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Exploring the potential of *Nauclea latifolia* for sustainable synthesis of ZnO nanoparticles: characterization and antibacterial assessment

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Abstract

The work presents a report on Zinc oxide nanoparticles (ZnO NPs) synthesized through a green approach using *Nauclea latifolia* fruit extracts, with a view to investigating the prepared nanoparticles for their antimicrobial activities. The ZnO NPs synthesized were characterized using various analytical instruments, including X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR), Ultraviolet-Visible (UV-Vis) spectroscopy, Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). The instruments provided valuable information on the characteristics of the Zn ONPs. The antibacterial activities of the synthesized ZnO NPs were evaluated with *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). The maximum absorption was observed at 379 nm. The average hydrodynamic size and the polydispersity index (PDI) were measured as 81.77 nm and 0.401, respectively. The nanomaterial has a hexagonal wurtzite structure, and the Zn–O bond was detected at 537 cm⁻¹. The nanoparticles were in the nano range with sizes ranging from 10.02 nm to 28.50 nm. The *N. latifolia* fruit extract-mediated ZnO NPs showed excellent performance against the two bacteria at all concentrations of ZnO NPs. The highest inhibition zones for *E. coli* and *S. aureus* at 8 mg/L of ZnO NPs are 21 and 16 mm, respectively. This study provides valuable insights into an efficient, simple, and environmentally friendly route for synthesizing ZnO NPs with a potential application in the biomedical field.

Keywords ZnONPs, *Nauclea latifolia*, Antibacterial, Zone of inhibition

Introduction

Nanotechnology is one area with the most advancing technology in recent times. It is innovative research that embraces nano-sized materials production, characterization, and engagement. Recently, nanoparticles have emerged as the topmost area of attention in the engineering, cosmetics, pharmaceutical, and textile industries due

to their manipulative and unique properties [1–3]. Metal oxide nanoparticles are receiving overwhelming attention from the research world due to essential qualities, such as availability, a broad range of radiation absorption, high chemical stability, light stability, safety, and cost-effectiveness [4–6]. Noble metal nanoparticles have exhibited remarkable chemical and physical properties, surpassing their larger-scale counterparts [7, 8]. Zinc oxide nanoparticles are nanoparticles that have attracted great interest in recent applications. It is considered one of the most employed nanoparticles, alongside Ag, TiO₂ and SiO₂ nanoparticles [9]. ZnO nanoparticles have received that much attention due to their distinctive features, including high adsorptive activity, a broad band

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gap (3.17 eV), non-hazardous nature, and a low-cost preparation method [10]. Nanomaterials' size, shape, and surface qualities affect their cellular absorption, which determines their suitability for medical engagements and demands. ZnO NPs have drawn intensive research due to their unique attributes and wide applications in medicine, agriculture, corrosion, environment, electronics, and material science [11]. ZnO NPs can be prepared by biological, chemical or physical methods. However, complex procedures, high energy demand, high production cost, and safety bias inherent in chemical and physical processes [12] make them fall behind in use compared to the biological methods of nano-synthesis techniques. Synthesis of nanoparticles *via* biological method is a green technology involving bioactive materials of plants' and animals' origins (such as various plants' parts and wastes). This method is safe, cost-effective, simple, and nature-friendly.

In previous investigations, ZnO NPs have been derived from bio-sources such as *Ocimum basilicum* [13], *Cocos nucifera* leaves [2], *Aeromonas hydrophila* [14], *Poncirus trifoliata* [15], *Parthenium hysterophorus* [16], *Aloe vera* peel [17], Blackberry nightshade [18] *Coriandrum Sativum* [19], *Caltropis procera* [20], *Garcinia mangostana* [21], *Allium sativum* and *Zingiber officinale* [1]. The bioactive compounds such as flavonoids, alkaloids, tannins, saponins, proteins, phenolic compounds, carbohydrates, quinine, glycosides, and steroids, found in plant materials [22], play a crucial role in serving as effective agents for reducing and stabilizing the process of nanoparticles formation. Additionally, these bioactive compounds significantly impact nanoparticles' antimicrobial properties [23].

Nauclea latifolia (African peach) belongs to the family of Rubiaceae, commonly found in tropical Nigeria, Ghana, DR Congo and Asia [24]. It is a deciduous flowering plant with an open canopy and can grow up to 9 m tall. The fruit is a red or pinkish compound fruit with a round shape and contains tiny seeds. *Nauclea latifolia* is synergistic and protective against specific hepatocellular injury [25] and possesses antioxidant activity [26].

Aqueous extract of *Nauclea latifolia* fruit has been reported to contain important phytochemicals, including alkaloids, saponins, flavonoids, terpenoids, tannins, steroids, and glycosides [27]. These phytochemicals play a pivotal role in the reduction and stabilization of nanoparticles during green synthesis. *N. latifolia* extract is known for its composition of flavonoids and polyphenols, containing phenolic hydroxyl groups and conjugated double bonds [25]. These moieties play a crucial role in electron transfer during nano synthesis. This transformative process reduces metal ions to their zero-valent state, leading to the formation of metallic nanoparticles. Additionally, these compounds contribute to the formation

of a protective layer through coordination bonds with the nanoparticles' surface. This layer serves to prevent aggregation, ensuring the maintenance of their small size and distinctive properties. Moreover, integrating plant bioactive materials into nanoparticles holds significant promise for pioneering therapeutic approaches, thereby fostering advancements in antimicrobial strategies [28–31]. However, despite the existing literature documenting numerous plant-mediated ZnO nanoparticles, as well as the presence of various phytochemicals in *Nauclea latifolia* fruit extract, the potential of the *Nauclea latifolia*-ZnO NPs as effective, and low-cost antibacterial agent remains untapped. Therefore, the present work aimed to synthesize ZnO nanoparticles using *Nauclea latifolia* fruit extract and evaluate its antimicrobial properties.

Experimental

Materials used and sample preparation

Fresh *Nauclea latifolia* fruits were obtained at the Ureje area of Ado-Ekiti, Nigeria and transported to the Chemistry Laboratory at the Federal University of Technology, Akure, Nigeria. The fresh fruits were washed thoroughly, dried for 20 days, crushed to powder, sieved and stored in a plastic container for further use. Zinc nitrate hexahydrate (precursor) was procured from MoliChem, India. Other materials used were ethanol, deionized water, a magnetic stirrer, measuring cylinders, beakers, a thermometer, a ceramic crucible, analytical balance (Metler Toledo ME4002 ME Series), Whatman No. 1 filter paper, a drying oven, and an electric furnace. All chemicals are of analytical grades and were used with no prior treatments.

Extraction procedure

The *N. Latifolia* fruit powder (25 g) was boiled in 500 ml of distilled water for 30 min at 60°C. The mixture was filtered, and the extract was used immediately.

ZnO NPs synthesis

For ZnO NPs synthesis, 50 ml of freshly prepared *N. latifolia* fruit extract was heated in a 250 ml beaker at 80°C and stirred at 200 rpm. A 10 g of zinc salt was added to the extract at 60°C; the stirring and heating continued until precipitation began to form. The mixture was oven-dried at 60°C for 4 days and later calcined at 400°C for 2 h in a furnace. The flow chart for the synthesis of the ZnO NPs is shown in Fig. 1.

Characterization of material

The instruments employed to characterize the ZnO NPs include UV-Vis spectrophotometer (UV-1800 series, Shimadzu, Japan) for optical analysis within the 300–600 nm range. Fourier transform infrared (Agilent Cary 630 FTIR spectrometer) spectroscope to reveal

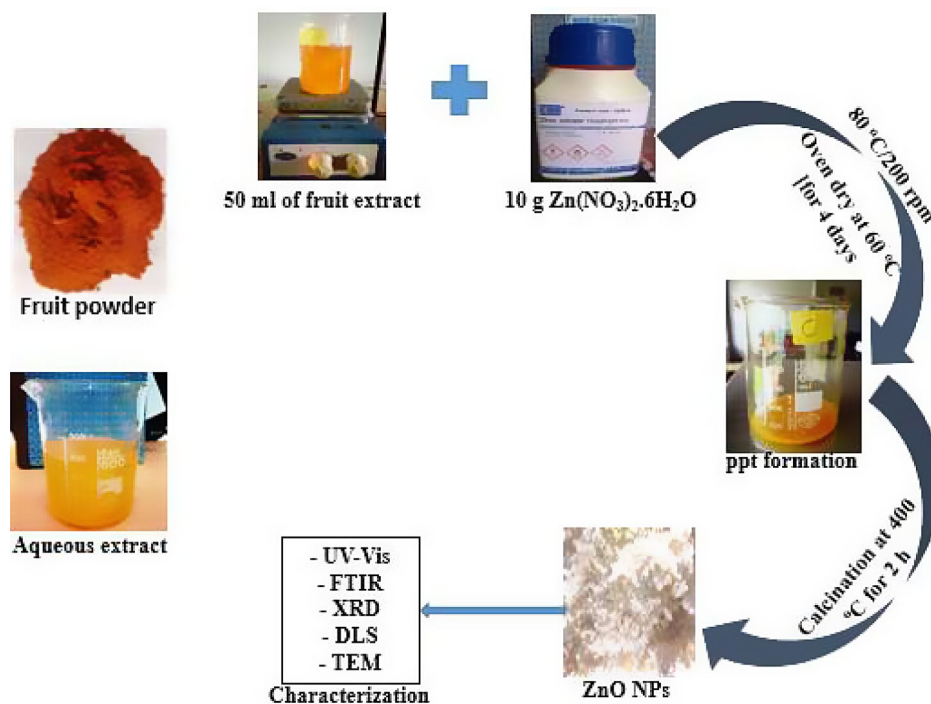


Fig. 1 The flow chart of the process of synthesis

the functional groups on the synthesized nanoparticles. X-ray diffraction was engaged in examining the crystalline structure of the ZnO NPs. Dynamic light scattering (Malvern Zetasizer, Nano ZS Series) was employed to assess the average hydrodynamic size and polydispersity index of ZnO nanoparticles, while the surface morphology and particle size of the ZnO NPs were studied using a transmission electron microscope.

Determination of average crystallite size

The average crystal size of the zinc oxide crystallites was calculated using the Scherrer equation [22] (Eq. 1).

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

Where D represents the particle diameter, K is the shape factor (0.9), λ corresponds to the X-ray wavelength (1.5419 Å) used, β is the FWHM (full width at half maximum), and θ represents the Bragg angle.

Antibacterial study

The ZnO NPs' antibacterial potency was investigated using the disc dilution method [32, 33] with *Escherichia coli* (ATCC 25,922) and *Staphylococcus aureus* (ATCC 12,598) strains. *Escherichia coli* and *Staphylococcus aureus* were selected as model organisms for antimicrobial studies, representing Gram-negative and Gram-positive bacteria, respectively. Bacterial cultures were grown

and kept at 25 °C on Luria-Bertani (LB) medium for 24 h and maintained at 4 °C in a refrigerator. Both bacterial pathogens were cultured in nutrient agar overnight to produce 10⁶ colony-forming units (CFU) per millilitre. One hundred microlitres of each bacteria culture was spread on the LB agar plates. A disc (sterile filter paper) was prepared before soaking the disc with 2, 4, 6 and 8 mg/L of ZnO NPs. Water was employed as a control for the present evaluation. Prepared discs were placed carefully on the surface of the agar plates while ensuring they adhered firmly and were spaced adequately apart. The plates were incubated for 18–24 h at 37 °C, and millimetre (mm) measurements of the zone of inhibition were made. The experiments were done in triplicate and the average was determined.

Results and discussion

Physical assessment of ZnO-NPs

The product's physical characteristics at various stages of the synthetic process confirmed the synthesis of ZnO NPs using *Nauclea latifolia* fruit extracts. Cloudy yellow suspension was observed when the fruit extract reacted with Zn(NO₃)₂·6H₂O at 60–80 °C with continuous agitation at 200 rpm. The formation of the tiny particles can be attributed to some plant bioactive compounds acting as the reducing agent on the zinc nitrate [28]. Subsequently, after drying at 60 °C in an oven for four days, the material developed a pronounced deep yellow hue, and calcination at 400 °C for 2 h resulted in a whitish final

product. The calcination of ZnO nanoparticles is crucial for achieving proper crystallinity, controlled particle size, and dispersion. The choice of 400 °C calcination temperature helps to prevent agglomeration, as well as to manipulate the size, morphology, and structure of ZnO. The changes observed at the different stages of the synthetic process were in accordance with the early report of the biosynthesis of ZnO-NPs [34].

Material characterization

UV-Vis spectroscopy analysis: The identity of the material synthesized using *N. latifolia* was pre-confirmed as zinc oxide nanoparticles by UV-Vis spectrophotometer. Fig. 2a shows the FTIR analysis done between 300 and 600 nm. The figure shows that the ZnO NPs demonstrated a broad UV absorption peak at 379 nm. A slight shift in the ZnO NPs' maximum wavelength from the standard (360–365 nm) can be attributed to some functional groups from plant biomolecules, leading to a slight shift in the maximum absorption wavelength. The result was in agreement with similar reports [32, 35].

X-ray Diffraction (XRD) analysis was done to understand the crystallinity of the *N. latifolia*-mediated ZnO NPs. The XRD pattern in Fig. 2b was examined to authenticate the phase of ZnO NPs. The scanning was

performed in the range of 2θ between 20 and 70°. The analysis displayed distinct diffraction peaks occurring at 2θ of 31.83, 34.45, 36.29, 47.62, 56.64, 62.88, 66.47, 67.94, and 69.14° which correspond to the crystallographic planes of 100, 002, 101, 102, 110, 103, 112, 200, and 201, respectively, as indexed by the Joint Committee on Powder Diffraction Standards (JCPDF) file no. 00-036-1451. The peaks were familiar with those of ZnO NPs and showed a hexagonal wurtzite crystal structure [18, 40]. The distinct and noise-free peaks revealed the pure status and nature of crystalline of the ZnO NPs. The average crystallite size of the zinc oxide nanoparticles was calculated to be 31.25 nm. This measurement has implications for the structural and functional characteristics of the nanoparticles. Nanoparticles with larger crystallites may exhibit less resistance to agglomeration over time. It is worth noting that the recorded peaks and the average crystallite size in the present work align with the previous reports on the biosynthesis of ZnO NPs [1, 19].

The FTIR analysis was conducted on the ZnO NPs in the range 4000–400 cm^{-1} to examine the functional groups in the nanoparticles. As presented in Fig. 2c, the results revealed several FTIR absorption peaks peculiar to ZnO nanoparticles of plant origin. The bands include a 3534 cm^{-1} peak corresponding to water's O–H stretching

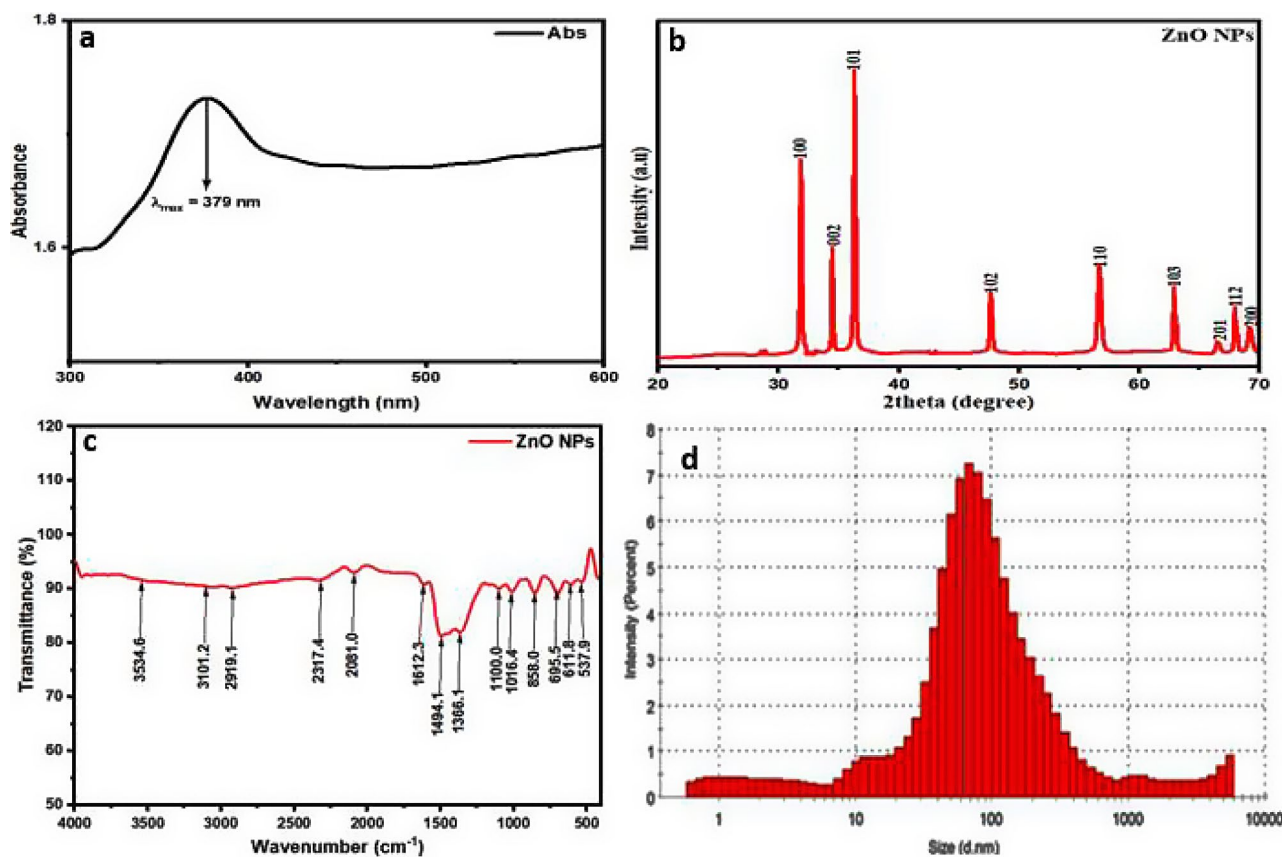


Fig. 2 The UV-Vis spectrum (a), XRD pattern (b), FTIR spectrum (c) and size distributions (d) of ZnO NPs

vibration. The glycosidic linkages of C–O–C and secondary alcohol were observed at 1612 and 1100 cm^{-1} , respectively. The peak at 1016 cm^{-1} corresponds to the stretching bonds of –C–O–C. Bands at about 3101 and 2919 cm^{-1} can be attributed to =C–H of an aromatic compound. The band 2317 is typically associated with C=O stretching vibrations carboxylic. The IR peak at about 537 cm^{-1} can be linked to the stretching vibration of the Zn–O bond. Several absorption peaks observed in the ZnO-NPs can be traced to active metabolites in the plant material used as reducing and stabilizing agents [21, 25].

Dynamic Light Scattering technique measured the average hydrodynamic size of ZnO NPs, revealing an average size of 81.77 nm. The size distribution graph (Fig. 2d) shows that the particle size is polydispersed. It is important to note that the DLS-derived average particle size exhibits significant variation compared to the one determined through the Scherrer equation using XRD data. This discrepancy can be attributed to the distinct principles governing these two measurement techniques. Dynamic Light Scattering primarily analyzes particles in a solution, considering their hydrated state and potential influence from solvent molecules or stabilizers, which can increase the hydrodynamic size. In contrast, XRD measures dry samples, providing information about particle size and crystal structure without the influence of a surrounding medium. The polydispersity index (PDI) is primarily used to measure the average uniformity of a particle in solution [23], with applications in evaluating nanoparticles for drug loading, encapsulation efficiency, bioavailability, and efficacy [36]. The PDI of *Nauclea latifolia* ZnO NPs was measured to be 0.401, which indicates a moderate to high PDI [37]. This value indicates a range of particle sizes within the material, suggesting a possibility for the particle to agglomerate over time.

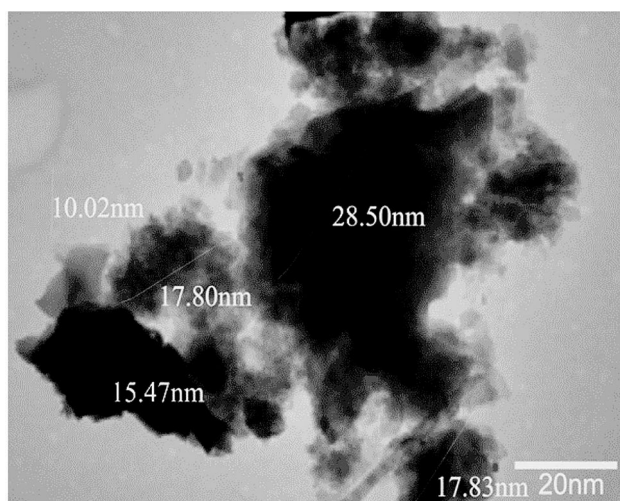


Fig. 3 TEM image of the ZnO NPs

This attribute is particularly pertinent to semiconductor nanoparticles such as ZnO NPs, which may exhibit heightened susceptibility to agglomeration due to their usual high surface energy, leading to the attraction of particles with opposing charges and propelling particles to aggregate [38].

Transmission Electron Microscopy (TEM) was used to visualize the morphology and size of the material. The TEM image presented in Fig. 3 revealed the presence of relatively small, irregular, and spherical to hexagonal particles. The average size of the ZnO nanoparticles fell within the range of 10.02 to 28.50 nm. The cloudy appearance observed in some sections can be attributed to particle overlapping, a result of particle agglomeration as indicated by the PDI value. The TEM image confirmed the presence of nanoparticles (NPs) with properties similar to those reported by other researchers using different plant extracts [5, 38].

Antimicrobial activities

The inhibitory effects (Fig. 4a and b) of ZnO NPs synthesized using *Nauclea latifolia* fruit extract were studied using *Escherichia coli* and *Staphylococcus aureus*. Fig. 4c presents the zone of inhibition for the interaction of bacteria with different concentrations (2, 4, 6 and 8 mg/L) of the ZnO NPs. From the figure, the ZnO NPs exhibited good constraint on the two bacteria. The efficiency increased as the ZnO NPs concentration increased. The plant nanoparticles were more potent on *E. coli* than on *S. aureus*. The highest inhibition zones were measured as 21.00 and 16.00 mm for *E. coli* and *S. aureus*, respectively, at the highest concentration (8 mg/ml) of ZnO NPs. The size of the ZnO NPs combined with the antimicrobial activity of *N. latifolia* might have been responsible for their excellent antibacterial performance in this study [36]. The small size of the NPs promotes their absorption by bacterial cells and interacts with bacterial DNA and proteins, causing significant damage. This damage eventually leads to the death of bacterial cells. However, *E. coli* was more susceptible to ZnO-NPs than *S. aureus* at all concentrations. This could be a result of *E. coli* (gram-negative bacteria) with a more porous outer membrane compared to *S. aureus* (gram-positive bacteria), causing an increased permeability in *Escherichia coli*, allowing ZnO nanoparticles to penetrate more quickly [39] and disrupt cellular processes, leading to an elevated susceptibility. The efficiency of the ZnO NPs as antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus* can be attributed to the bioactive molecules in the *Nauclea latifolia* extract incorporated into the nano-sized particles. The performance recorded with *Nauclea latifolia*-ZnO NPs in the present work was similar to those previously reported [40, 41].

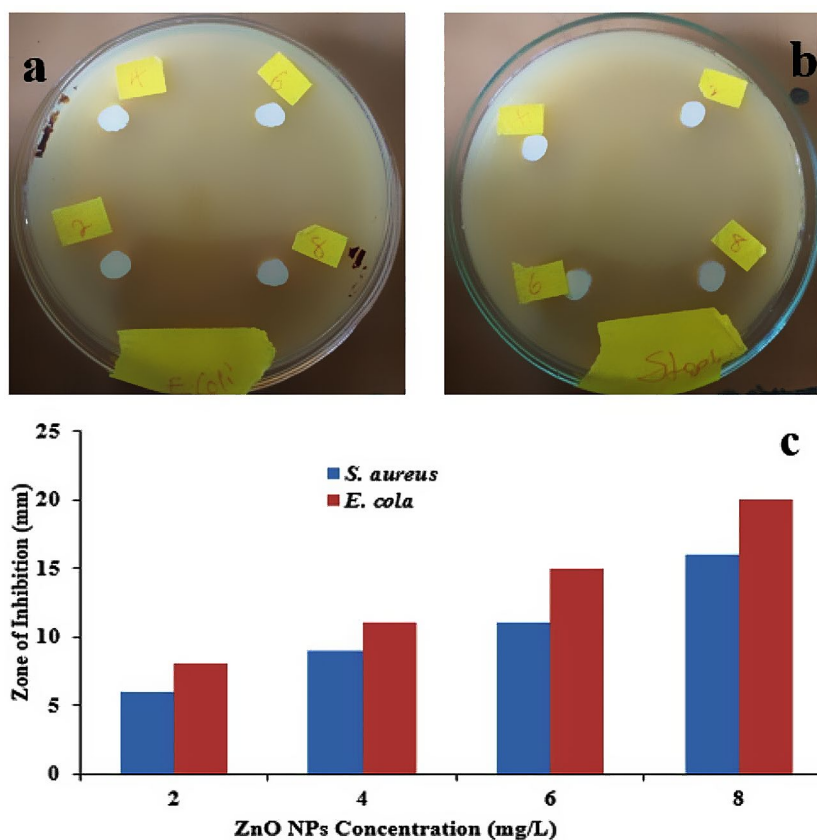


Fig. 4 Experimental set-up antimicrobial activities of ZnO NPs against *E. coli* (a), *S. aureus* (b) and the zone of inhibition for *E. coli* and *S. aureus*(c)

In this study, ZnO nanoparticles were synthesized using *Nauclea latifolia* fruit extracts and utilized as antimicrobial agents against *S. aureus* and *E. coli*. UV-Vis spectroscopic measurement revealed the maximum absorption of 379 nm for the ZnO NPs. X-ray diffraction analysis of the synthesized plant-mediated Zn ONPs confirmed the formation of a hexagonal structure and crystalline nature of the ZnO NPs with an average crystallite size of 31.25 nm. The FTIR evaluation showed a band at 537 cm^{-1} to confirm the synthesized material as ZnO NPs. Dynamic Light Scattering revealed a PDI value of 0.401, indicating a tendency of the particles to agglomerate over time. The antimicrobial evaluations of the prepared ZnO NPs showed that the *Nauclea latifolia*-ZnO nanoparticles are highly potent against *E. coli* and *S. aureus*. The highest zones of inhibitions of 16 and 21 mm were observed for *S. aureus* and *E. coli*, respectively, using 8 mg/L of the ZnO NPs. This research further proved that simple, effective, low cost and environmentally friendly nanoparticles could be bio-synthesized. Furthermore, we also conclude that the prepared ZnO NPs could be used as antimicrobial agents.

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Author contributions

All authors conceived the research idea, participated in experimental work, involved in manuscript writing and editing, and approved the final manuscript for submission.

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Data availability

Not applicable.

Declarations

Ethics approval and consent to participate.

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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