

Green Synthesis and Characterization of Cobalt Oxide Nanoparticles using *Eucalyptus Globulus* Leaf Extract: In Vitro Antibacterial and Antifungal Activity

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ABSTRACT

The rise of antibiotic resistance has intensified the search for novel antimicrobial agents. Green synthesis of metal oxide nanoparticles using plant extracts offers an eco-friendly alternative to conventional methods. This study reports the green synthesis and characterization of cobalt oxide nanoparticles (CoNPs) using an aqueous leaf extract of *Eucalyptus globulus* and cobalt (II) chloride, and evaluates their *in vitro* antibacterial and antifungal activity. Nanoparticle formation was confirmed by UV-Vis, FTIR, XRD, and SEM. UV-Vis spectra showed an absorption band at 220 nm, consistent with the $O^{2-} \rightarrow Co^{2+}$ charge-transfer transition for Co_3O_4 . FTIR bands at 660–690 and 570–660 cm^{-1} confirmed Co–O lattice vibrations. XRD revealed a crystalline structure with an average particle size of 72 nm, while SEM showed an irregular, clustered morphology. Antimicrobial activity was evaluated by agar well diffusion against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*. The CoNPs produced the largest inhibition zones against *C. albicans* (19.9 ± 0.34 mm) and *S. aureus* (18.5 ± 0.13 mm), and were active against all organisms except *S. typhi* (9.7 ± 0.22 mm). Notably, the crude n-hexane extract (15 mg/mL) outperformed the CoNPs against *S. aureus* (23.7 mm). These findings indicate that *E. globulus*-mediated cobalt oxide nanoparticles are promising antimicrobial candidates, pending *in vivo* evaluation.

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INTRODUCTION

Nanotechnology is an emerging scientific field focused on the study and manipulation of materials at the nanoscale, typically ranging from 1 to 100 nanometres, and represents an advanced interdisciplinary domain that integrates principles from materials science, biology, and chemistry (Das et al., 2025). Its practical applications include the development and utilization of nanoscale materials, components, and devices such as biosensors, antimicrobial agents, and water purification systems.

The physical characteristics of metal and metal oxide nanoparticles — size, shape, composition, and internal structure, whether in alloy or core-shell form — greatly influence their functionality (Gong et al., 2020). Tailoring nanoparticles to achieve precise structural features therefore remains a major challenge in nanoscience. Owing to their catalytic, electrical, optical, and structural properties, metal and metal oxide nanoparticles have attracted extensive scientific interest, with applications spanning biotechnology, electronics, optics, biomedicine, catalysis, and magnetism (Singh et al., 2023).

Researchers have increasingly turned to green nanotechnology, an approach rooted in the principles of green chemistry, which guides the sustainable synthesis and stabilization of metal-based nanoparticles (Soltys et al., 2021). Biosynthesis using non-toxic chemicals, sustainable solvents, and renewable resources has proven valuable for eco-friendly nanoparticle production (Abdullahi et al., 2026,

Chopra et al., 2022), with plant-derived phytochemicals rich in antioxidants facilitating the reduction of metal ions into nanoparticles (Adeyemi et al., 2022).

There is growing interest in natural products with medicinal properties for use in food and medicine, driven partly by concerns over the adverse effects of synthetic drugs (Beura & Raul, 2024). Natural substances and traditional remedies have contributed significantly to modern medicine, beginning with the isolation of morphine from opium in 1805 (Narwariya, 2023), and plant-derived bioactive compounds continue to be investigated for conditions ranging from cancer to Alzheimer's disease (Shoaib et al., 2023).

The *Eucalyptus* tree is a diverse genus of flowering trees in the Myrtaceae family, native to Australia and also found across Southeast Asia (Jo et al., 2022). Eucalyptus leaves and the essential oil derived from them possess antiseptic and antibacterial properties and are widely used to manage respiratory and cold-related illnesses (Sabo & Knezevic, 2019).

Despite growing interest in green nanotechnology, there remains a notable gap in research on the synthesis of cobalt-based nanoparticles using leaf extracts of *Eucalyptus globulus* and the evaluation of their antimicrobial activity. This study addresses that gap by synthesizing cobalt oxide nanoparticles using *E. globulus* leaf extract, characterizing their physicochemical properties, and evaluating their *in vitro* antibacterial and antifungal activity, with a view to informing the future development of sustainable antimicrobial agents.



Plate 1: *Eucalyptus globulus* leaf

MATERIALS AND METHODS

Materials

The equipment and materials used include cobalt (II) chloride, a Hanna Instruments 2211 pH meter, a Jenway 6400 UV-visible spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) equipment, an ultrasonic bath, a measuring cylinder, an analytical balance, beakers, conical flasks, a magnetic stirrer, a heating mantle, a spatula, a funnel, reagent bottles, a burette, a retort stand, test tubes, a test tube rack, Whatman No. 1 filter paper, a centrifuge, methanol, and distilled water.

Sample Collection and Preparation

Leaves of *Eucalyptus globulus* were collected from the Modibbo Adama University, Yola area of Adamawa State. The leaves were washed thoroughly with distilled water to remove dust and debris, then air-dried at room temperature on clean polythene sheets for a minimum of two weeks. The dried leaves were mechanically ground using a pestle and mortar, and the resulting powder was sieved and stored in labelled polyethylene bags for further use.

Preparation of Eucalyptus Globulus Leaf Extract

10 g of the dried, powdered leaves was added to 100 mL of deionized water, and the mixture was heated at 60°C for 30 minutes with constant stirring to facilitate extraction of the bioactive compounds. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtrate was used as the reducing and stabilizing agent in the bio-reduction of cobalt (II) chloride (Mamuru et al., 2019).

Preparation of Cobalt Oxide Nanoparticles

A 0.504 M cobalt (II) chloride solution was prepared by dissolving 6 g of CoCl_2 in 50 mL of distilled water. Fifty millilitres of the *E. globulus* leaf extract was placed in a beaker, and the cobalt chloride solution was added dropwise with continuous stirring (Amin et al., 2021). The pH of the mixture was adjusted to 9–10 (Bachheti et al., 2021), and the mixture was heated at 60°C for 3 hours (Govindasamy et al., 2022). A colour change to dark brown was observed, and the resulting product was centrifuged at 8000 rpm for 45 minutes, filtered, rinsed thoroughly with distilled water, and dried at room temperature (Mamuru et al., 2015). The synthesis was repeated nine times (9x) so as to have enough nanoparticles for further analysis.

Spectroscopic and Morphological Characterization

UV-Visible absorption spectroscopy was performed over the range 200–450 nm at 5 nm intervals, using distilled water as a blank for the extract and ethanol as the blank for the nanoparticle suspension (Mamuru et al., 2019). FTIR analysis was carried out with a PerkinElmer Spectrum instrument (Version 10.4.3) to identify functional groups associated with nanoparticle formation (Mamuru et al., 2015). For XRD analysis, powdered samples were pelletized, sieved to 0.074 mm, mounted on an aluminium alloy grid, and analysed with a Rigaku D/Max-IIIC X-ray diffractometer (Rigaku Int. Corp., Tokyo, Japan) at a scanning rate of 2°/min over 2–50° 2 θ , using $\text{CuK}\alpha$ radiation at 40 kV and 20 mA; diffraction data were compared with the ICDD mineral powder diffraction file (Mamuru et al., 2019). Surface morphology was examined by Scanning Electron Microscopy (SEM) with a secondary electron detector (Kachalla et al., 2022).

Antibacterial Activity

Antibacterial activity of the CoNPs and the crude *E. globulus* extract was evaluated against five bacterial isolates *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi* using the agar well diffusion method (Khanam et al., 2024). The extract and nanoparticle were dissolved in ethanol at concentrations of 25, 50, 75, and 100 mg/mL. Mueller-Hinton Agar (MHA) plates were used, with Gentamicin 10 $\mu\text{g}/\text{mL}$ and methicillin 5 $\mu\text{g}/\text{mL}$ as the positive control, and plates were incubated at 35–37°C for 24 hours. Inhibition zone diameters were measured in millimetres. The experiment was conducted in triplicate.

Antifungal Activity

Antifungal activity was evaluated against *Candida albicans*, *Aspergillus niger*, and a further *Aspergillus flavus*, following the agar well diffusion method described by Daoud et al. (2021). A 6 mm well was made in potato dextrose agar (PDA) plates and inoculated with freshly prepared spore suspensions. The extract was tested at 100, 200, and 300 mg/mL, using DMSO as solvent control and ketoconazole as the positive standard. Plates were incubated at 30°C for 72 hours, and inhibition zone diameters were measured in triplicate.

RESULTS AND DISCUSSION

Formation of Cobalt Oxide Nanoparticles

The development of a dark brown colouration (Figure 2C) indicated the formation of cobalt-based nanoparticles, with

colour intensity increasing with contact time. Colour change of this type has been attributed by Mamuru et al. (2019) to collective oscillation of conduction-band electrons at the nanoparticle surface, a phenomenon absent in bulk material

or isolated atoms. The reducing and capping role of *Eucalyptus* extract phytochemicals in metal nanoparticle synthesis is consistent with previous reports (Gingasu et al., 2023; Barzinjy & Azeez, 2020; Dauda et al., 2021).

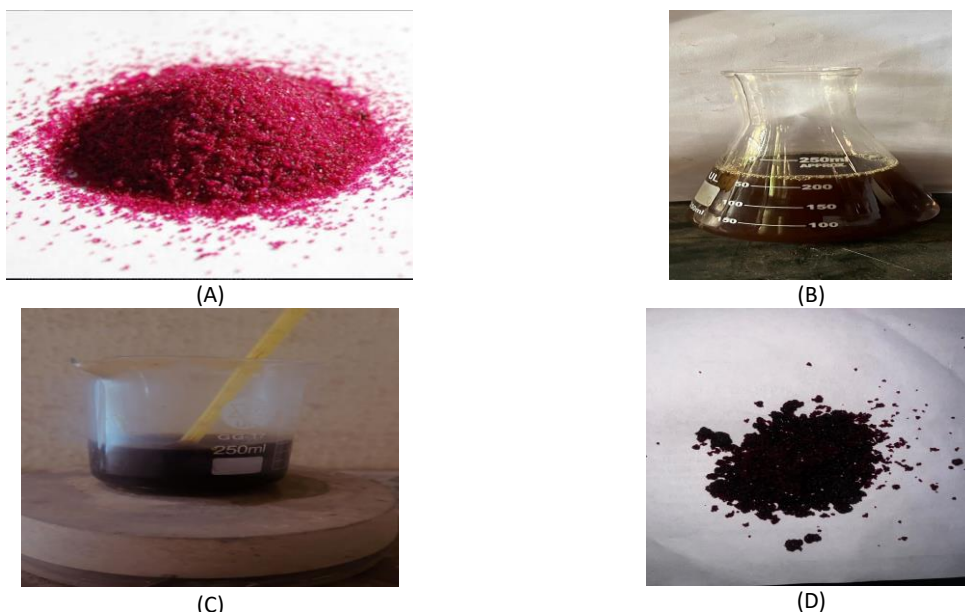


Plate 2: Formation of cobalt oxide nanoparticles

UV-Visible Spectroscopy

Figure 1 shows an absorption band at approximately 220 nm. Since metallic cobalt nanoparticles typically show SPR bands in the 350–500 nm region or, in some radiolytic preparations, closer to 517 nm (Anjali et al., 2010; Simulation and Synthesis of Cobalt Nanoparticles by Gamma Radiation Technique). A band at approximately 220 nm is instead

consistent with the $O^{2-} \rightarrow Co^{2+}$ ligand-to-metal charge-transfer transition reported for Co_3O_4 (cobalt oxide) nanoparticles, which typically show absorption in the 200–350 nm and 380–600 nm ranges (Farhadi et al., 2013; Al-Qasbi, 2022). This reassignment is corroborated by the present FTIR data, in which bands at 660–690 and 570–660 cm^{-1} (see below) are attributable to Co–O lattice vibrations characteristic of Co_3O_4 .

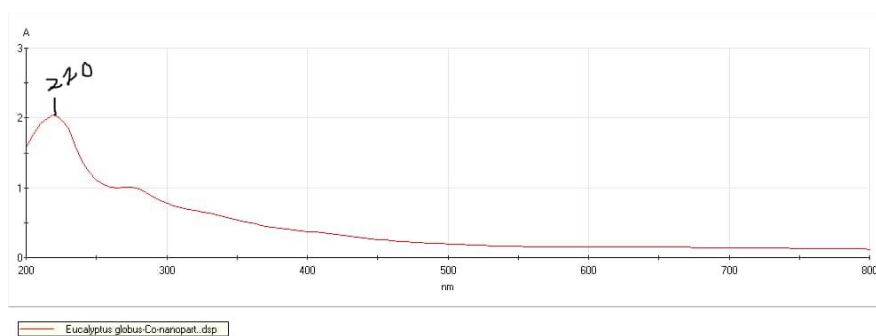


Figure 1: UV-Visible spectrum of cobalt oxide nanoparticles synthesized using *Eucalyptus globulus* extract

FTIR Spectroscopy Analysis

Figures 2 and 3 show the FTIR spectra of the *E. globulus* extract and the synthesized nanoparticles, respectively, over the region 350–4400 cm^{-1} . Seven distinctive peaks were resolved for the nanoparticles and eight for the plant extract, indicating a measurable change in surface chemistry upon nanoparticle formation. The band at 3300–3400 cm^{-1} , present in both spectra, corresponds to O–H stretching of adsorbed water and alcohols. Bands at 2920 and 2850 cm^{-1} , corresponding to C–H stretching of alkanes/terpenoids, appeared only in the extract and were absent in the nanoparticle spectrum, consistent with consumption of these functional groups during bio-reduction, as similarly reported by Siddiqi et al. (2020). The band at 1610–1660 cm^{-1} is assigned to O–H bending (water) in the nanoparticles and to

C=O stretching (ketones) in the extract. The band at 1510–1600 cm^{-1} , observed only in the extract, corresponds to aromatic C=C stretching and suggests the presence of flavonoids, consistent with Mishra et al. (2015).

The band at 1380–1400 cm^{-1} was present in both spectra. In the extract, this band is attributed to nitrate residuals from the cobalt salt retained on the leaf surface/matrix; in the nanoparticle spectrum, the same region is attributed to O–H bending, since the nitrate-associated component would not be expected to persist through the wash and centrifugation steps. These are presented as two distinct, mutually exclusive assignments for the two different samples rather than a dual assignment for a single spectrum. The band at 1030–1150 cm^{-1} , seen in the extract, corresponds to C–O stretching of esters, ethers, or alcohols (Siddiqi et al., 2020). Bands at 660–

690 and 570–660 cm^{-1} , observed only in the nanoparticle spectrum, are assigned to Co–O stretching at the tetrahedral and octahedral sites of Co_3O_4 , respectively, and directly support the Co_3O_4 assignment discussed above. The band near

900 cm^{-1} , present in both spectra, is attributed to aromatic C–H bending in the extract and to Co–O lattice vibration in the nanoparticles (Alpuche-Aviles & Wudl, 2002).

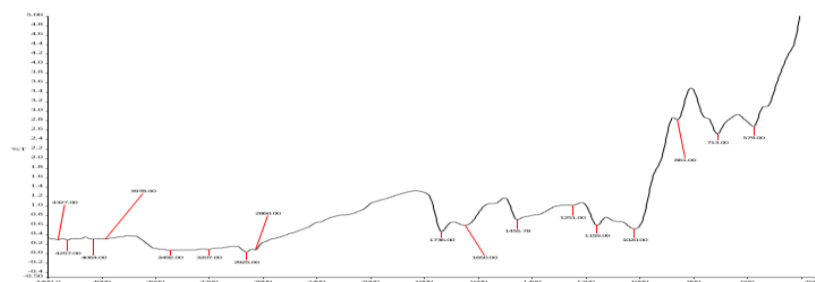


Figure 2: FTIR spectrum of the cobalt oxide nanoparticles

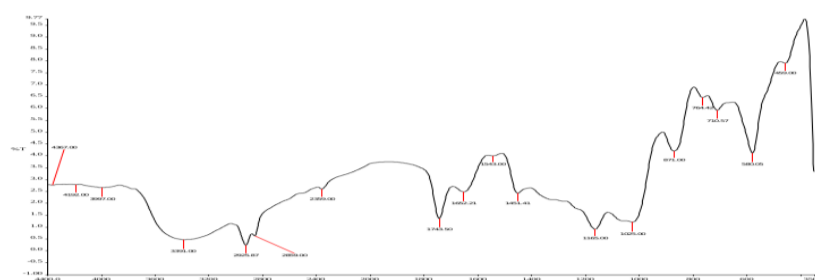


Figure 3: FTIR spectrum of Eucalyptus globulus extract

X-Ray Diffraction (XRD) Analysis

The XRD pattern of the synthesized nanoparticles showed broad peaks at 2θ values of 25.28°, 30.30°, 35.29°, and 50.18°, consistent with a cobalt-based nanostructure; the notable broadening of these peaks indicates that the material is in the nanometre size range. The average crystallite size, calculated from the Debye-Scherrer equation, $D \text{ (nm)} = K\lambda/\beta\cos\theta$ (where D is the mean crystallite diameter, β is the full width at half maximum, λ is the X-ray wavelength, θ is the Bragg angle, and K is the Scherrer constant, taken as 0.94), was 72 nm.

This value falls within the range reported for biosynthesized cobalt/cobalt oxide nanoparticles. Cherepanova et al. (2025) characterized the domain structure of metallic cobalt nanoparticles obtained by reduction of Co_3O_4 and reported nanoheterogeneous crystallite domains in the nanometre range; Parveen et al. (2025) note that plant-mediated cobalt-based nanoparticles typically range from 20–100 nm depending on phytochemical composition and synthesis conditions; and Sivakumar et al. (2020) reported XRD-

derived sizes of approximately 70 nm for plant-extract-synthesized cobalt oxide nanoparticles, attributing stabilization to flavonoids and phenolic compounds. The 72 nm particle size obtained here is therefore consistent with values reported in the literature for green-synthesized cobalt oxide nanoparticles, supporting the interpretation that the extract's phytochemicals both reduced the cobalt ions and stabilized the resulting nanoparticles.

Scanning Electron Microscopy (SEM) Analysis

Figure 4 shows the surface morphology of the nanoparticles, including aggregates at approximately 200, 150, and 100 μm . The particles displayed an irregular, clustered morphology rather than well-defined spherical particles, with brighter regions representing agglomerated nanostructures composed of primary particles that were either fused or loosely packed. This structure suggests a high surface area, which is advantageous for adsorption-based and antimicrobial applications (Govindasamy et al., 2022).

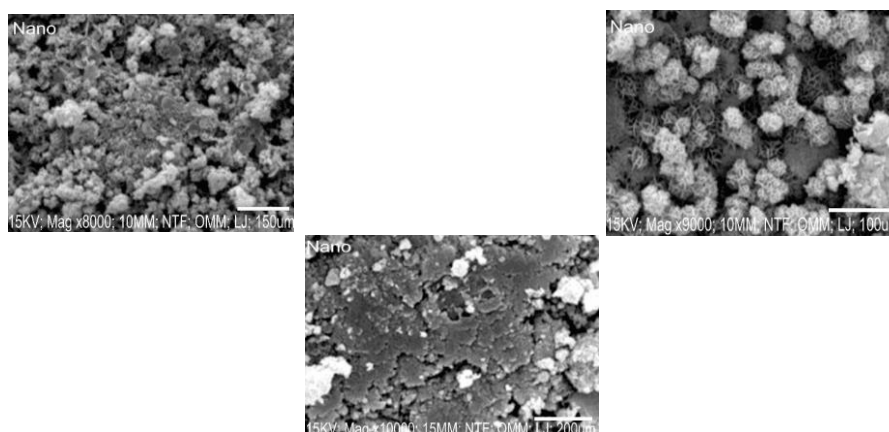


Figure 4: SEM images of the cobalt oxide nanoparticles

Antimicrobial Activity

The antimicrobial activities of the cobalt oxide nanoparticles ($\text{Co}_3\text{O}_4\text{NPs}$) and the crude *Eucalyptus* extract were evaluated against a panel of bacterial and fungal isolates using the agar well diffusion method. As shown in Table 1, both agents exhibited varying degrees of activity depending on the solvent fraction, concentration, and target organism. Overall, the $\text{Co}_3\text{O}_4\text{NPs}$ were active against a broader range of organisms than the crude extract and generally produced larger inhibition zones, but this was not uniform across all organisms: for *S. aureus* specifically, the crude extract's *n*-hexane fraction (15 mg/mL) produced a larger zone of inhibition (23.7 mm) than the $\text{Co}_3\text{O}_4\text{NPs}$ (18.5 mm) (Table 2). The nanoparticles' advantage was therefore concentrated in broad-spectrum coverage and in activity against fungi, rather than in outperforming the extract against every organism tested.

Comparative Susceptibility of Microorganisms

Table 1 summarizes the susceptibility ranking of the tested microorganisms to the most active agent. *S. aureus* and *C. albicans* were the most susceptible organisms, with inhibition zones of 18.5 ± 0.13 mm and 19.9 ± 0.34 mm, respectively, when treated with $\text{Co}_3\text{O}_4\text{NPs}$. This is consistent with reports that Gram-positive bacteria and yeasts tend to be more susceptible to nanoparticles owing to the absence of an outer membrane barrier (Hajipour et al., 2012). In contrast, *S. typhi* was the least susceptible organism, with only 9.7 ± 0.22 mm inhibition even against the most active agent; this reduced susceptibility may be attributable to the protective lipopolysaccharide layer characteristic of Gram-negative *Salmonella* species (Nikaido, 2003).

Table 1: Comparative Susceptibility of Microorganisms to the Most Active Agent Tested

Microorganism	Type	Most Active Agent	Max (mm)	Zone	Susceptibility Ranking
<i>S. aureus</i>	Gram-positive bacterium	CoNPs (n-hexane fraction)	18.5 ± 0.13		Most susceptible
<i>C. albicans</i>	Fungus	$\text{Co}_3\text{O}_4\text{NPs}$ (ethanolic fraction)	19.9 ± 0.34		Most susceptible
<i>Aspergillus flavus</i>	Fungus	$\text{Co}_3\text{O}_4\text{NPs}$ (ethanolic fraction)	17.8 ± 0.41		Highly susceptible
<i>B. cereus</i>	Gram-positive bacterium	$\text{Co}_3\text{O}_4\text{NPs}$ (n-hexane fraction)	16.3 ± 0.57		Highly susceptible
<i>P. aeruginosa</i>	Gram-negative bacterium	$\text{Co}_3\text{O}_4\text{NPs}$ (n-hexane fraction)	16.1 ± 0.46		Moderately susceptible
<i>A. niger</i>	Fungus	$\text{Co}_3\text{O}_4\text{NPs}$ (ethanolic fraction)	16.6 ± 0.37		Moderately susceptible
<i>E. coli</i>	Gram-negative bacterium	$\text{Co}_3\text{O}_4\text{NPs}$ (n-hexane fraction)	14.2 ± 0.61		Moderately susceptible
<i>S. typhi</i>	Gram-negative bacterium	$\text{Co}_3\text{O}_4\text{NPs}$ (n-hexane fraction)	9.7 ± 0.22		Least susceptible
Ketoconazole	Positive control (fungus)		24.5 ± 0.4		
Methicillin	Positive control		24.8 ± 0.6		
/Gentamicin	(bacteria)		24.5 ± 0.4		

Overall Potency Ranking

Table 2 ranks the test agents by overall potency. $\text{Co}_3\text{O}_4\text{NPs}$ in the ethanolic fraction ranked first, owing to strong activity against *C. albicans* (19.9 mm) and broad-spectrum coverage across both bacteria and fungi. $\text{Co}_3\text{O}_4\text{NPs}$ in the *n*-hexane fraction ranked second, with strong antibacterial activity. The crude extract (15 mg/mL) in the ethanolic fraction ranked third, with strong antifungal activity (20.5 mm) but a

narrower spectrum, while the same extract in the *n*-hexane fraction achieved the single largest inhibition zone recorded in this study (23.7 mm against *S. aureus*), ranking fourth overall because this activity did not extend broadly across the other test organisms. At lower concentrations, the crude extract showed only low to moderate activity across both fractions.

Table 2: Overall Potency Ranking of Test Agents

Rank	Test Agent	Solvent	Best Activity (Organism)	Max Zone (mm)	Broad-Spectrum
1	Cobalt Nano	Ethanoic	<i>C. albicans</i>	19.9	High
2	Cobalt Nano	N-Hexane	<i>S. aureus</i>	18.5	High
3	Eucalyptus (15 mg/ml)	Ethanoic	<i>A. flavus</i> / <i>C. albicans</i>	20.5	High (fungi)
4	Eucalyptus (15 mg/ml)	N-Hexane	<i>S. aureus</i>	23.7	Moderate
5	Eucalyptus (low conc.)	Both	Various	<10	Low
6	Ketoconazole	Positive control (fungus)	All	24.5 ± 0.4	High
7	Methicillin /Gentamicin	Positive control (bacteria)	Various	24.8 ± 0.6 24.5 ± 0.4	High

Conclusion

This study demonstrated an eco-friendly, green synthesis of cobalt oxide nanoparticles ($\text{Co}_3\text{O}_4\text{NPs}$) using the leaf extract of *Eucalyptus globulus*. Phytochemicals present in the extract

served as reducing and capping agents, facilitating the formation of stable nanoparticles. UV-Vis spectroscopy showed an absorption band at 220 nm consistent with the $\text{Co}^{2+} \rightarrow \text{O}$ charge-transfer transition of Co_3O_4 , and FTIR

analysis confirmed key functional groups, including Co–O bands, supporting this assignment. XRD analysis showed the nanoparticles to be crystalline with an average size of 72 nm, and SEM imaging revealed an irregular, clustered morphology consistent with a high surface area.

The nanoparticles showed measurable antibacterial activity against *S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa*, and antifungal activity against *C. albicans* and *A. niger*, generally outperforming the crude extract in breadth of activity, though not in every individual comparison (Table 2). *S. typhi* was comparatively resistant to both agents.

These findings suggest that *E. globulus*-mediated cobalt oxide nanoparticles are a promising, sustainable platform for further antimicrobial development. However, this study was limited to *in vitro* testing; no *in vivo* efficacy, pharmacokinetic, or cytotoxicity data are presented, and the nanoparticles should not be characterized as a treatment at this stage. Further work is needed to evaluate *in vivo* efficacy, cytotoxicity, and stability, and to determine whether these nanoparticles could be formulated into topical or other delivery systems for future therapeutic exploration.

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