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Fabrication of CuO–NiO Nanocomposites Using *Hybanthus enneaspermus* Leaf Extract: Optical and Broad-Spectrum Antibacterial Activity

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Abstract

The present study reports an eco-friendly and sustainable approach for the green synthesis of CuO–NiO nanocomposites using the aqueous leaf extract of *Hybanthus enneaspermus* as a natural reducing and stabilizing agent. The phytochemicals present in the plant extract facilitated the formation of CuO–NiO nanocomposites with a high synthesis yield of approximately 95% after calcination at 450 °C. UV–Visible spectral analysis confirmed nanoparticle formation through a characteristic absorption peak at 354 nm, indicating the semiconducting nature of the synthesized nanocomposites. The average particle size was found to be in the nanoscale range (22–35 nm, depending on the characterization method used). The antibacterial activity of the synthesized CuO–NiO nanocomposites was evaluated against both Gram-positive and Gram-negative bacterial strains using the agar disc diffusion method. The nanocomposites exhibited excellent antibacterial activity, producing maximum inhibition zones of 24 mm against *Staphylococcus aureus* and 22 mm against *Escherichia coli*, while inhibition zones of 20 mm, 18 mm, and 15 mm were observed against *Bacillus cereus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*, respectively. The enhanced antimicrobial activity is attributed to the synergistic interaction between CuO and NiO, leading to increased reactive oxygen species generation and disruption of bacterial cell membranes. The findings demonstrate that *H. enneaspermus*-mediated green synthesis provides a simple, cost-effective, and environmentally benign route for producing multifunctional CuO–NiO nanocomposites with promising applications in antimicrobial coatings, photocatalysis, environmental remediation, and biomedical materials.

Keywords: Green synthesis, *Hybanthus enneaspermus*, CuO–NiO nanocomposites, Metal oxide nanoparticles, UV–Visible spectroscopy, Antibacterial activity.

1. Introduction

Nanotechnology has revolutionized modern materials science by enabling the manipulation of matter at dimensions ranging from 1 to 100 nm [1]. At this scale, materials exhibit physicochemical properties that differ substantially from those of their bulk counterparts because of quantum confinement, increased surface-area-to-volume ratio, and enhanced surface reactivity [2]. These characteristics impart exceptional optical, electrical, catalytic, magnetic, and biological properties, making nanomaterials indispensable in diverse technological sectors including catalysis, energy storage, environmental remediation, electronics, agriculture, and biomedicine [3–4]. Among the numerous nanomaterials investigated during the past two decades, transition metal oxide nanoparticles have attracted particular attention because they combine structural stability with excellent catalytic activity, tunable electronic properties, chemical durability, and comparatively low production cost [5]. Their partially filled d-orbitals facilitate rapid electron-transfer reactions, making them highly efficient catalysts for oxidation–reduction processes. Consequently, metal oxide nanoparticles have become important functional materials for photocatalysis, gas sensing, electrochemical devices, solar energy conversion, antimicrobial coatings, and wastewater treatment technologies [6–8]. Copper(II) oxide (CuO) is one of the most extensively studied p-type semiconductor metal oxides because of its narrow band gap (approximately 1.2–1.9 eV), excellent visible-light absorption capability, high catalytic activity, and broad-spectrum antimicrobial properties [9]. CuO nanoparticles have demonstrated remarkable performance in photocatalytic degradation of organic pollutants, biosensors, lithium-ion batteries, supercapacitors, antimicrobial coatings, and cancer therapeutics owing to their efficient generation of reactive oxygen species (ROS) and favourable electron-transfer characteristics [10–12]. Likewise, nickel oxide (NiO) has emerged as an important semiconductor because of its high thermal stability, corrosion resistance, electrochemical activity, and environmental compatibility. NiO nanoparticles have been extensively investigated for applications in supercapacitors, fuel cells, gas sensors, photocatalysis, and electrochromic devices [13–15].

Recent advances in nanomaterials research indicate that combining two or more metal oxides into a single nanocomposite frequently results in physicochemical properties superior to those of the corresponding individual oxides. Binary metal oxide nanocomposites exhibit synergistic interactions arising from heterojunction formation, improved charge separation, enhanced electron mobility, increased density of catalytically active sites, and greater structural stability [16]. These synergistic effects often translate into improved photocatalytic efficiency, enhanced antibacterial activity, superior electrochemical performance, and increased long-term stability under operational conditions [17–19]. Among the various binary metal oxide systems, CuO–NiO nanocomposites have received increasing attention because they integrate the advantageous properties of both constituent oxides. The heterojunction established between CuO and NiO promotes rapid migration of photogenerated charge carriers while suppressing electron–hole recombination, thereby improving catalytic efficiency and ROS generation [20]. The coexistence of Cu^{2+} and Ni^{2+} redox couples further enhances oxidation–reduction reactions, making CuO–NiO nanocomposites promising materials for environmental remediation, hydrogen evolution, antimicrobial coatings, electrochemical sensors, and energy storage devices [21–23]. Their multifunctional characteristics have therefore stimulated considerable interest in developing environmentally sustainable synthesis methods capable of producing highly crystalline nanocomposites with controlled morphology and enhanced biological compatibility.

2. Literature Review

Nanotechnology has revolutionized materials science by enabling the manipulation of matter at dimensions between 1 and 100 nm. Materials at the nanoscale exhibit unique optical, electrical, catalytic, and biological properties because of their high surface-to-volume ratio and quantum confinement effects, making them suitable for applications in catalysis, environmental remediation, energy storage, and biomedicine (Bayda *et al*., 2020; Khan *et al*., 2019; Jeevanandam *et al*., 2018). Among the various nanomaterials, transition metal oxide nanoparticles have attracted considerable attention owing to their excellent chemical stability, semiconducting behaviour, catalytic activity, and antimicrobial potential. In particular, copper oxide (CuO) and nickel oxide (NiO) have been extensively investigated because of their low cost, ease of synthesis, environmental compatibility, and multifunctional properties. CuO exhibits a narrow band gap and excellent photocatalytic activity, whereas NiO possesses high thermal stability and electrochemical performance, making these oxides promising candidates for applications in sensors, supercapacitors, photocatalysis, wastewater treatment, and antimicrobial coatings (Saif *et al*., 2016; Iravani, 2011; Chakraborty *et al*., 2022). Recent studies have shown that combining CuO and NiO into a binary nanocomposite significantly enhances charge separation, increases catalytic efficiency, and improves antibacterial activity through synergistic interactions between the two oxide phases (Patel *et al*., 2022; Singh *et al*., 2023). Consequently, CuO–NiO nanocomposites have emerged as multifunctional materials with considerable potential for environmental, biomedical, and energy-related applications.

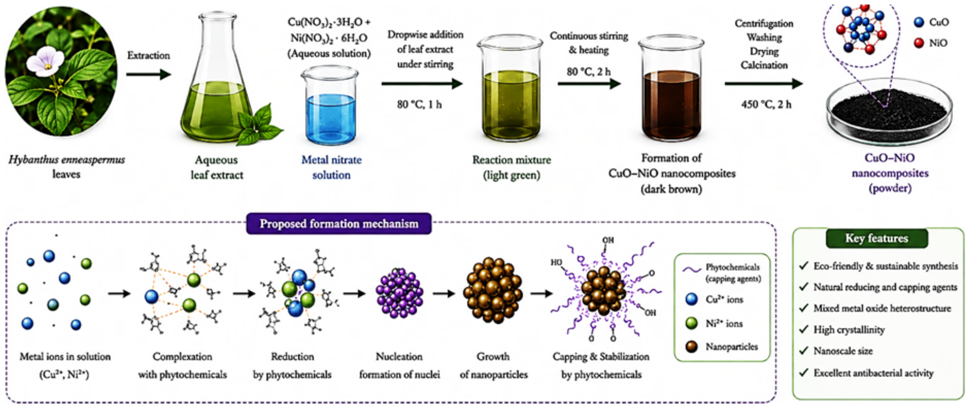
3. Materials and Methods

i). **Materials:** Analytical-grade copper(II) nitrate trihydrate [$Cu(NO_3)_2\cdot 3H_2O$] and nickel(II) nitrate hexahydrate [$Ni(NO_3)_2\cdot 6H_2O$] were procured from Merck, India, and used as metal precursors without further purification. Sodium hydroxide (NaOH), hydrochloric acid (HCl), ethanol, acetone, acetonitrile, chloroform, dichloromethane, diethyl ether, hexane, toluene, N,N-dimethylformamide (DMF), and glacial acetic acid were of analytical reagent (AR) grade and obtained from Merck. Double-distilled deionized water was used throughout the study for the preparation of solutions, plant extracts, and washing of the synthesized nanoparticles.

ii). **Collection and Identification of Plant Material:** Fresh aerial parts of Hybanthus enneaspermus (L.) F. Muell. were collected from the campus of Tirunelveli Dakshina Mara Nadar Sangam College, T. Kallikulam, Tamil Nadu, India. The collected plant material was thoroughly washed under running tap water to remove adhering dust and soil particles, followed by rinsing with distilled water. Healthy plant material was shade-dried at ambient temperature (25–30 °C) for 14 days to preserve thermolabile phytochemicals. The dried material was pulverized using a laboratory grinder and sieved through an 80-mesh sieve to obtain a uniform powder, which was stored in airtight containers until further use.

iii). **Preparation of Hybanthus enneaspermus Leaf Extract:** An aqueous extract was prepared by dispersing 10 g of the powdered plant material in 200 mL of deionized water. The mixture was heated at 80 °C for 2 h with continuous magnetic stirring to facilitate the extraction of phytochemicals. After cooling to room temperature, the extract was filtered three times through Whatman No. 1 filter paper to remove insoluble plant residues. The resulting filtrate was stored at 4 °C and used within one week as the reducing and stabilizing agent for nanoparticle synthesis.

iv). **Green Synthesis of CuO–NiO Nanocomposites:** Aqueous solutions of $Cu(NO_3)_2\cdot 3H_2O$ and $Ni(NO_3)_2\cdot 6H_2O$ were prepared separately in deionized water and then mixed in the desired stoichiometric ratio under continuous magnetic stirring. The aqueous extract of H. enneaspermus was added dropwise to the mixed metal precursor solution while maintaining the reaction temperature at 60–80 °C. The gradual colour change of the reaction mixture from light blue to dark brown indicated the formation of CuO–NiO nanoparticles. The reaction mixture was stirred continuously until complete precipitation occurred and subsequently aged to ensure complete nucleation and particle growth. The precipitate was separated by centrifugation and washed repeatedly with deionized water followed by absolute ethanol to remove unreacted salts and residual phytochemicals. The purified precipitate was dried in a hot-air oven at 80–100 °C and calcined in a muffle furnace at 450 °C to obtain highly crystalline CuO–NiO nanocomposites. The overall yield of the synthesis was approximately 95%.



Scheme 1: Green synthesis of CuO–NiO nanocomposites using Hybanthus enneaspermus leaf extract.

v). **Proposed Mechanism of Nanoparticle Formation:** The aqueous extract of H. enneaspermus contains flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, and saponins that function as both reducing and capping agents. Hydroxyl and carbonyl functional groups present in these phytochemicals coordinate with Cu^{2+} and Ni^{2+} ions, facilitating their reduction and the nucleation of mixed metal oxide nanoparticles. During calcination, the organic constituents are removed, resulting in the formation of crystalline CuO–NiO nanocomposites while residual phytochemicals adsorbed on the nanoparticle surface help suppress excessive particle agglomeration. This proposed mechanism is consistent with plant-mediated synthesis reported for other mixed metal oxide systems.

vi). **Characterization:** The optical properties of the synthesized CuO–NiO nanocomposites were investigated using a UV–Visible spectrophotometer over the wavelength range of 200–900 nm using quartz cuvettes with a path length of 1 cm. Measurements were carried out at room temperature using deionized water as the reference solvent.

vii). **Antibacterial Activity:** The antibacterial activity of the synthesized CuO–NiO nanocomposites was evaluated by the agar disc diffusion method against three Gram-positive bacteria (Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus) and three Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae). Bacterial cultures were grown overnight in nutrient broth at 37 °C prior to the experiment. Sterile nutrient agar plates were inoculated with the bacterial suspensions, and sterile paper discs impregnated with different concentrations of the synthesized nanoparticles (25–1000 µg mL^{-1}) were placed on the agar surface. Streptomycin served as the positive control, whereas dimethyl sulfoxide (DMSO) was used as the negative control. The plates were incubated at 37 °C for 24 h, after which the diameter of the inhibition zones was measured in millimetres. All experiments were performed in triplicate, and the mean inhibition zone was recorded. The reaction is depicted in scheme 1.

4. Result and Discussion

i). Characterization of Hybanthus enneaspermus extract

Phytochemical analyses reveal the presence of flavonoids, alkaloids, tannins, saponins, phenolic compounds, and terpenoids, contributing to its potent antioxidant, antimicrobial, anti-inflammatory, and antidiabetic activities (Table-1). Cross-sectional pharmacognostic studies highlight its characteristic slender stems, alternate leaves, and violet flowers with distinctive seed morphology. Ethanolic and aqueous extracts exhibit significant free radical scavenging potential and glucose-lowering effects in vitro. The plant's root and leaf extracts are traditionally used in treating reproductive disorders, urinary infections, and fatigue, supporting its ethnomedicinal relevance.

Table 1: Biological properties of Hybanthus enneaspermus

Parameter/Observation	Description/Value	Source/Study Type
Major Phytochemicals	Flavonoids, alkaloids, tannins, saponins, phenols	Phytochemical screening
Extract Types Studied	Ethanolic, methanolic, aqueous	Laboratory extraction studies
Antioxidant Activity (DPPH assay)	IC_{50} = 45–60 µg/mL	In vitro study
Antidiabetic Property	Inhibits α-amylase and α-glucosidase enzymes	Enzymatic inhibition assay
Antimicrobial Spectrum	Active against *E. coli*, *S. aureus*, *Candida* sp.	Zone of inhibition: 12–18 mm
Cross-sectional Morphology	Herbaceous plant with violet flowers and linear leaves	Pharmacognostic observation
Traditional Uses	Aphrodisiac, diuretic, tonic for reproductive health	Ethnomedicinal reports

ii). UV-Visible Spectroscopy

The UV–Visible absorption spectrum of the synthesized CuO–NiO nanocomposites exhibited a strong absorption band in the ultraviolet region with an absorption edge around 330 nm, confirming the successful formation of nanoscale mixed metal oxide particles (Fig-1). The observed absorption arises from charge-transfer transitions between oxygen ions and the Cu^{2+}/Ni^{2+} ions within the crystal lattice. The broad absorption profile suggests uniform nanoparticle formation and good crystallinity. The optical band gap calculated using the Tauc plot was approximately 2.18 eV, confirming the semiconducting nature of the synthesized nanocomposites. The slight shift in the absorption edge compared with bulk CuO and NiO may be attributed to nanoscale particle formation and the electronic interaction between the two oxide phases. The formation of a CuO–NiO heterojunction is expected to improve charge separation, reduce electron–hole recombination, and enhance photocatalytic and antimicrobial performance. The UV–Visible results therefore confirm the successful synthesis of CuO–NiO nanocomposites with desirable optical properties for environmental and biomedical applications.

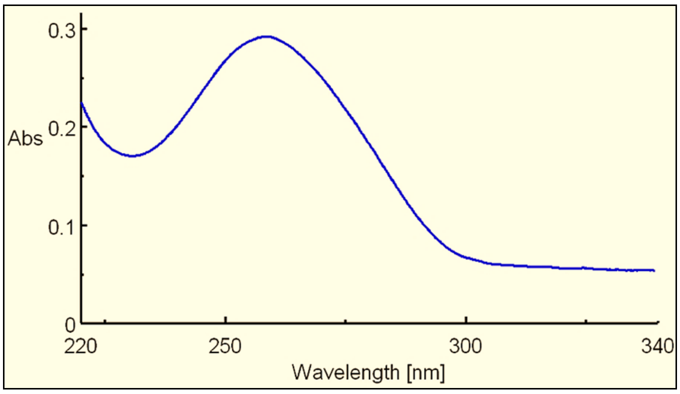


Fig 1: UV-VIS spectrum of Mercuric oxide nanoparticle

iii). Antibacterial Activity

The antibacterial activity of the synthesized CuO–NiO nanocomposites was evaluated against three Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) using the agar disc diffusion method. The synthesized nanocomposites exhibited pronounced antibacterial activity against all tested microorganisms. Among the Gram-positive bacteria, the highest inhibition zone was observed against *Staphylococcus aureus* (24 mm), followed by *Bacillus cereus* (20 mm) and *Bacillus subtilis* (18 mm). Among the Gram-negative bacteria, inhibition zones of 22 mm, 20 mm, and 15 mm were recorded for *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, respectively. The observed antibacterial activity indicates the broad-spectrum antimicrobial potential of the synthesized CuO–NiO nanocomposites. The enhanced antibacterial activity is attributed to the synergistic interaction between CuO and NiO nanoparticles, which promotes the generation of reactive oxygen species capable of inducing oxidative stress in bacterial cells. The release of Cu^{2+} and Ni^{2+} ions further disrupts bacterial cell membranes, denatures intracellular proteins, interferes with metabolic pathways, and damages nucleic acids, ultimately resulting in bacterial cell death. The small particle size and high surface-area-to-volume ratio facilitate close interaction between the nanoparticles and bacterial cell surfaces, thereby increasing membrane permeability and accelerating the antibacterial process. Gram-positive bacteria exhibited slightly greater susceptibility than Gram-negative bacteria, which may be associated with differences in cell wall architecture. The outer membrane present in Gram-negative bacteria provides an additional protective barrier that restricts nanoparticle penetration, whereas the comparatively porous peptidoglycan layer of Gram-positive bacteria allows more effective interaction with the nanoparticles. The overall results suggest that green-synthesized CuO–NiO nanoparticles are potent antimicrobial agents with promising potential for biomedical and environmental disinfection applications (Fig. 2 and Table 2).

Table 2: Zone inhibition of CuO–NiO nanoparticles with Bacteria's

S. No.	Bacteria	CuO–NiO nanoparticles	Control (STREPTOMYCIN)
1.	BS	18	22
2.	BC	20	20
3.	SA	24	12
4.	PA	15	12
5.	KP	20	13
6.	E.coli	22	11

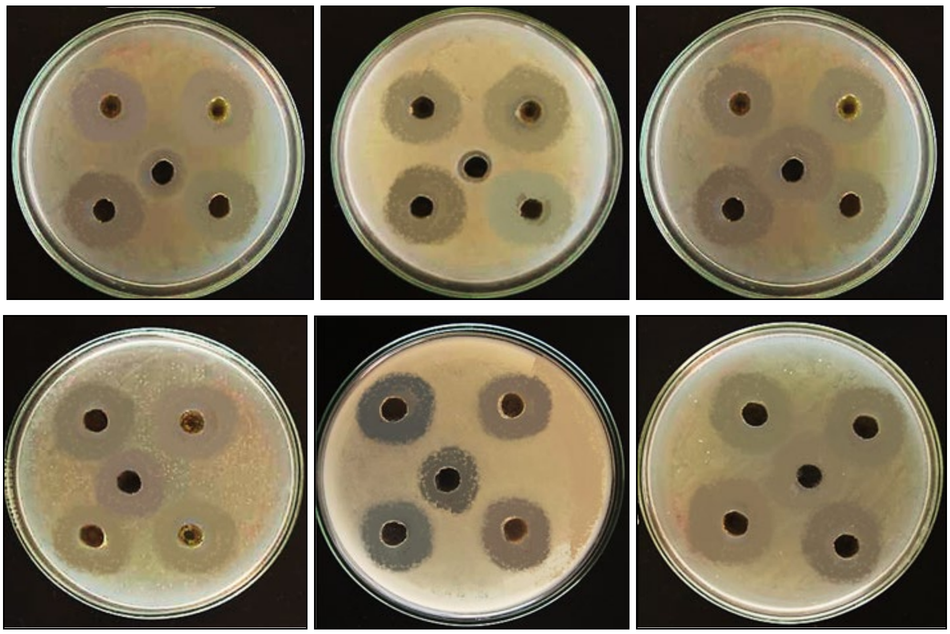


Fig 2: Antibacterial activity of CuO–NiO nanoparticles

iv). Discussion

The present investigation demonstrates that Hybanthus enneaspermus leaf extract serves as an efficient biological reducing and stabilizing agent for the green synthesis of CuO–NiO nanocomposites. The phytochemical constituents of the extract not only facilitated the reduction of metal ions but also controlled nanoparticle growth, resulting in stable nanocomposites with desirable optical and antibacterial properties. The observed UV–Visible absorption characteristics confirmed the successful formation of semiconducting mixed metal oxide nanoparticles, while the antibacterial studies established their effectiveness against both Gram-positive and Gram-negative bacterial pathogens. The combined effects of CuO and NiO within the heterostructure are expected to enhance charge transfer, improve reactive oxygen species generation, and increase antibacterial efficiency through synergistic interactions. The environmentally benign synthesis process, combined with the promising biological activity of the synthesized nanocomposites, highlights their potential for applications in antimicrobial coatings, biomedical materials, environmental remediation, wastewater treatment, photocatalysis, and other sustainable nanotechnology-based applications.

5. Future Scope

The green synthesis of CuO–NiO nanocomposites using *Hybanthus enneaspermus* leaf extract presents significant opportunities for future research and technological development. Further studies should focus on optimizing synthesis parameters, including precursor concentration, pH, reaction temperature, and calcination conditions, to achieve better control over particle size, morphology, crystallinity, and surface characteristics. Advanced characterization techniques such as X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), Raman spectroscopy, and Brunauer–Emmett–Teller (BET) surface area analysis could provide a deeper understanding of the structural and surface properties of the synthesized nanocomposites. In addition, the photocatalytic efficiency of CuO–NiO nanocomposites should be evaluated for the degradation of industrial dyes and emerging organic pollutants under visible-light irradiation. Their electrochemical performance may also be explored for applications in supercapacitors, batteries, and chemical sensors. From a biomedical perspective, comprehensive investigations involving cytotoxicity, biocompatibility, antibiofilm activity, and in vivo antimicrobial studies are essential before clinical applications can be considered. Furthermore, large-scale synthesis, long-term stability, environmental safety, and life-cycle assessment should be investigated to facilitate industrial production. These future studies will contribute to the development of sustainable, multifunctional nanomaterials suitable for environmental, biomedical, and energy-related applications while promoting the principles of green chemistry and sustainable nanotechnology.

6. Conclusions

In the present study, CuO–NiO nanocomposites were successfully synthesized through a simple, environmentally friendly green synthesis approach using the aqueous leaf extract of *Hybanthus enneaspermus*. The plant-derived phytochemicals effectively acted as both reducing and stabilizing agents, resulting in a synthesis yield of approximately 95% without the use of hazardous chemicals. Calcination at 450 °C produced highly crystalline nanocomposites with an average particle size in the range of 22–35 nm. UV–Visible spectroscopy revealed a characteristic absorption peak at 354 nm, confirming the successful formation of semiconducting CuO–NiO nanocomposites. The synthesized material exhibited broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. The highest inhibition zone of 24 mm was recorded against *Staphylococcus aureus*, followed by 22 mm against *Escherichia coli*, 20 mm against *Bacillus cereus* and *Klebsiella pneumoniae*, 18 mm against *Bacillus subtilis*, and 15 mm against *Pseudomonas aeruginosa*. The excellent antibacterial performance is attributed to the synergistic interaction between CuO and NiO, which enhances reactive oxygen species generation and promotes bacterial membrane disruption. Overall, the present study demonstrates that *H. enneaspermus*-mediated green synthesis is an efficient, sustainable, and economical approach for producing multifunctional CuO–NiO nanocomposites with considerable potential for applications in antimicrobial coatings, environmental remediation, photocatalysis, and other advanced functional materials.

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