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Bioinspired NiO nanoparticles from *Bauhinia tomentosa* with improved antimicrobial and antioxidant properties

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Abstract

Green synthesis approach of nanomaterials inspired by biological materials, in particular, extracts from plants, has become a preferred method to synthesize nanoparticles (NPs). This is mainly due to the improved therapeutic activity of the NPs informed by phytochemicals found in plant extracts, and the environmental friendliness and cost effectiveness of the method. The present work reports the bioinspired synthesis of nickel oxide NPs (NiO NPs) from the aqueous extract of *Bauhinia tomentosa* as reducing and capping agent. The morphology, functional groups, elemental composition, and size of the NiO NPs were characterized by scanning electron microscopy (SEM), Fourier transform Infrared spectroscopy (FTIR), energy dispersive X-ray (EDX), and dynamic light scattering (DLS), respectively. The antioxidant and antimicrobial activities of the NPs were also investigated to test their therapeutic potential. The synthesized NiO NPs were spherically shaped and appropriately dispersed with a size distribution of 30–100 nm, and absorb at 346 nm in the ultraviolet region. The FTIR spectra of the NiO NPs revealed a stretching vibration band at 601 nm, representing the Ni-O bond. The bioinspired NiO NPs showed improved antioxidant activities over the extracts by scavenging the free radicals generated by hydrogen peroxide (H₂O₂) and 2,2-diphenyl-2-picrylhydrazyl (DPPH). Furthermore, the NiO NPs showed outstanding activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*, comparable to popularly used antibiotics and antimycotics.

Keywords Nickel oxide nanoparticle, *Bauhinia tomentosa*, Green synthesis, Antimicrobial, Antioxidant

1 Introduction

Nanotechnology is a rapidly evolving area of research, particularly in the field of medicine, where they are employed for the development and delivery of new therapeutics [1]. Nanoparticles (NPs) are so called due to the relatively small size of the materials (within the 1–100 nm range), which make them easily diffuse through the cell membrane to the



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regions where they efficiently perform their duties [2]. NPs have become very useful in cancer research, protein research, cosmetics development, sensors, and for building storage devices [3–6]. NPs can be developed as metal and metal oxides [1, 4, 7, 8], polymers [9–11], quantum dots [12, 13], among others.

Metal oxide NPs, such as those of NiO, SnO₂, CuO, ZnO, BaO, Fe₂O₃, etc., have become widely adopted, arousing the interest of researchers as a result of their fascinating physical, optical, chemical, and biological properties [14–16]. Metal oxide NPs have found vast application in drug discovery and delivery, environmental remediation, catalysis, tissue engineering, food technology, magnetism, imaging, among others [17–20]. Nickel oxide NPs (NiO NPs) for example, is popular and vital metal oxide nanoparticle that has attracted interest due to their robust application. In drug discovery, NiO NPs have been broadly researched for their efficiency as antibacterial, antifungal, antioxidant, anticancer, and antiviral agents [7, 21–25].

These NPs are synthesized through a chemical or biological means. Chemical synthesis of NPs can be done using pyrolysis [26], sol – gel [27], electrospray [28], coprecipitation [29], laser ablation [30], and hydrothermal [31] methods. Chemical synthesis of NPs suffer from the disadvantage of the need for costly chemicals and environmental pollution due to release of toxic chemicals to the environment. Biological synthesis, also known as green synthesis of NPs has been preferably adopted over chemical methods majorly because of their eco-friendliness and cost-effectiveness [1, 23]. Biological synthesis of NPs uses extracts from plants, or microorganisms to reduce metal salts into metal oxide NPs. This method is energy efficient, non-toxic, easy, and cheap [32, 33]. Biological synthesis using plant extracts is preferred due to the robust and cheap availability of different plant species and how the phytochemicals, such as alkaloids, flavonoids, tannins, glycerides, etc [34], which are present in these plant extracts are able to improve their overall efficiencies and activities.

Using biological synthesis approach, NiO NPs have been synthesized using the extracts of different plants such as *Sageritia thea* [35], *Stevia* [36], *Phytolacca dodecandra* [23], *Elettaria cardamomum* [37], *Croton macrostachyus* [1], *Arundo donax* [22], *Phoenix dactylifera* [25], *Berberis pachyacantha* [20], *Prunus armeniaca*, among others. These bio-synthesized NPs showed improved properties and biological activities over their chemically synthesized counterparts or the pristine extracts from which they were synthesized from.

Although several plants have been employed to biologically synthesize NiO NPs, one of the many plants whose potential in the synthesis of NiO NPs has not been explored is *Bauhinia tomentosa* Linn. *Bauhinia tomentosa* L. is a popular flowering plant of the *Cesalpinoideae* subfamily known for its robust richness in phytochemicals [39]. Evidence from previous researches on this plant shows that it is rich in bioactive secondary metabolites, including phenolic compounds, flavonoids, and tannins [39, 40], which have functional groups that can aid the reduction of metal salts to their oxides, and has found use in traditional medicine to treat different conditions, such as abscesses, liver inflammation, tumor, hyperlipidemia, diabetes, diarrhea, helminthiasis, and fever, as well as abdominal, skin, and urinary tract illnesses [39–41]. The extracts of *Bauhinia tomentosa* have been tested for their anti-inflammatory, antimicrobial, anti-tumor, anti-diabetic, and anti-rheumatic properties [42–44], as well as an analgesic and hypo-cholesterolemic agent [43]. In addition, the extracts have been successfully employed to produce

biologically important NPs, such as silver oxide NPs [43] and zinc oxide NPs [46]. Till date, no study has reported on the exploitation of the extracts of *Bauhinia tomentosa* for the synthesis of NiO NPs.

In the present study, we report the first-hand biosynthesis of NiO NPs using extracts from *Bauhinia tomentosa* L. Aqueous extract of the seedpod and leave of the plant was extracted by cold maceration, tested for their phytochemical composition, and employed as reducing and capping agent for the bioinspired synthesis of the NPs. The plant extracts and their biosynthesized NPs were characterized for their optical properties by UV-Vis spectroscopy. The functional groups present in the extracts and their respective NPs was analyzed by Fourier transform Infrared spectroscopy (FTIR). The morphology and elemental composition of the NPs was analyzed by energy dispersive X-ray-coupled scanning electron microscopy (SEM-EDX). Dynamic light scattering (DLS) was employed to analyze the size distribution of the NPs. The antioxidant properties of the NPs was tested using the hydrogen peroxide (H_2O_2) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Finally, the activity of the NPs against Gram-negative and Gram-positive bacteria, as well as a fungus was evaluated.

2 Materials and methods

2.1 Chemicals and reagents

Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) (Burgoyne Burbidges and Co, Mumbai, India); potassium hydroxide (KOH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrochloric acid (HCl), copper sulphate (CuSO_4), and sodium hydroxide (NaOH) (Molychem, Mumbai, India); dimethyl sulfoxide (DMSO) (Guandong Sci-Tech Co, Ltd, Guandong, China); ethanol, hydrogen peroxide and methanol (BDH Limited Poole, England); ascorbic acid (Kermel Reagent Co, Ltd; Tianjin, China).

2.2 Preparation and extraction of *Bauhinia tomentosa*

The *Bauhinia tomentosa* Linn seedpod (BTSP) and leaf (BTLE) used in this work was collected from Dominion University, Ibadan, Nigeria. The flowering parts (seedpod, flowers, and leaves) of the plant was identified and authenticated by Mr Esimekhuai at the herbarium unit of the Department of Botany, University of Ibadan, Nigeria (Authentication number UIH-23360). The samples were prepared as previously reported [47]. After 2 weeks of airdrying followed by pulverization, the BTSP and BTLE were cold-extracted using double-distilled water (ddH_2O) using a modified protocol [48]. Briefly, 100 g of BTSP and BTLE samples were suspended separately in 1 L of ddH_2O and extracted at room temperature for 5 days with constant stirring. The crude extract was separated using a muslin bag and further filtered using a Whatman filter paper 1.0 before use for green synthesis.

2.3 Bioinspired synthesis of NiO nanoparticle

Bioinspired NiO nanoparticle was synthesized from BTSP and BTLE extracts using the established protocol [23] with modifications. Briefly, 20 mL of BTSP or BTLE were added to 50 mL of a 0.1 M nickel nitrate solution drop wisely and constantly stirred at room temperature for 30 min. pH adjustment of the mixture was done using 5 M NaOH followed by stirring for another 2 h. The precipitate formed was collected by centrifugation and washed severally with ddH_2O and ethanol to remove unreacted salts and

impurities. The NiO nanoparticle synthesized from the leaves (NiO-BTLE) and seed-pods (NiO-BTSP) was oven-dried for 8 h at 70 °C.

2.4 Phytochemical screening of BTSP and BTLE extracts

The BTLE and BTSP plant extract were screened for their phytochemical composition using already established protocol [49]. The presence of *saponins* was tested by shaken the extracts in a test tube. Foaming of the extract, which remained persistent when heated confirms that saponin is present. *Steroids* was tested by adding 4 mL of plant extracts and 2 mL of chloroform into a test tube, followed by a few drops of concentrated H_2SO_4 along the wall of the test tube. Appearance of red colour indicates that steroid is present. The presence of *alkaloids* was tested by adding 5 mL of 2% HCl to 1 mL plant extract. The mixture was heated for 10 min, filtered, and a few drops of Mayer's reagent was added to 1 mL of the filtrate. Whitish precipitate confirms that alkaloids are present. The presence of *flavonoids* was tested by adding a few drops of 2% NaOH to 1 mL plant extract. Yellow coloration which disappears in the presence of HCl shows that flavonoids are present. *Tannins and Phenols* were tested by adding few drops of 5% FeCl_3 to 1 mL of extract. Formation of a dark blue or dark green precipitate indicates that phenol or tannins are present. *Protein* was tested by adding two drops of 3% CuSO_4 and a few drops of 10% of NaOH to 1 mL plant extract. Violent or red precipitate indicates that protein is present. *Carbohydrates* was tested by adding 1 mL each of Fehling's solution A and B to 1 mL of the extract, and heating the mixture 5 min. A red or dark red precipitate indicates that carbohydrate is present. The presence of *cardiac glycosides* was tested by adding 0.4 mL of glacial acetic acid, 2 to 3 drops of 10% FeCl_3 , and 2 mL of plant extract to a test tube, and 1 mL of concentrated H_2SO_4 along the wall of the test tube. A deep-blue colour indicates the presences of cardiac glycosides.

2.5 Physicochemical characterization of extracts and nanoparticles

The extracts and their corresponding NPs were characterized for their optical, morphological, and compositional properties. FTIR was used to characterize the functional groups present in the samples using a PerkinElmer FT-IR Spectrum 2 spectrometer (PerkinElmer, USA) [47]. UV-Vis spectroscopy was used to analyze the absorption spectra of the samples using a Mettler Toledo UV5Bio UV-Vis spectrophotometer (Mettler Toledo, USA). A Zetasizer Nano S90 (Malvern instruments, U.K) was used to measure the size distribution of the NPs based on DLS technique [50]. A Hitachi SU 3500 scanning microscope (Hitachi, Japan) coupled to an energy dispersive X-ray (EDX) spectroscope was used to characterize the surface morphology and elemental composition of the nanoparticles [51].

2.6 Antioxidant activity assays

2.6.1 DPPH assay

The DPPH radical scavenging activity of the extracts and NPs was analyzed using established protocols [52, 53]. Stock solutions of 1 mM of DPPH and 1 mg mL^{-1} of extracts or NPs was prepared separately in DMSO. 0.5 mL of the DPPH stock was then mixed with varying concentrations ($30\text{--}150 \text{ }\mu\text{g mL}^{-1}$) of the extracts or NPs and allowed to react in the dark for 30 min. A negative control containing only DPPH in DMSO, and a positive control containing varying concentrations ($30\text{--}150 \text{ }\mu\text{g mL}^{-1}$) of ascorbic acid in DMSO

was also prepared. The absorbance of the mixture was measured by UV-Vis at a wavelength of 517 nm. All experiments were carried out in duplicates and the percentage Radical Scavenging Activity (% RSA) was calculated using Eq. 1.

$$\% \text{ RSA} = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

Where A_c is the absorbance of negative control and A_s is the absorbance of the sample.

2.7 Hydrogen peroxide assay

The hydrogen peroxide scavenging activity of the extracts and their NPs was analyzed using established protocol [52]. A 1 mg mL^{-1} of the samples and 100 mM of H_2O_2 was freshly prepared in phosphate buffer solution (PBS). 1.2 mL of the extracts or their nanocomposites of varying concentrations ($30\text{--}150 \text{ }\mu\text{g mL}^{-1}$) was prepared in 2.4 mL of freshly prepared 100 mM H_2O_2 solution. The volume of the solution was scaled up to 4 mL using 0.4 mL of PBS and the mixture was allowed to equilibrate for 30 min in the dark. A negative control containing only H_2O_2 in PBS, and a positive control containing varying concentrations ($30\text{--}150 \text{ }\mu\text{g mL}^{-1}$) of ascorbic acid in PBS was also prepared. The absorbance of the samples was measured by UV-Vis at wavelength of 600 nm . All experiments were carried out in duplicates and the percentage Radical Scavenging Activity (% RSA) was calculated using Eq. 1.

2.8 Antimicrobial assay

2.8.1 Preparation of test organisms

Pure isolates of Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria, and fungus (*Candida albicans*) were aseptically sub-cultured into nutrient agar and sabouraud dextrose agar, respectively. The cultures were incubated for $18\text{--}24 \text{ h}$ at $37 \text{ }^\circ\text{C}$ (bacteria) or $28 \text{ }^\circ\text{C}$ (yeast) and the resulting colonies were then used for sensitivity analysis. The bacterial and fungal inoculums were prepared by transferring the microorganisms in their exponential phase into sterile normal saline and standardizing them by adjusting the concentration to match the 0.5 McFarland standard (equivalent to $1.5 \times 10^6 \text{ CFU mL}^{-1}$) [54].

2.8.2 Antibacterial and antifungal activity assay

The antibacterial activity of BTLE, BTSP, and their corresponding NPs (NiO-BTLE and NiO-BTSP) was tested individually using the well diffusion method [54–56]. Standardized bacterial or fungal inoculum was spread uniformly on sterilized and solidified Mueller Hinton agar (bacteria) and Sabouraud Dextrose agar (fungi) on petri plates using a sterile swab. 5 mm diameter wells were made in each plate, into which $50 \text{ }\mu\text{L}$ of varying concentrations ($125\text{--}500 \text{ }\mu\text{g mL}^{-1}$) of the samples were introduced before incubation for 24 h at $37 \text{ }^\circ\text{C}$ (bacteria) or $28 \text{ }^\circ\text{C}$ (fungi). A well containing DMSO was used as a negative control (NC), while $5 \text{ }\mu\text{g}$ Ciprofloxacin (CIP), $30 \text{ }\mu\text{g}$ of Amoxicillin and Clavulanic Acid (AMC), and $25 \text{ }\mu\text{g}$ of Fluconazole (FLC) were used as positive control. After incubation, the petri plates were read by measuring clear zones of inhibition (in millimeters) around the well that contains each sample. All experiments was performed twice.

3 Results and discussion

3.1 Phytochemical screening of BTSP and BTLE extracts

The various phytochemicals and bioactive compounds in the leaves and seedpods of *Bauhinia tomentosa* was qualitatively analyzed to test their potential as reducing or capping agents for nanoparticle synthesis, as well as their potential as antimicrobials. The result confirmed that different phytochemicals, such as alkaloids, steroids, and phenols (Table 1) are present in the aqueous extracts of BTLE and BTSP, which suggests that the extract is a good source of secondary metabolites. The phytochemicals also carry hydroxyl and carbonyl groups that will be important in reducing nickel salts to nickel NPs similar to previous reports [23, 58, 59].

3.2 Physicochemical characterization of extracts and nanoparticles

On addition of the light brown aqueous extract of BTSP and BTLE, the bright green nickel nitrate solution turned darkish green, indicating the formation of NiO-BTSP and NiO-BTLE NPs. After synthesis, 0.435 g of NiO-BTSP was obtained, while only 0.319 g of NiO-BTLE was obtained, indicating that the synthesis of NPs from the seed pod of *Bauhinia tomentosa* is more effective than from the leaf. The phytochemicals which are in the extracts effectively reduced the nickel salt to its NPs, while also acting as a capping agent to prevent aggregation of the NPs [60].

The UV-Vis spectra of the extracts and NPs showed an absorption peak at 280 nm in both the extracts and their NPs (Fig. 1). This peak probably indicates proteins which are present in the extracts. In addition to the peak at 280 nm, the NPs displayed a surface plasmon resonance (SPR) band at 346 nm, which was absent in the extracts (Fig. 1). This peak has been previously reported for NiO NPs [23, 38, 61] and suggests successful bio-synthesis of NiO NPs.

The functional groups in the extracts and their NPs were further characterized using FTIR at a frequency range of 400–4000 cm^{-1} . The FTIR spectra (Fig. 2) showed different peaks indicative of the presence of phenols, alcohols, alkanes, carboxylic acids, etc. The band at 3304 cm^{-1} , which is present in the extracts and their NPs, can be attributed to the stretching vibrations from N-H and/or O-H groups suggesting the presence of amines, amides, and alcohols. This is consistent with our qualitative phytochemical screening of the extracts that revealed that alkaloids and phenolic phytochemicals are present in the extracts, and is consistent with previous studies [23, 62]. In addition, two bands at 2980 and 2852 cm^{-1} were present in the spectra of all the samples, indicative of the sp^2 hybridization of an alkene. Furthermore, the bands at 1626 and 1034 cm^{-1} are indicative of the presence of C = O and C-O stretching vibrations, respectively, which may be suggestive of alcohols, esters, and carboxylic acid groups [62]. Most importantly,

Table 1 Phytochemical screening of extracts

Phytochemicals	BTLE	BTSP
Saponin	+	+
Steroid	+	+
Flavonoid	—	—
Alkaloid	—	+
Tannin/Phenol	+	+
Protein	+	+
Carbohydrate	+	+
Glycosides	-	-

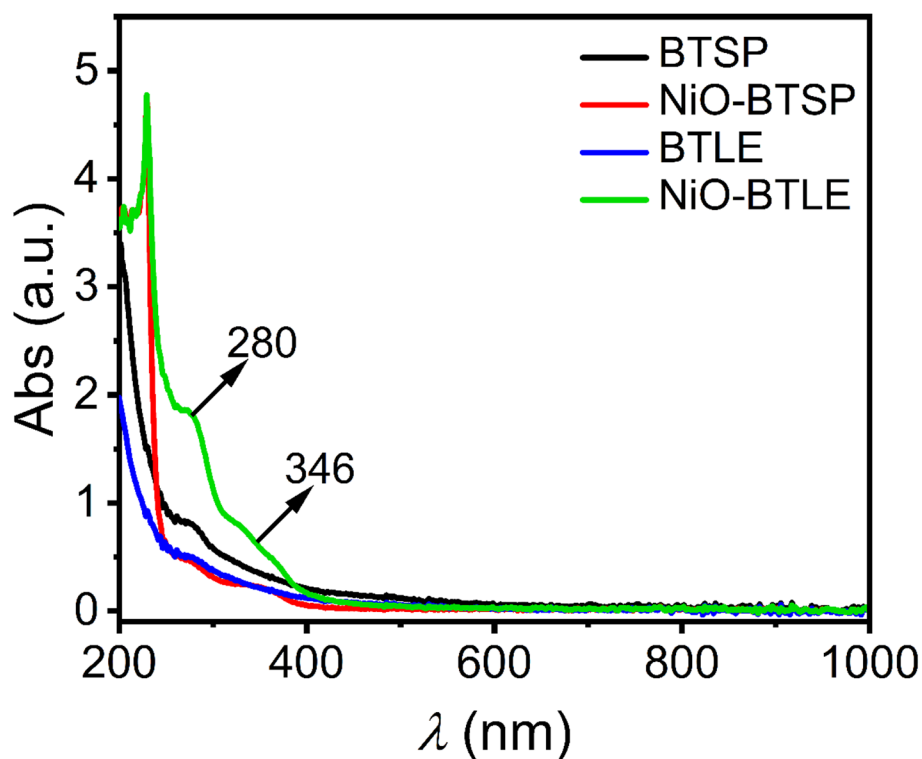


Fig. 1 The UV-Vis spectra of aqueous extracts of BTSP and BTLE and their corresponding bioinspired NiO NPs

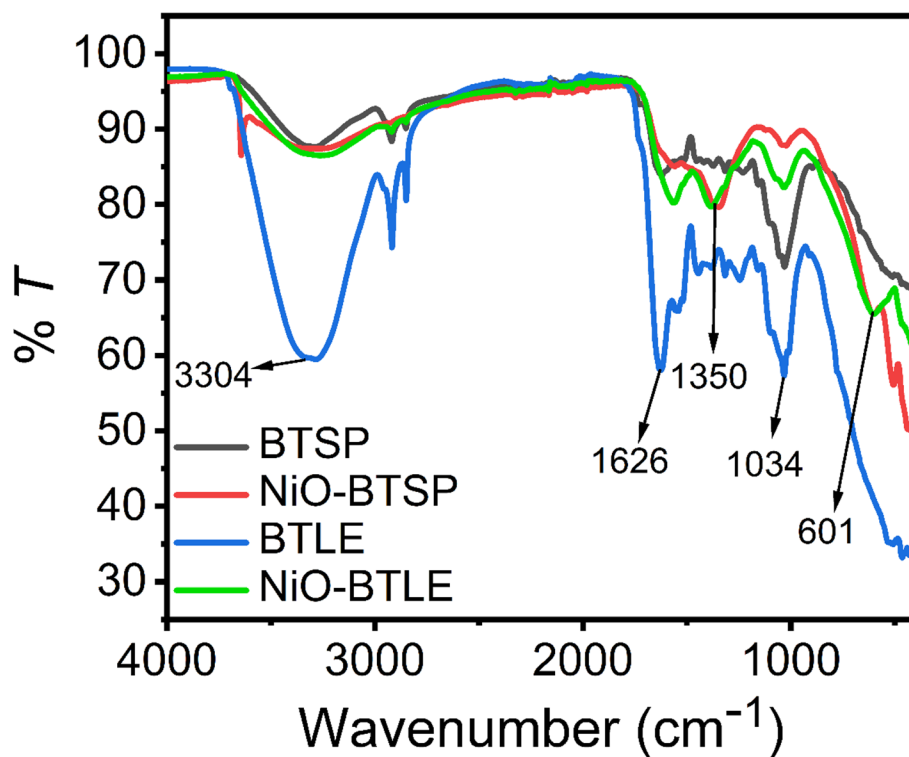


Fig. 2 FTIR spectra of aqueous extracts of BTSP and BTLE and their corresponding bioinspired NiO NPs

the band at 601 cm^{-1} , which is present only in the NPs, is attributed to Ni-O stretching vibrations [23], further confirming the successful biosynthesis of NiO NPs from the plant extracts.

The morphology of the biosynthesized NPs was further analyzed by SEM at an imaging magnification of 500x and 1000x (Fig. 3a-d). The micrographs showed that the biosynthesized NPs are appropriately dispersed. The NiO-BTSP NPs (Fig. 3a-b) showed irregular disc-shaped particles with cavities and pore spaces, while the NiO-BTLE NPs (Fig. 3c-d) showed a disrupted network of agglomerated particles with larger and more irregular cavities. Elucidation of the size distribution of the NPs compared to their respective aqueous extracts by DLS clearly distinguished between the size of the particles present in the extract and those of the NPs. As can be seen in Fig. 3e, while

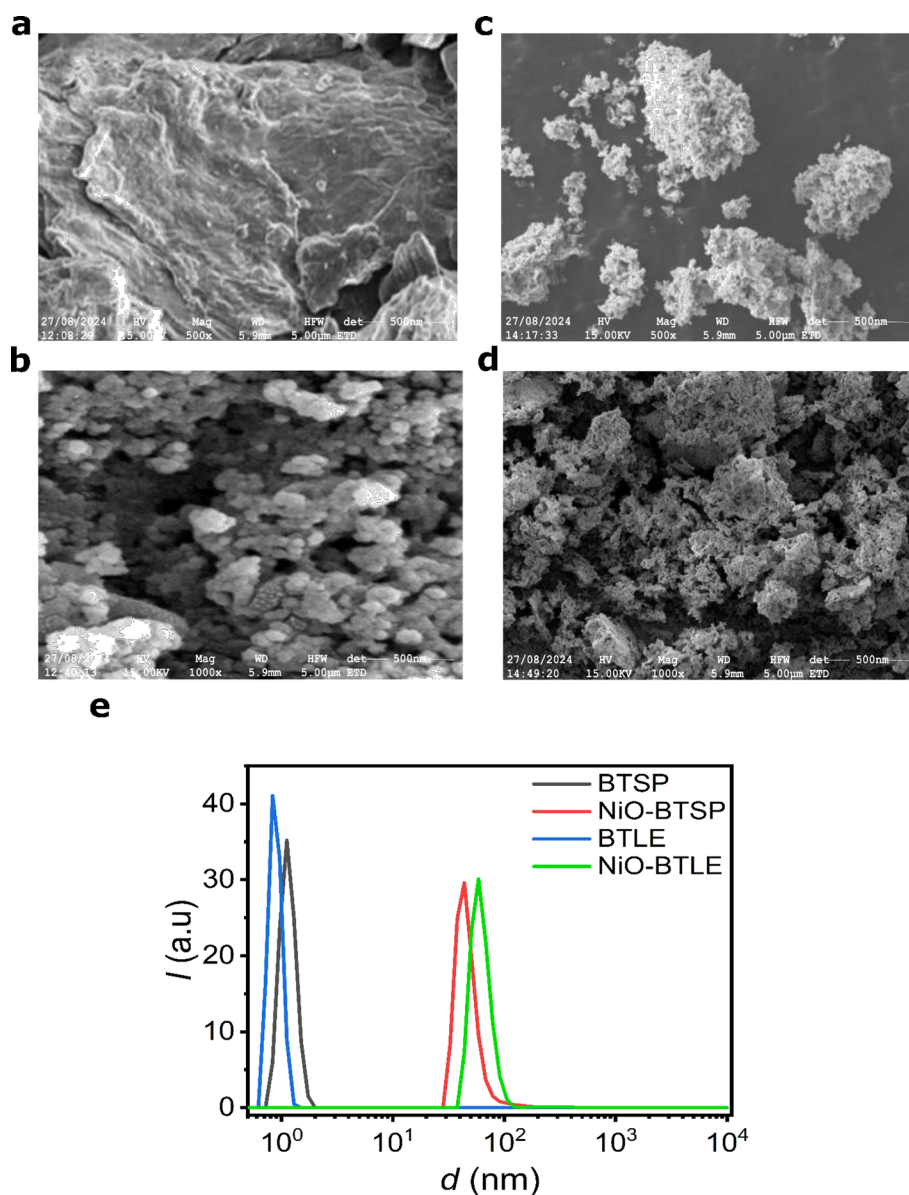


Fig. 3 Morphology characterization of NiO nanoparticles. **a** SEM micrograph of NiO-BTSP at 500x magnification. **b** SEM micrograph of NiO-BTSP at 1000x magnification. **c** SEM micrograph of NiO-BTLE at 500x magnification. **d** SEM micrograph of NiO-BTLE at 1000x magnification. **e** Size distribution of extracts and NiO nanoparticles measured by DLS

the aqueous extracts of BTSP and BTLE showed a relatively small size distribution of ~ 1 nm, confirming a good level of purity of the extracts, their respective NPs showed larger size distributions, with NiO-BTSP NPs having a size distribution of 30–80 nm and an average size of 45 nm, and NiO-BTLE NPs having a size distribution of 40–100 nm and an average size of 70 nm. This shows that the NiO-BTSP NPs is smaller than NiO-BTLE NPs, suggesting the possible advantage of NiO-BTSP NPs over NiO-BTLE NPs in drug delivery.

The elemental composition of the nanoparticles was characterized by EDX and showed a strong Ni peak at 1.2 keV and 2 keV, with a percentage composition of 52.68 (NiO-BTLE NPs) (Fig. 4a) and 53.03 (NiO-BTSP NPs) (Fig. 4b). In addition, an oxygen peak at 0.8 keV, with a percentage composition of 41.08 (NiO-BTLE NPs) and 42.15 (NiO-BTSP NPs) (Fig. 4) was detected, which was contributed by the phytochemicals in the plant extracts reduce the nickel salt to nickel NPs [23, 63].

3.3 Antioxidant activity assays

Antioxidants scavenge the free radicals present in a medium through the transfer of hydrogen or electrons to oxidants [64]. The properties of the extracts and NPs as antioxidant agents were tested using in vitro DPPH assay and the hydrogen peroxide radical scavenging assay following established protocols [52]. The DPPH assay was used to investigate the capacity of the extracts and their NPs to scavenge the unstable DPPH radicals that are released in the reaction medium, while the H_2O_2 assay was used to investigate the ability of the samples to scavenge hydroxyl radicals in the reaction medium [65]. Varying concentrations ($30\text{--}180\text{ }\mu\text{g mL}^{-1}$) of the samples were prepared in DMSO (DPPH assay) or PBS (H_2O_2 assay) and tested for their capacity to scavenge free radicals in comparison to ascorbic acid (AA).

DPPH assay of the aqueous extracts of *Bauhinia tomentosa* and their NPs showed a dose-dependent increase in average RSA similar to AA (Fig. 5). As sample concentration increased from $30\text{ }\mu\text{g mL}^{-1}$ to $180\text{ }\mu\text{g mL}^{-1}$, the ability of the samples to scavenge the free radicals also experienced a corresponding increase, with optimum activity obtained using $180\text{ }\mu\text{g mL}^{-1}$ of the samples (Fig. 5). In all cases, the NPs synthesized from the extracts of *Bauhinia tomentosa* showed significant activity against DPPH radicals compared to the extracts and positive control. The average RSA of NiO-BTLE increased

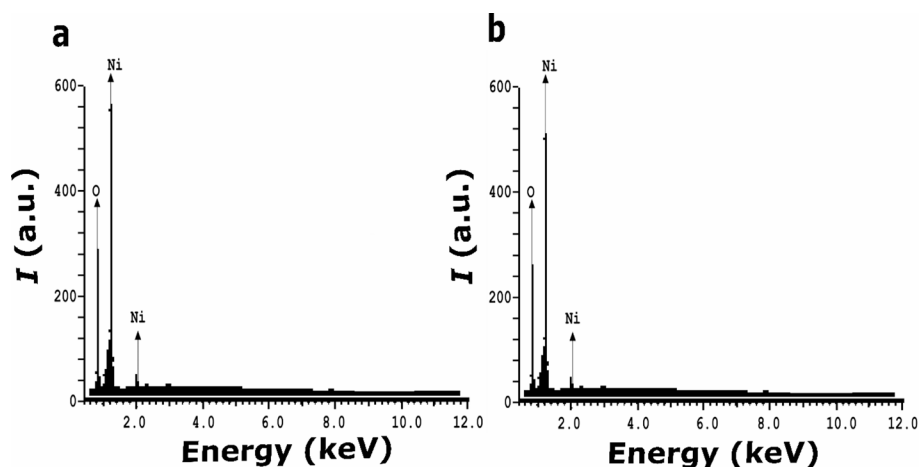


Fig. 4 EDX spectra of NiO nanoparticles. **a** NiO-BTSP NPs. **b** NiO-BTLE NPs

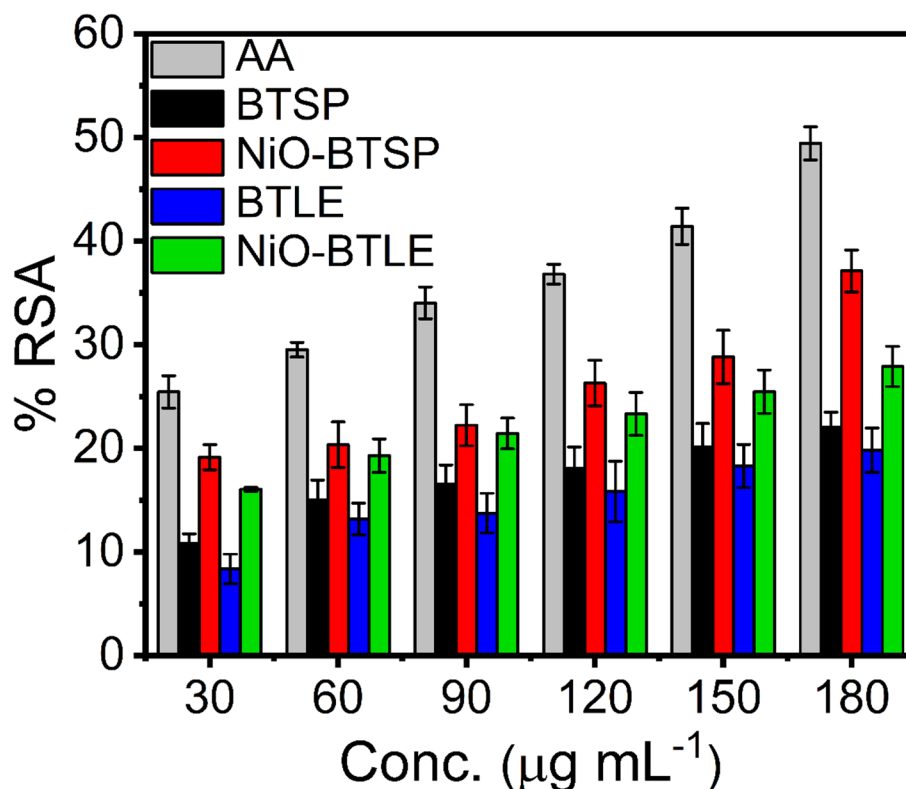


Fig. 5 The scavenging potential of DPPH free radicals by BTSP, BTLE and their corresponding bioinspired NiO NPs. Standard errors of duplicate experiments are shown as error bars

from $\sim 16\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 30\%$ at $180 \mu\text{g mL}^{-1}$, with an IC_{50} of $63.95 \mu\text{g mL}^{-1}$, compared to the BTLE extract, which increased from $\sim 8\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 20\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $69.76 \mu\text{g mL}^{-1}$. Similarly, the average RSA of NiO-BTSP increased from $\sim 19\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 37\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $63.37 \mu\text{g mL}^{-1}$, compared with its corresponding extract whose average RSA increased only from $\sim 10\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 22\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $68.54 \mu\text{g mL}^{-1}$ (Fig. 5). These results are consistent with previous reports [23, 52, 66, 67] and indicate the superior property of the NPs over their corresponding extracts. The enhanced DPPH radical scavenging activity of the NPs may be due to the presence of metal ions, which is usually employed by enzymes as cofactors to breakdown radicals [23, 68]. The AA used as positive control increased in average RSA activity from $\sim 25\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 49\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $51.80 \mu\text{g mL}^{-1}$. Among all the samples studied, NiO-BTSP showed the highest DPPH scavenging activity at all concentrations, suggesting its superior activity compared to NiO-BTLE, which may be related to the improved activity of BTSP over BTLE.

The activity of the extracts and their corresponding NPs against reactive oxygen species [69] was tested using a H_2O_2 radical scavenging assay. H_2O_2 assay of the aqueous extracts of *Bauhinia tomentosa* and their NPs showed a dose-dependent increase in average RSA similar to AA (Fig. 6). As sample concentration increased from $30 \mu\text{g mL}^{-1}$ to $180 \mu\text{g mL}^{-1}$, the average RSA of the samples experienced a corresponding increase, with optimum activity obtained using $180 \mu\text{g mL}^{-1}$ of the samples. In all cases, the bio-synthesized NPs showed significant activity against DPPH radicals when compared to

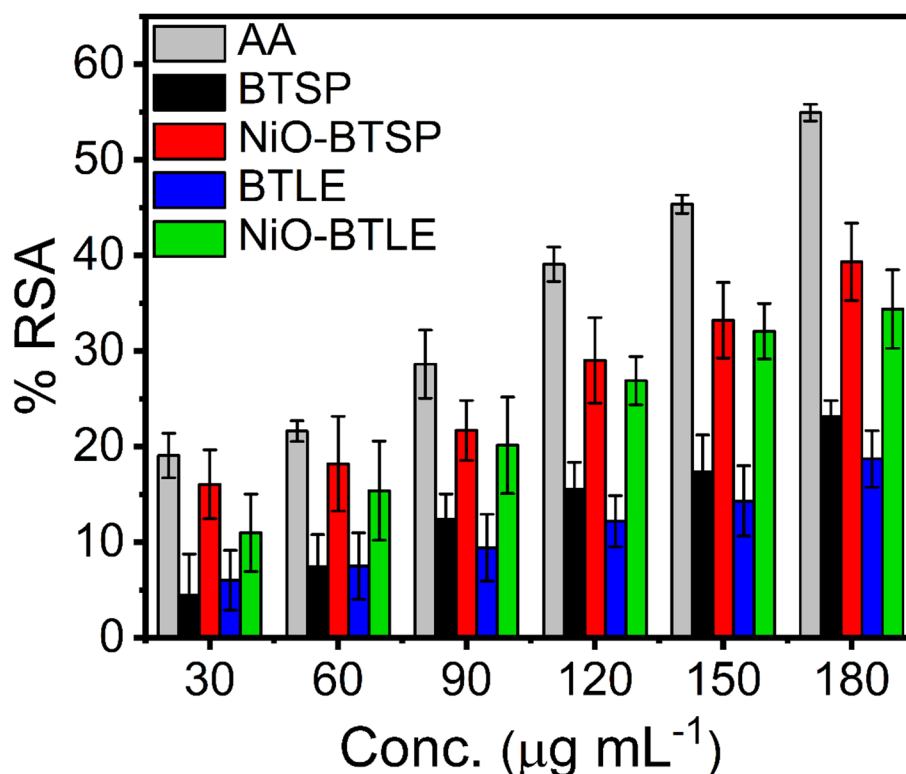


Fig. 6 The H_2O_2 free radical scavenging potential of BTSP, BTLE and their corresponding bioinspired NiO NPs. Standard errors of duplicate experiments are shown as error bars

the extracts. The activity of NiO-BTLE against H_2O_2 radicals increased from $\sim 11\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 34\%$ at $180 \mu\text{g mL}^{-1}$, with an IC_{50} of $295.80 \mu\text{g mL}^{-1}$, compared to the BTLE extract, which increased from $\sim 6\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 19\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $325.50 \mu\text{g mL}^{-1}$. Similarly, the activity of NiO-BTSP against H_2O_2 radicals increased from $\sim 16\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 39\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $245.70 \mu\text{g mL}^{-1}$, compared with its corresponding extract whose average RSA increased only from $\sim 4\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 23\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $309.10 \mu\text{g mL}^{-1}$ (Fig. 6). The positive control, AA, showed a similar pattern, increasing in activity from $\sim 18\%$ at $30 \mu\text{g mL}^{-1}$ to $\sim 55\%$ at $180 \mu\text{g mL}^{-1}$ with an IC_{50} of $206.4 \mu\text{g mL}^{-1}$ (Fig. 6). These results align well with existing reports [23, 52] and indicate the superior property of the NPs over their corresponding extracts. The improved quality and activity of the NPs at scavenging radicals can be attributed to the presence of metal ions, which is usually employed by enzymes as cofactors to breakdown radicals [68]. Similar to the observation from DPPH assay, NiO-BTSP showed the greatest activity against free radicals produced by H_2O_2 at all concentrations compared to NiO-BTLE. This may be related to: (i) its smaller size compared to NiO-BTLE (Fig. 3E), which allows it to more easily diffuse, capture, and scavenge the free radicals, or (ii) the improved activity of BTSP over BTLE.

3.4 Antimicrobial assay

The antimicrobial potential of the extracts of *Bauhinia tomentosa* and their corresponding NPs was tested against bacteria and fungi. Specifically, the samples were tested against Gram-positive bacterium (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungus (*Candida albicans*), which

are important to clinical and public health. The standard antibiotics, amoxicillin and clavulanic acid (AMC) and ciprofloxacin (CIP), as well as the standard antimycotic drug, fluconazole (FLC) were used as positive controls. The results (Table 2) revealed that the antimicrobial activity of the bioinspired NiO nanoparticles synthesized from *Bauhinia tomentosa* increased relatively with increasing concentration of NPs, with optimum activity obtained using $500 \mu\text{g mL}^{-1}$ of the samples. The NiO NPs displayed outstanding and improved antibacterial and antifungal properties compared to the respective extracts. While the aqueous extract of BTLE showed no antifungal activity against *Candida albicans* at operational concentrations, its green-synthesized NiO NPs displayed outstanding activity against the microorganism. In addition, although the aqueous extract of BTSP could not show activity against *E. coli* at concentrations below $500 \mu\text{g mL}^{-1}$, its corresponding bioinspired NPs displayed outstanding activity against the bacteria at low concentrations. These results are similar to previous reports from literature [1], and further confirm the superiority of these NPs over the aqueous extracts. The superiority of the NPs can be associated with their improved potency due to the metal ions present in them, and their ease of penetration through the cell membrane of the microorganisms [52, 67]. It is worthy of mention that the aqueous extract of BTSP possessed relatively greater potential over BTLE against the microorganisms, particularly against the Gram-positive bacteria, *S. aureus*, and the fungus, *C. albicans* (Table 2). This may be due to the fact that alkaloids, an important secondary metabolite known for its antimicrobial properties, is absent in the BTLE extract as shown in the phytochemical screening of the extracts (Table 1). This aligns well with previous findings [69–71]. It is also important to state that the bioinspired NiO NPs from *Bauhinia tomentosa* at $500 \mu\text{g mL}^{-1}$ have activities that are close to that of ciprofloxacin against *E. coli*, suggesting that these NPs could be suitable in the treatment of *E. coli*-related diseases. Furthermore, the activity of the bioinspired NiO NPs against the isolates supersedes that of AMC at all concentrations, and displayed interesting activity against *Staphylococcus aureus* and

Table 2 Antimicrobial screening of BTSP, BTLE, NiO-BTSP, NiO-BTLE, and controls. Standard errors of duplicate experiments are indicated

Sample	Concentration($\mu\text{g mL}^{-1}$)	Zone of inhibition (mm)			
		Gram-Negative Bacteria		Gram-Positive Bacteria	Fungi
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>
NC	0	0	0	0	0
CIP	5	22.85 ± 0.95	27.50 ± 0.50	26.10 ± 0.30	32.50 ± 0.50
AMC	30	11.60 ± 0.80	8.60 ± 0.60	0	0
FLC	25	*nt	*nt	*nt	32.75 ± 0.25
BTLE	500	14.85 ± 1.65	13.20 ± 1.90	0	0
	250	12.05 ± 0.35	14.85 ± 1.65	0	0
	125	0	12.25 ± 0.95	0	0
BTSP	500	11.40 ± 0.70	15.10 ± 0.30	11.90 ± 1.60	15.95 ± 0.55
	250	0	14.20 ± 1.60	10.10 ± 1.20	13.05 ± 1.45
	125	0	11.50 ± 2.40	0	9.45 ± 0.95
NiO-BTLE	500	20.40 ± 1.90	17.35 ± 2.15	15.80 ± 0.70	17.55 ± 1.15
	250	17.40 ± 0.90	14.65 ± 0.55	13.70 ± 0.60	16.05 ± 0.75
	125	15.85 ± 0.55	15.40 ± 0.70	12.45 ± 1.05	11.75 ± 0.55
NiO-BTSP	500	17.90 ± 0.80	20.10 ± 0.60	15.90 ± 0.80	19.05 ± 0.45
	250	15.35 ± 0.85	18.40 ± 0.90	12.75 ± 1.45	16.90 ± 0.60
	125	15.05 ± 0.75	15.80 ± 0.60	11.70 ± 0.80	10.70 ± 1.00

*nt not tested. NC Negative Control. CIP Ciprofloxacin. AMC Amoxicillin and Clavulanic Acid. FLC Fluconazole

Table 3 Comparison between different biosynthesized NiO NPs

Sample	Particle size (nm)	Antimicrobial activity (zone of inhibition (mm))					Antioxidant activity (IC ₅₀ (μg mL ⁻¹))		Refs.
		Conc (mg mL ⁻¹)	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>C. albicans</i>	DPPH	H ₂ O ₂	
NiO NP from <i>Phytolacca dodecandra</i> leaf extract	< 10	–	nt	nt	nt	nt	nt	364	[23]
NiO NP from <i>Ocimum sanctum</i> leaf extract	12–36	0.1	12.25	nt	nt	37.23	nt	nt	[73]
NiO NP from <i>Calendula officinalis</i> leaf extract	60.39	–	nt	nt	nt	nt	192	nt	[74]
NiO NP from Okro plant extract	31.2	nr	0	0	10	0	nt	nt	[75]
NiO NP from <i>Stevia rebaudiana</i> leaf extract	20–50	0.1	16	nt	nt	nt	nr	nr	[76]
NiO NP from <i>Aegle marmelos</i> leaf extract	8–10	nr	11	nt	nt	nt	nt	nt	[77]
NiO NP from <i>Populus ciliata</i> leaf extract	58.18	2	13.2	nt	nt	nt	nt	nt	[78]
NiO NP from <i>Moringa oleifera</i> leaf extract	nr	nr	10	nt	15	nt	nt	nt	[79]
NiO NP from <i>Acacia nilotica</i> leaf extract	16	20	11.5	10.5	8.7	nt	nt	nt	[80]
NiO NP from <i>Desmodium gangeticum</i> root extract	2695	1	nt	6.04	5.67	nt	nt	nt	[81]
NiO NP from <i>Salvadora persica</i> root extract	25.15	nr	20	18	22	26	51.45	nt	[82]
NiO NP from <i>Bauhinia tomentosa</i> seedpod (NiO-BTSP)	~ 45	0.125	15.05	15.8	11.7	10.7	63.37	245.7	This work
NiO NP from <i>Bauhinia tomentosa</i> leaf extract (NiO-BTLE)	~ 70	0.125	15.85	15.4	12.45	11.75	63.95	295.8	This work

*nr = not reported; nt = not tested

Candida albicans, where AMC displayed no activity (Table 2). This suggest that these NPs could be a good replacement to AMC, although further study is needed to ascertain this.

Put together, aqueous extracts from the seedpod and leaves of *Bauhinia tomentosa* proved to be very effective in synthesizing NiO NPs via a green ecofriendly route. The synthesized NiO NPs are nano-sized and in most cases, disc-shaped, showing morphological properties similar to NiO NPs produced from other plants (Table 3). When compared to other NiO NPs synthesized via a green route, NiO NPs from BTSP and BTLE showed great antimicrobial properties against Gram-positive and Gram-negative bacteria, as well as fungus. Furthermore, the NiO NPs from BTSP and BTLE showed antioxidant properties that are, in most cases, superior to NiO NPs synthesized from other plant extracts (Table 3). These results suggest the potential of *Bauhinia tomentosa* in synthesizing ecofriendly NiO NPs that have potential for pharmaceutical applications, particularly, in the development of new therapeutics.

4 Conclusion

This study reports on the easy, cheap, and eco-friendly biosynthesis of NiO NPs using aqueous extracts of the leaves and seedpods of *Bauhinia tomentosa*. The extracts used for the biosynthesis contains secondary metabolites like phenols, tannins, steroids, and alkaloids, which contain active groups that reduces metal salts into metal oxide NPs. The bioinspired NPs, which are appropriately dispersed and spherical in shape, have a size distribution of 30–100 nm, absorbs at 346 nm in the ultraviolet (UV) region, and showed stretching vibrations at a frequency of 601 cm^{-1} in IR, confirming successful production of NiO NPs. The bioinspired NiO NPs possessed improved antioxidant activities over the extracts by scavenging free radicals generated by DPPH and H_2O_2 . Furthermore, the NiO NPs showed outstanding activities against clinically relevant microorganism, such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*, comparable to the activities of popularly used antibiotics and antimycotics. The results indicate that NiO NPs produced via a green route using aqueous extracts of the seedpods and leaves of *Bauhinia tomentosa* have a great potential to be useful in the development of new antibacterial, antioxidant, and antimycotic therapeutics. It is, however, recommended that further research be carried out on these NPs to ascertain their robust applications, particularly in human health improvement.

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Author contributions

M.T.A.: Conceptualization, supervision, investigation, data curation, data analysis, writing, review, and editing. D.A.A.: Supervision, review, and editing. F.O.O.: Supervision, review, and editing. I.F.T.: Investigation and data curation. O.M.O.: Investigation and data curation. I.D.K.: Investigation and data curation. O.M-A.: Data curation, review and editing. J.O.B.: Conceptualization, review, and editing. All authors approved the manuscript for publication.

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Data availability

All data used to produce this article will be made available upon request to the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable. This work does not experiment on humans or animals.

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