Moringa Oleifera Root Extract for Sustainable Copper Oxide and Cobalt Oxide Nanoparticle Fabrication and Analysis Via Environmentally Friendly Synthesis

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Abstract
Presently, scientists commonly employ natural substances as reducing and stabilizing agents for the synthesis of copper oxide nanoparticles (CuO-NPs) and cobalt oxide nanoparticles (CoO NPs) due to their environmentally friendly, convenient, cost-effective, and feasible attributes. Using an eco-friendly approach, non-toxic and economical CuO nanoparticles were synthesized using moringa oleifera root extract. The optical properties of the copper oxide and cobalt oxide nanoparticles, synthesized via green methods, were elucidated using UV-visible spectroscopy. Fourier transform infrared spectroscopy (FTIR) was employed to investigate the active biomolecules and functional groups engaged in the biological reduction of metal ions into nano-scale CuO and CoO particles. X-ray diffraction analyses were conducted to scrutinize the structure, phase, and crystalline planes of the nanoparticles. The experimental results underscore that *moringa oleifera* root holds promise as a cost-effective and beneficial bio-resource for producing metal oxide nanoparticles. These standardized nanoparticles exhibit potential applications across various fields such as medicine, industry, and the environment. The study establishes the bioactivity and potential applications of environmentally friendly metal oxide nanoparticles.

Keywords: *Moringa Oleifera root*, XRD-EDX, Copper oxide nanoparticles, Cobalt oxide nanoparticles

1. Introduction:

Nanotechnology generates, characterizes, speeds up, and uses nano-sized (1-100 nm) materials to regulate their size and properties. Nanotechnology is being studied for electronics, industry, imaging, and medicine [1-5]. Because of their vast surface area, strong reactivity, and unique particle form, nanoparticles are intriguing. Due to their unique properties and many applications, metal oxide nanoparticles have received interest recently. Nanotechnology research has focused on nanomaterial synthesis due to industrial and medical needs for fillers, disinfectants, optics, antibacterial agents, drug delivery, and catalytic products [6]. CeO_2, TiO_2, ZnO, and Fe_2O_3 nanoparticles are being researched for environmental and medicinal applications. Researchers are interested in using stable, biocompatible copper oxide nanoparticles (CuO-NPs) and cobalt oxide nanoparticles (CoO-NPs) in catalysis, photocatalysis, cells, batteries, sensor fabrication, therapeutic agents, and drug delivery [7-10]. Nanoparticles are synthesized by co-precipitation [11], solvothermal [12], template-assisted precipitation [13], spray drying [14], microwave [15], hydrothermal [16], and sol-gel [17]. Many of these therapies are complicated, expensive, time-consuming, and employ hazardous chemicals that affect the ecology. Researchers recently employed green synthesis, a simple, safe, less damaging, and eco-friendly process. Plant extracts, microorganisms, vitamins, carbohydrates, and other derivatives can be used to make green nanoparticles [18-23]. The biological extracts comprise carbohydrates, saponins, amino acids, flavonoids, terpenoids, and proteins. These chemicals may reduce and stabilize bulk salts into nanoparticles. Fruit extracts have been used to biosynthesize CuO-NPs and CoO-NPs, according to a literature study.
This process is simple and viable compared to chemical and physical synthesis.
This research covers green CuO and CoO nanoparticle production using *moringa oleifera* root extract and their properties. No literature exists on green CuO and CoO nanoparticle production utilizing moringa *oleifera root* extract. The therapeutic herb *Moringa oleifera* root belongs to the *Moringaceae* family and genus Moringa. This plant's leaves are greenish or reddish underneath and darker above. They are lanceolate and opposed. Thin stem with yellowish bristly hairs and scarlet tint. Cut or wounded aerial parts produce milky fluid. Traditional medicine uses the herb to treat cough, coryza, asthma, digestive difficulties, worm infestations, and kidney stones. Bioactive components of *Moringa oleifera* root include alkaloids, flavonoids, saponins, carbohydrates, terpenoids, amino acids, and polyphenols, which suppress corrosion. *Moringa oleifera* is antibacterial, antioxidant, antifungal, and anti-inflammatory.
This study uses *moringa oleifera* root extract to make CuO and CoO nanoparticles in a cost-effective, simple, fast, and ecologically friendly method. The CuO and CoO nanoparticles were examined by XRD, UV-Vis, FTIR, and FE-SEM.

2. Experimental:

2.1. Materials:
Analytical reagent-grade copper chloride pentahydrate and cobalt chloride hexahydrate served as the precursor materials for the synthesis of metal oxide nanoparticles. Freshly harvested *Moringa oleifera roots* were obtained from the college premises. All aqueous solutions were prepared using triple-distilled water.

2.2. Preparation of *Moringa oleifera root* extract:
The harvested fruit of the selected weed plant underwent cleaning to remove any extraneous dust particles and was subsequently air-dried in shade at ambient temperature for a defined duration. Following drying, the roots were finely crushed and ground into a powdered form. This powdered material derived from the fruit of Moringa oleifera was employed to produce an extract using a Soxhlet extractor, with triple-distilled water serving as the solvent. The resulting colloidal solution was subjected to centrifugation and filtration through Whatman paper to remove any residual plant matter, after which it was stored in a refrigerator at 4°C for future utilization.

2.3. Synthesis of Copper Oxide Nanoparticles (CuO-NPs):
Copper oxide nanoparticles (CuO-NPs) were synthesized using an eco-friendly approach. Initially, a 0.01M solution of [$Cu(Cl)_2 \cdot 5H_2O$] was prepared in triple-distilled water. Subsequently, *moringa oleifera* root extract was gradually introduced into the solution at a predetermined ratio while being continuously agitated with a magnetic stirrer until a brown colloidal solution formed. The resulting colloidal solution underwent centrifugation at 10,000 rpm for a specific duration. The centrifuged particles were then washed 2-3 times with distilled water and subsequently separated and dried in a hot air oven at 60°C. The resultant CuO-NPs were stored in a plastic tube vial at room temperature.

2.4. Synthesis of Cobalt Oxide Nanoparticles (CuO-NPs):
Cobalt oxide nanoparticles (CoO-NPs) were synthesized using an environmentally sustainable approach. Initially, a 0.01M solution of [$Cu(Cl)_2 \cdot 5H_2O$] was prepared in triple-distilled water. Moringa oleifera root extract was then gradually added to the solution in a predetermined ratio while being continuously stirred with a magnetic stirrer until a brown colloidal solution formed. The resulting colloidal solution underwent centrifugation at 10,000 rpm for a specified duration. The centrifuged particles were rinsed two to three times with distilled water. Following separation, the centrifuged particles were dried at 60°C in a hot air oven. The resulting CuO-NPs were stored in a plastic tube vial at room temperature.

2.5. Characterization of Synthesized Nanoparticles:
The nanoparticles underwent analysis utilizing established standard analytical methodologies.

2.5.1. UV visible spectrum:
UV-visible absorption spectra were obtained employing a JASCO V650 UV spectrometer.

2.5.2. FT-IR spectroscopy:
FT-IR spectra of the synthesized metal oxide nanoparticles were acquired using an IR Brucker instrument. The spectra were generated by scanning the material across the 500-4000 cm^{-1} range.

2.5.3: X-ray diffraction spectra:
The X-ray diffraction spectra were obtained using the X-PERT PRO diffractometer, with the powdered raw material employed for analysis. X-ray diffraction (XRD) measurements were conducted in the 2θ range of 10-90 ° using Cu Kα radiation having a wavelength of 1.5406 Å. The instrument was operated at a voltage of 40 kV and a current of 40 mA at room temperature.

2.5.4 FE-SEM and EDS analysis:
The SEM pictures of the produced nanoparticles were obtained using the Nova Nano FEI Nova Nano-SEM 450 device. The nanoparticle's elemental composition was determined using Energy Dispersive X-ray Spectroscopy with FE-SEM equipment.

2.6: Antimicrobial activities:
The synthesized Cu and Co oxide nanoparticles were evaluated for their antimicrobial efficacy against four bacterial strains: *B. subtilis*, *P. aeruginosa, E. coli*, and *S. aureus*. The antibacterial activity was assessed by employing a modified Kirby-Bauer disc diffusion technique [24]. A solvent blank served as the negative control, while a positive control was established using the antibiotic streptomycin.

An antifungal susceptibility assay was employed to assess the efficacy of moringa oleifera fruit extract encapsulated CuO and CoO nanoparticles against fungal organisms. Samples of the fungi *A. niger* and *S. cerevisiae* were utilized to evaluate the antifungal effectiveness of the nanoparticles.

3. Result and Discussion:

The synthesized CuO and CoO nanoparticles were assessed and characterized using standard instrumentation and methodologies in a greener approach. The initial observation involved detecting a change in color within the reaction mixture, signifying the formation of metal oxide nanoparticles. Analysis of UV-Vis and FTIR spectrum data confirmed the production of metal oxide nanoparticles.

3.1. UV-visible spectroscopy:

UV-visible spectroscopy was employed to verify the synthesis of nanoparticles. **Figure 1** illustrates the UV-Vis absorption spectra of both the extract and the CuO and CoO nanoparticles. In the spectra (**Figure 1**), CuO and CoO nanoparticles exhibited absorption peaks at 320 and 347 nm, respectively. The determination of the direct band gap energy of the synthesized copper oxide nanoparticles was achieved by fitting the absorption data to a designated equation.

$\alpha h\nu = K (h\nu - E^g)^2$.

The symbols α, hv, Eg, and K denote the optical absorption coefficient, photon energy, direct bandgap, and a constant, respectively. To determine the direct band gap energy value, $(\alpha h\nu)^2$ is plotted against photon energy (hv), and the linear section of the curve is extended to where absorption reaches zero. The direct band gap energies of the synthesized CuO and CoO nanoparticles were found to be 3.6 and 3.7 eV, respectively. These values fall within the range reported in existing literature.

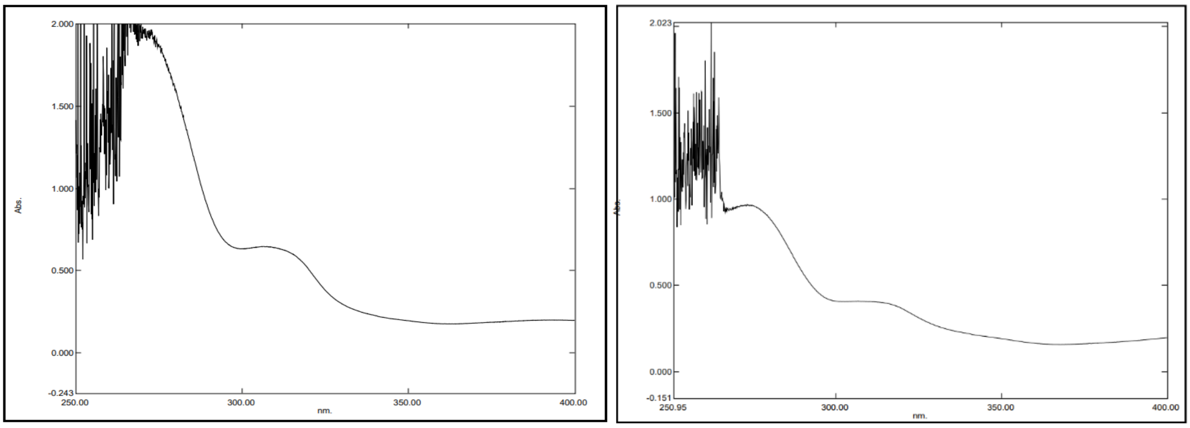


Figure 1. UV-Vis spectra of CuO nanoparticles and CoO nanoparticles synthesized using moringa oleifera.

3.2: FTIR analysis:

Infrared spectroscopy serves as a valuable tool for discerning chemical functional groups within a molecule and assessing the potential involvement of phyto-constituents in the capping and stabilization of nanoparticles. **Figure 2** presents the FTIR spectra of CuO and CoO nanoparticles synthesized using *moringa oleifera*. Notable peaks were observed in the spectra (**Figure 2**). The band observed at 3536-3553 cm^{-1} is attributed to the stretching vibration of hydroxyl (O–H) groups. The peak at 1617-1635 cm^{-1} corresponds to the bending vibrations of adsorbed –OH groups. Additionally, peaks at 982-988 cm^{-1} and 540-556 cm^{-1} in the spectra correspond to the vibration modes of O-M-O and M-O, respectively (where M = Cu, Co). The detection of M-O vibrational absorption bands indicates the formation of nanoparticles as MO.

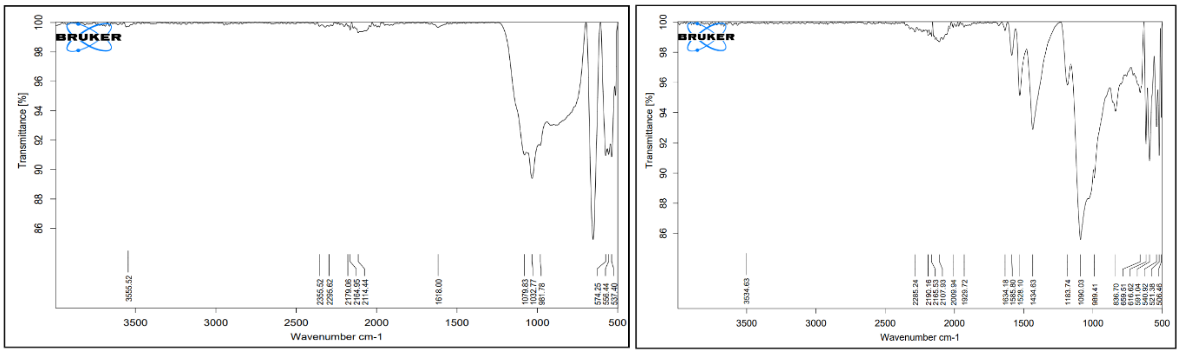


Figure 2. FTIR spectra of CuO nanoparticles and CoO nanoparticles synthesized using moringa oleifera.

3.3. XRD Analyses:

The X-ray diffraction (XRD) pattern of cobalt and copper oxide nanoparticles were acquired within the 20° to 80° range of 2θ values, as depicted in **Figure 3**. The diffraction peaks were observed at multiple 2θ values: 28.1-28.3°, 47.30-47.36°, 56.25-56.28°, and 76.83-76.85°, corresponding to the crystallographic planes (111), (022), (113), and (133) respectively [25-26]. These plane indices are consistent with the face-centered cubic (FCC) fluorite structure of CuO and CoO. The XRD patterns were analyzed via Rietveld refinement utilizing PANalytical HighScore software and were compared to the JCPDS reference code 98-062-1704. The crystal structure of CuO and CoO exhibits a cubic arrangement with Fm-3m as the space group and a lattice constant of 5.411 Å. The volume of CuO and CoO produced using moringa oleifera was determined to be 156.92 cubic angstroms. The size of the crystallites within the nanoparticles was determined using Debye-Scherrer's formula.

Average crystallite size (D) = $0.9\lambda/\beta\cos\theta$.

The formula incorporates λ as the wavelength of the X-ray source utilized for analysis, β representing the angular peak width at half-maxima in radians, and θ as Bragg's angle of diffraction. Utilizing the Scherrer formula outlined above, the average size of the crystallites was computed to be 16 nm.

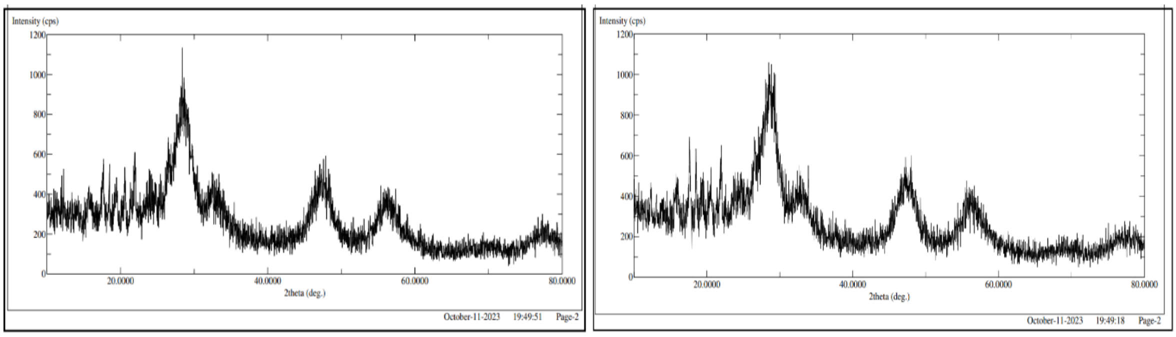


Figure 3: XRD spectra of *moringa oleifera* mediated CuONPs and CoONPs

3.4 FE-SEM and EDS analysis:

The structural analysis of the synthesized copper and cobalt oxides was conducted via FE-SEM, and the outcomes are illustrated in **Figures 4** to **7**. The images distinctly depict the heterogeneous morphology of the copper and cobalt oxide nanoparticles, featuring several spherical particles. Observations of CuO and CoO nanoparticles were made at magnifications of 10 µm and 1 µm, respectively. Figures **4** to **7** also exhibit the EDS spectrum of CuO and CoO nanoparticles synthesized using *moringa oleifera root* extract, revealing the presence of copper, cobalt, and oxygen constituents in the sample composition. The spectrum peaks indicate the presence of copper (Cu) and oxygen (O) at energy levels of 8.052 and 0.534 kilo electronvolts (keV), respectively. Similarly, the presence of cobalt (Co) and oxygen (O) is indicated by the spectrum peak at 5.396 and 0.509 keV, respectively.

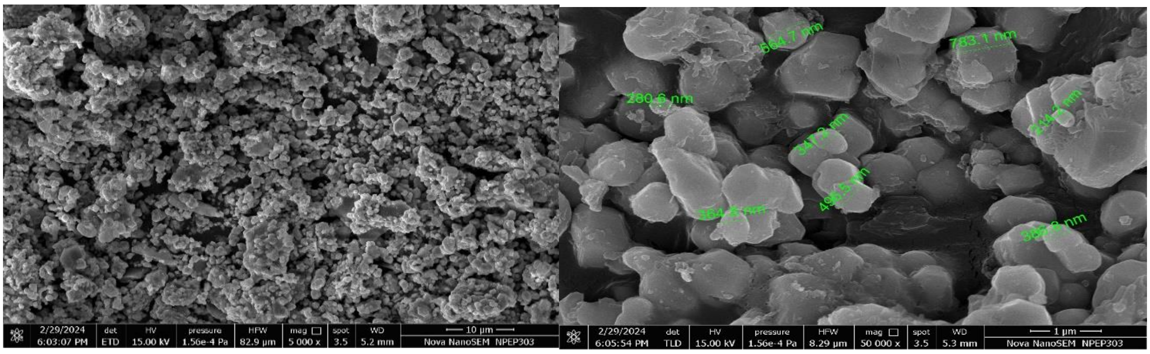
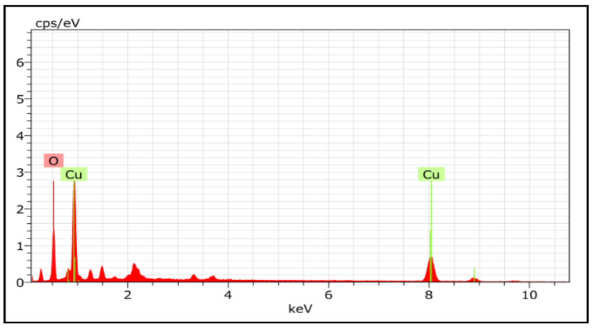


Figure 4: Field emission scanning electron microscopy (FE-SEM) micrographs of CuO nanoparticles synthesized with moringa oleifera, captured at magnifications of 10 µm and 1 µm.



EDS Report

Spectrum: OBJECT1 727

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Cu	29	K-series	52.31	86.17	61.07	1.74
O	8	K-series	8.40	13.83	38.93	1.20
		Total:	60.70	100.00	100.00	

Figure 5: Energy-dispersive X-ray spectroscopy (EDX) spectrum for CuO nanoparticles synthesized with moringa oleifera.

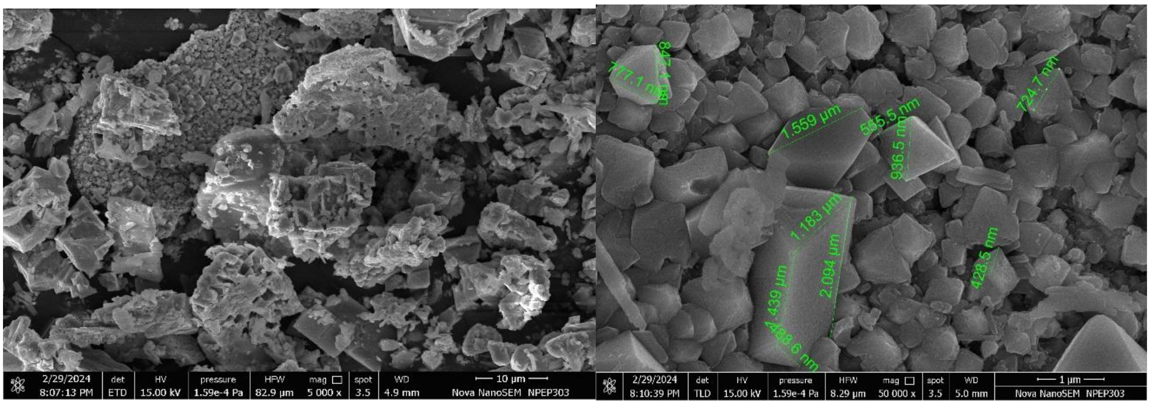
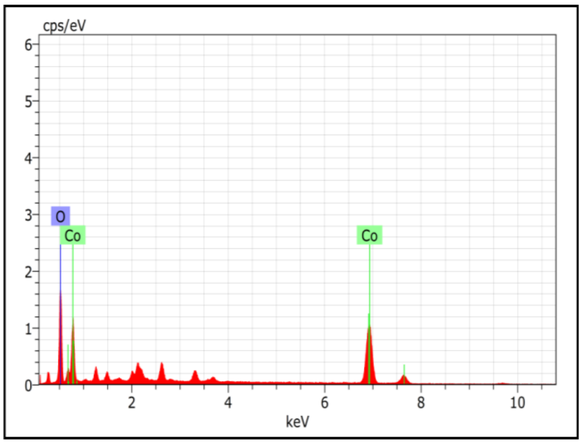


Figure 5: FE-SEM micrographs of CoO nanoparticles synthesized using moringa oleifera, captured at magnifications of 10 µm and 1 µm.



EDS Report

Spectrum: OBJECT1 735

El	AN	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (1 Sigma) [wt.%]
Co	27	K-series	57.62	82.86	56.75	1.78
O	8	K-series	11.92	17.14	43.25	1.60
		Total:	69.54	100.00	100.00	

Figure 7: EDX spectrum for CoO nanoparticles synthesized with moringa oleifera.

3.5: Antimicrobial activities:

The inhibitory effect of CuO and CoO nanoparticles at doses of 25 µg, 50 µg, and 75 µg on bacterial growth on agar plates. An augmentation in antibacterial activity was observed with escalating concentrations of CuO and CoO nanoparticles. Upon adsorption onto the bacterial cell, nanoparticles undergo dehydrogenation through the respiratory mechanism occurring at the bacterial cell membrane. Before interacting with the nanoparticles, the bacteria had already deactivated their enzymes, resulting in the generation of hydrogen peroxide, which ultimately eradicates the bacteria [27].

4. Conclusions:

Copper and cobalt oxide nanoparticles were synthesized utilizing moringa oleifera root extracts via an eco-friendly approach that minimizes the usage of chemicals. Preliminary confirmation of CuO-NPs and CoO-NPs formation was established through FTIR spectra analysis, wherein observed peaks corroborate the presence of M-O bonds, implicating the involvement of moringa oleifera root extract in nanoparticle generation. Morphological, compositional, and optical attributes of the synthesized CuO-NPs and CoO-NPs nanoparticles were evaluated employing FE-SEM, EDAX, and UV-visible spectroscopy. XRD analysis unveiled the presence of a face-centered cubic phase in CuO-NPs and CoO-NPs, with an average crystallographic size of 16 nm. The band gap energy of CuO-NPs and CoO-NPs nanoparticles was determined via the Tauc plot method, yielding a value of 3.8 eV. This rapid, straightforward, and environmentally benign synthetic procedure presents a highly appealing and efficient means of producing nano-scale metal oxide particles applicable across diverse industrial sectors.

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