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PHYTOFABRICATION OF NICKEL OXIDE NANOPARTICLES AND THEIR ANTIOXIDANT, ANTIBACTERIAL, AND ANTICANCER ACTIVITIES

Medari Ravinder[1], Vellanki Ragha Suma[2] and Md. Moulana Kareem[3,✉]
[1]Department of Botany, GDC, Tandur, Telangana
[2]Department of Chemistry, GDC, Bhadrachalam, Telangana
[3]Department of Chemistry, GDC, Manuguru. Telangana
✉Corresponding Author: kareemgdc@gmail.com

ABSTRACT

In this study, we employed an eco-friendly approach to produce nickel oxide nanoparticles (NiO NPs) employing an aqueous leaf extract derived from *Alternanthera sessilis*. The resulting NiO NPs exhibited a monodispersed nature, as verified through UV-Vis spectroscopy, which exposed a clear peak at λmax 280 nm and stabilizing agents during the synthesis of *A.S*-NiO NPs. The XRD examination asserted the Face Centre Cubic (FCC) crystallinity of the biogenic *A.S*-NiO NPs. TEM examination revealed a size of 18 nm and a spherical morphology for *A.S*-NiO NPs. The biogenic NiO NPs demonstrated noteworthy antioxidant, antibacterial, and anticancer activities. The DPPH scavenging activity was shown to be maximum at an amount of 100 µg/ml, at 62.72%, and an IC_{50} value of 86.46 µg/ml. Additionally, *A.S*-NiO NPs exhibited superior antibacterial efficacy P. aeruginosa, E. coli, and other bacteria showed the strongest antibacterial activity. S. pyogenas, and S. aureus, resulting in Zone of Inhibition values of 16 mm, 14.5 mm, 10.6 mm, and 6.5 mm, respectively. Furthermore, *A.S*-NiO NPs demonstrated potential dose-dependent anticancer efficacy against HeLa and MCF-7 cancer cell lines.

Keywords: Anticancer, Antibacterial, Aqueous Extract, Eco-Friendly, Nanoparticles, Scavenging Activity.
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INTRODUCTION

Global multidrug resistance (MDR) in bacteria claims 700,000 lives annually, potentially rising to 10 million deaths if current antibiotic practices persist. This crisis impacts healthcare and demands the exploration of alternative strategies. Cancer cases doubled from 1975 to 2000, reaching over 18 million in 2018 with 9.6 million fatalities. Future projections exceed 29.5 million cases by 2040, underscoring the need for novel anticancer drugs. Metal nanoparticles, with unique characteristics like light absorption and surface Plasmon resonance, are gaining attention.[1–3] Their nanometer size facilitates easy penetration of the cell walls of multidrug-resistant bacteria, surpassing conventional antibiotics in antibacterial efficacy. Additionally, these nanoparticles induce oxidative strain by producing reactive oxygen species, boosting the demise of cancerous cells. NiO nanoparticles, particularly notable for electron transfer ability, super conductance, anticancer, and antibacterial properties, also exhibit environmental benefits through the adsorption and photocatalysis of heavy metals and synthetic dyes.[4–8] Diverse methods, including bio-fabrication with plant-extract mediation, are utilized for NiO nanoparticle production. This approach, especially using plant extracts, stands out for simplicity, eco-friendliness, cost-effectiveness, scalability, and applicability in aqueous environments at room temperature.[9,10] Researchers have successfully biosynthesized NiO nanoparticles using various plant materials, highlighting their versatility.[1] *Alternanthera sessilis,* a Southeast Asian weed from the *Amarantheacea* family, is utilized as a vegetable. Rich in active compounds like alkaloids, flavonoids, tannins, polyphenols, and terpenoids, it exhibits pharmacological properties, including anti-tumor effects.[11,12] NiO nanoparticles exhibited anticancer effects on HeLa cells, suggesting potential in cancer therapy.

The study aimed to create an affordable, user-friendly, one-step green synthesis method for NiO nanoparticles from plant sources with biological efficacy.

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EXPERIMENTAL

Material and Methods

All chemicals and reagents employed in this investigation were analytical grades procured from Hi Media. The glassware employed in this study was carefully cleansed twice with distilled water and dried in an oven before use.

Preparation of Leaf Aqueous Extract

Leaves of *Alternanthera sessilis* collected from Osmania University's Botanical Garden, Hyderabad, underwent a cleaning process with tap water and distilled water, followed by two weeks of shade drying. The dried leaves were ground using a mixer grinder, and one gram was mixed with 100 ml of distilled water. After heating on a magnetic stirrer. The mixture was filtered using a Whatman filter after 15 minutes at room temperature. Paper, resulting in the preservation of the aqueous extract.

Phyto-fabrication NiO NPs

To synthesize NiO nanoparticles, 80 ml of 5 mM nickel nitrate ($NiNO_3$) was added to 20 ml of *Alternanthera sessilis* leaf aqueous extract, maintaining a pH of 12 with 0.1 M NaOH. The reaction blend was heated on a magnetic stirrer for 20 minutes at ambient temperature. Then the colour turned from light green to dark brown, demonstrating NiO NP formation. The recovered residue was dried at 70°C following 5 minutes of centrifugation at 1000 rpm for 4 hours. The resulting dried residue was finely powdered for further characterization and assessment of biological efficacy.

Detection Method

A LAB INDIA UV-3000+ spectrophotometer operating in the 200–800 nm range was used to analyze the UV/visible properties of the biosynthesized NiO nanoparticles. Spectral data was plotted using Origin 2019b software. The dried NPs were analyzed using a Shimadzu FT-IR spectrophotometer, and their crystallinity was confirmed using a Bruker D8 Advance Diffractometer. The surface morphology of NiO nanoparticles was examined using Scanning Electron Microscopy (SEM) using a Carl Zeiss Ultra 55 microscope. The study utilized Energy dispersive X-ray (EDX) and an Oxford Instruments X-MaxN SDD system for the elevation analysis. The study utilized TEM to characterize biosynthesized NiO nanoparticles using a JEOL JEM-2100F instrument at a 200 kV accelerating voltage.

General Procedure

Evolution of Antioxidant Activity of NiO NPs

The antioxidant activity of A. sessilis NiO nanoparticles was assessed against DPPH, following a modified method by Khan *et al.*[13] A 4 percent methanolic DPPH solution was prepared and wrapped in aluminum foil for light sensitivity. Various reaction mixtures were created with different NiO NP concentrations (20, 40, 60, 80, and 100 mg) and 4 ml of DPPH, along with 4 ml of double-distilled water. Afterwards 25 min of gestation the scavenging activity was measured at 517 nm using a UV-visible spectrometer at room temperature. The percentage of DPPH scavenging activity is calculated by dividing the absorbance of the control (C) by the absorbance of the test sample (T). DPPH = $[(C - T)/C] \times 100$

Antibacterial Efficacy

The antibacterial efficacy of *A. sessilis* NiO NPs was evaluated against the well and was used to test Gram-positive (*S. aureus, S. pyogenas*) and Gram-negative (*E. coli, P. aeruginosa*) bacterial strains. diffusion method.[14] Bacterial inoculum attuned to 0.5 McFarland turbidity norms was spread on Muller-Hinton agar, and wells were created. *A. sessilis NiO NPs* at various dilutions (1, 5, 10, 50, and 100 µM/mL) were added. After incubation, antibacterial efficacy was assessed by measuring the inhibition zone around each well. Ampicillin served as a reference for comparing the antibacterial efficacy of *A. sessilis NiO NPs.*

In-vitro Cell Viability Assay

The anticancer efficacy of A.S. NiO NPs was evaluated on *HeLa and MCF-7* cancer cell lines through the MTT method. Cells were treated with various doses (1, 5, 10, 20, and 50 µM/mL) of *A. sessilis* NiO

NPs after seeding in 96-well plates. Following incubation, MTT solution was added, and formazan crystals generated by viable cells were solubilized. Absorbance measurements allowed the calculation of cell viability and determination of the IC50%. Inverted phase-contrast microscopy revealed morphological variations, facilitating the assessment of *A. sessilis* NiO NPs' impact on cell viability in HeLa and MCF-7 cell lines.

RESULTS AND DISCUSSION

UV-visible Analysis

The UV/Visible spectra of *A.S.* NiO NPs displayed a distinctive peak at λmax 280 nm, confirming the fabrication of *A.S.* NiO NPs.[13]

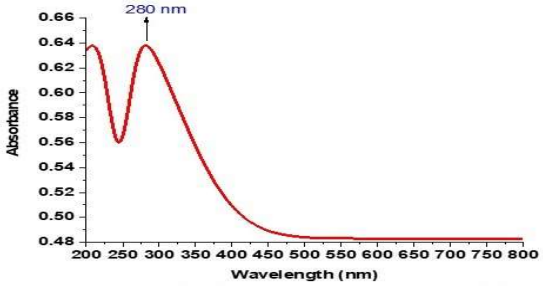

Fig-1: UV-Visible spectrum of *A.S.* NiO NPs,

FT-IR Spectroscopy Analysis

In Fig.-2, the FTIR spectra reveal multiple peaks corresponding to bio-constituents adsorbed by the synthesized NPs. NiO's FTIR profile exhibits vibrations at 3572 cm−1 for -OH groups, 2946 cm−1 for C-H stretching, and multiple peaks at 1648, 1416, and 1024 cm−1 indicating C=C and C-O stretching for aromatic ring and polyphenols. The 670 cm−1 peak signifies metal–oxygen stretching vibrations, confirming the presence of a Ni–O bond.[15]

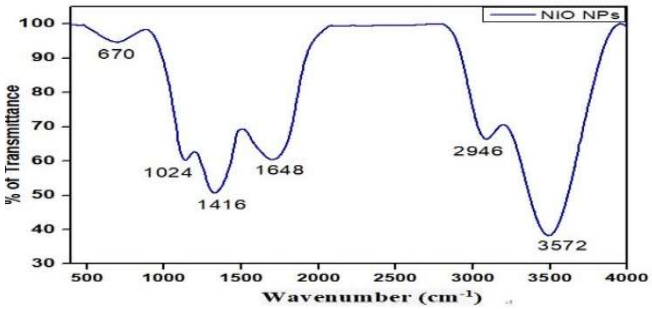

Fig-2: FTIR spectrum of *A.S.* NiO NPs,

XRD Analysis

In Fig.-3, the structural and crystallinity details of *A.S.* NiO NPs are presented. The XRD pattern confirms the formation of NiO nanoparticles, with strong reflection peaks at 2θ = 37.5, 43.4, 62.4, 75, 8, and 79.4 corresponding to indices (111), (200), (220), (311), and (222). The diffraction pattern for NiO NPs with an average lattice parameter of 4.17710Å16 is consistent with JCPDS card number 47-1049, confirming a face-cantered cubic nature. Using the traditional Debye–Scherrer estimate, the average crystallite size is found to be 16.5 nm.

SEM and EDX Analysis

Scanning electron microscopy (SEM) at 5.00 µM magnification was utilized to investigate the surface morphology of biosynthesized NiO NPs. The image (Fig.-4a) reveals significant agglomeration, forming large, and uneven nanoparticles with irregular shapes and clustered formations. This agglomeration is ascribed to high surface energy and surface area per unit volume ratio.[16] The energy-dispersive X-ray (EDX) spectrum (Fig.-4b) shows peaks at 0.85 KeV and 7.48 KeV, indicating the presence of nickel (Ni), while the peak at 0.52 KeV suggests the presence of oxygen (O). The confirmation of these peaks affirms the successful synthesis of NiO nanoparticles coated with phytomolecules.[13]

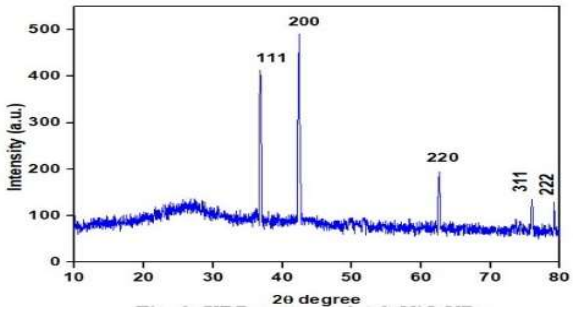

Fig-3: XRD pattern of *A.S.* NiO NPs,

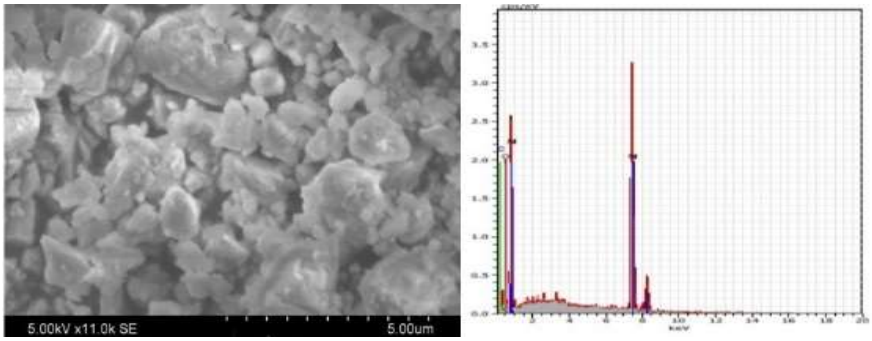

Fig-4: (a) SEM image of *A.S.* NiO NPs, (b) EDX of *A.S.* NiO NPs,

TEM and SAED Analysis

The images of Transmission Electron Microscopy (TEM) are shown in Fig.-5(a). The average size of NiOl NPs obtained by TEM was found to be 16 nm (Fig.-5c), which is according to XRD data. These particles are pseudo-spherical. Similar findings were reported by Fardin *et al.*[17] The crystallinity of NiO NPs also ascertained by SAED is shown in Fig.-5b. In this study, we found dazzling spherical shape rings. This might be reflected in the NiO nanoparticle lattice planes.

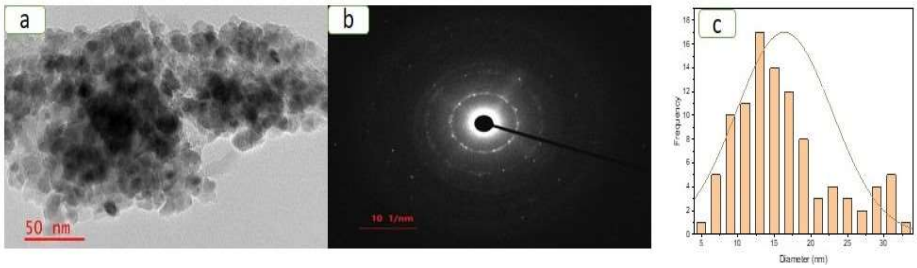

Fig-5: (a) TEM image of *A.S.* NiO NPs, (b) SAED pattern of *A.S.* NiO NPs, (c) Histogram of *A.S.* NiO NPs,

Antioxidant Properties of A.S. NiO NPs

The study investigated the antioxidant properties of *A.S.* NiO NPs and A.S. aqueous extract using DPPH, a stable free radical. Spectrophotometric analysis revealed that *A.S.* NiO NPs demonstrated superior scavenging activity against DPPH radicals (6.38% to 67.72%) compared to *A.S.* aqueous extract (3.34% to 42.44%). The concentration had a clear correlation with antioxidant activity. of *A.S.* NiO NPs. The effective concentration to inhibit 50% of free radicals was 86.46 mg/mL for *A.S.* NiO NPs, 122.48 mg/mL for *A.S.* extract, and 52.688 mg/mL for ascorbic acid. *A.S.* NiO NPs exhibited dose-dependent scavenging against free radicals, though slightly lower than ascorbic acid. Overall, the scavenging effect ranking was as follows: Ascorbic acid > *A.S.* NiO NPs > *A.S.* aqueous extract.

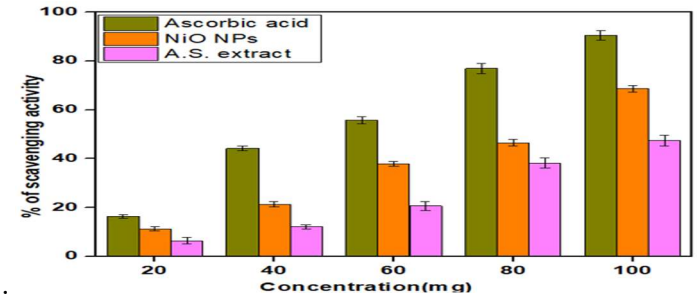

Fig-6: Scavenging activity of *A.S.* NiO NPs,

Antibacterial Activity of A.S. NiO NPs

The antibacterial activity of the *A. sessilis* leaf extract NiO-NPs was evaluated against four bacterial strains, with the resulting inhibition zone measurements (mm) presented in Fig.-7 and illustrated in Fig.-7. Analysis of the data revealed that the photogenic NiO-NPs exhibited greater efficacy at 100 mg/ml against the Gram-negative bacterium *Escherichia coli* (16.4 mm) related to the Gram-positive bacterial strains S. aureus (10.6 mm) and *S. pyogenas* (6.5 mm). In general, the antimicrobial potential was more pronounced against Gram-negative bacteria than Gram-positive bacteria. This discrepancy is explained by the fact that Gram-positive bacteria have a thick peptidoglycan layer, but Gram-negative bacteria lack one.[18]

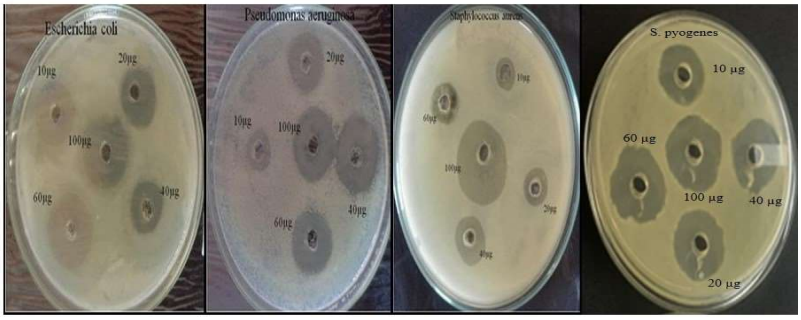

Fig-7: Antibacterial activity of *A.S.* NiO NPs.

Anticancer Activity of A.S. NiO NPs

The anticancer efficacy of *A.S.* NiO nanoparticles (NPs) against HeLa and MCF-7 cancer cell lines was investigated employing an MTT-based cytotoxicity test, as depicted in Fig.-8. Various concentrations of *A.S.* NiO NPs (ranging from 10 to 50 µM/mL) were employed during a 24-hour incubation period. The outcome indicated a significant decline in cell viability with increasing amounts of *A.S.* NiO NPs (Fig-8) in both *HeLa* and *MCF-7* cells. Particularly, at concentrations ranging from 20 to 50 µM/mL, A.S. NiO NPs exhibited a more pronounced cell death in *HeLa* cells compared to *MCF-7* cells.

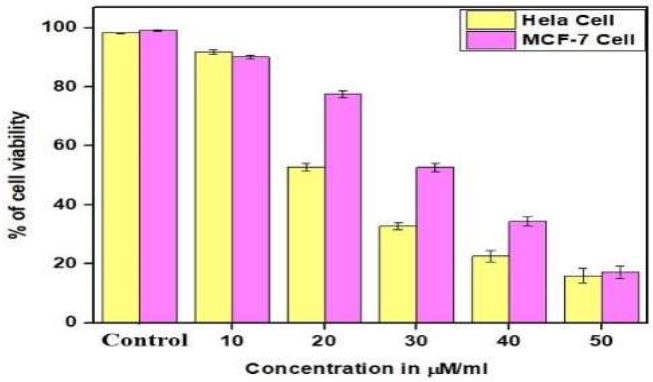

Fig.-8: Cytotoxic potential of NiO NPs in HeLa & MCF-7 cancer cell lines

CONCLUSION

This study successfully synthesized and characterized NiO nanoparticles (NiO NPs) using *Alternanthera sessilis* leaf aqueous extract. The dark brown blend and UV absorption spectra with the highest absorbance at 280 nm confirmed the synthesis. Crystallinity verification was achieved through XRD and SAED analyses, while TEM images provided insights into size and morphological distribution. These nanoparticles exhibited substantial DPPH scavenging activity and higher susceptibility to Gram-negative bacterial strains. Furthermore, NiO NPs induced cytotoxicity and apoptosis in cervical cancer cells, showing dose-dependent anticancer efficacy against *HeLa and MCF-7* cell lines. The green synthesis approach using *Alternanthera sessilis* proved to be facile, scalable, and environmentally friendly, highlighting the potential of biogenic NiO NPs across various applications.

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CONFLICT OF INTERESTS

The authors affirm that there are no conflicts of interest related to the publication of this article.

AUTHOR CONTRIBUTIONS

Each author made substantial contributions to this manuscript, participated in its review and editing, and endorsed the final draft for publication. The research profiles of the authors can be verified using their respective ORCID identifiers provided below:

Medari Ravinder https://orcid.org/0009-0005-5212-2565
Vellanki Ragha Suma https://orcid.org/0000-0002-3699-9296
Md. Moulana Kareem https://orcid.org/0000-0002-9545-7665

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