mg	Magnesium	0.00	0.00
47	Ag	Silver	0.00	0.00

Antimicrobial studies: Findings of the antimicrobial activity assay are shown in **Table 3**. The nickel NPs showed varying degrees of antimicrobial activity against the six bacterial strains tested. The highest inhibition zone diameter was observed for *Staphylococcus aureus* (28 mm), followed by *Escherichia coli* (15 mm), *Pseudomonas aeruginosa* (15 mm), *Bacillus subtilis* (12 mm), *Candida albicans* (13 mm), and *Aspergillus niger* (Nz).

Minimum inhibitory concentration: The MIC of NiO NPs lowest inhibitory concentrations against the tested four bacterial strains, showed no growth at concentrations of 1.25 mg/mL, 2.5 mg/mL, and 5.0 mg/mL of NiO NPs (**Table 4**). This indicates that NiO NPs have a potent antimicrobial effect against these bacterial strains [25, 26]. The MIC values obtained indicate NiO NPs are effective at inhibiting the growth of these bacterial strains at relatively low concentrations.

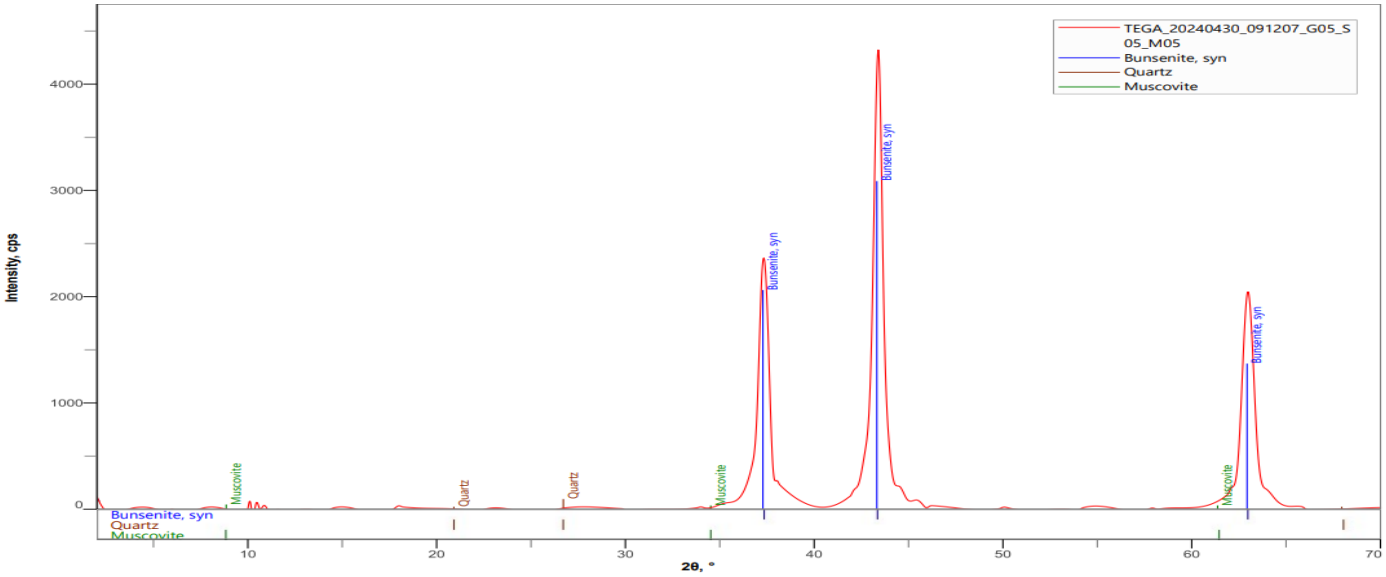

Figure 5: XRD image of NiO NPs

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Table 2: XRD data and crystallite size of biosynthesised ZnO NPs

Peak position (2θ,°)	FWHM,°	FWHM (Radians (nm))	Crystallite size diameter (nm)	Average crystallite size (nm)
37.31 (0.9475)	0.53	0.0093	15.735	
43.32 (0.9294)	0.50	0.0087	17.148	16.377
62.86 (0.8534)	0.57	0.0100	16.247	

Table 3: Antimicrobial activity of nickel oxide nanoparticles against bacterial strains

Organism	NiO-NP Inhibition (in mm)	Control inhibition (in mm)
Escherichia Coli	15	40
Staphylococcus aureus	28	40
Pseudomonas aeruginosa	15	40
Bacillus subtilis	12	30
Candida albicans	13	24
Aspergillus niger	Nz	21

Control = Ciprofloxacin (Bacterial), Ketoconazole (Fungi), Nz (no zone of inhibition)

Table 4: Minimum Inhibitory Concentration (MIC) of the NiO-NPs

Organism	1.25 mg/mL	2.5 mg/mL	5.0 mg/mL
E. coli	NG	NG	NG
S. aureus	NG	NG	NG
P. aeruginosa	NG	NG	NG
B. subtilis	NG	NG	NG

NG= No growth; G = Growth

Conclusion: The synthesis of nickel oxide nanoparticles is a cost-effective green synthesis route using *Bryophyllum pinnatum* leaf extract, and it is useful in inhibiting the growth of certain bacteria. The antimicrobial analysis carried out against different strains of bacterial and fungi strains exhibited the tested minimum concentration against all the bacterial strains. The synthesized nickel oxide nanoparticles are useful in inhibiting the growth of certain bacteria.

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Author contribution: OBO conceived and designed the study. OBO & TDO collected the data. OBO, IHI, MII & IE performed data analysis. OBO & TDO contributed to data interpretation. OBO drafted and revised the manuscript for important intellectual context. All the authors approved the final version of the manuscript and agreed to be accountable for its contents.
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Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that they have followed all relevant ethical guidelines and obtained any necessary IRB and/or ethics committee approvals.