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Green synthesis of cobalt oxide nanoparticles using *Andrographis paniculata* leaf extract: Characterization and antimicrobial evaluation

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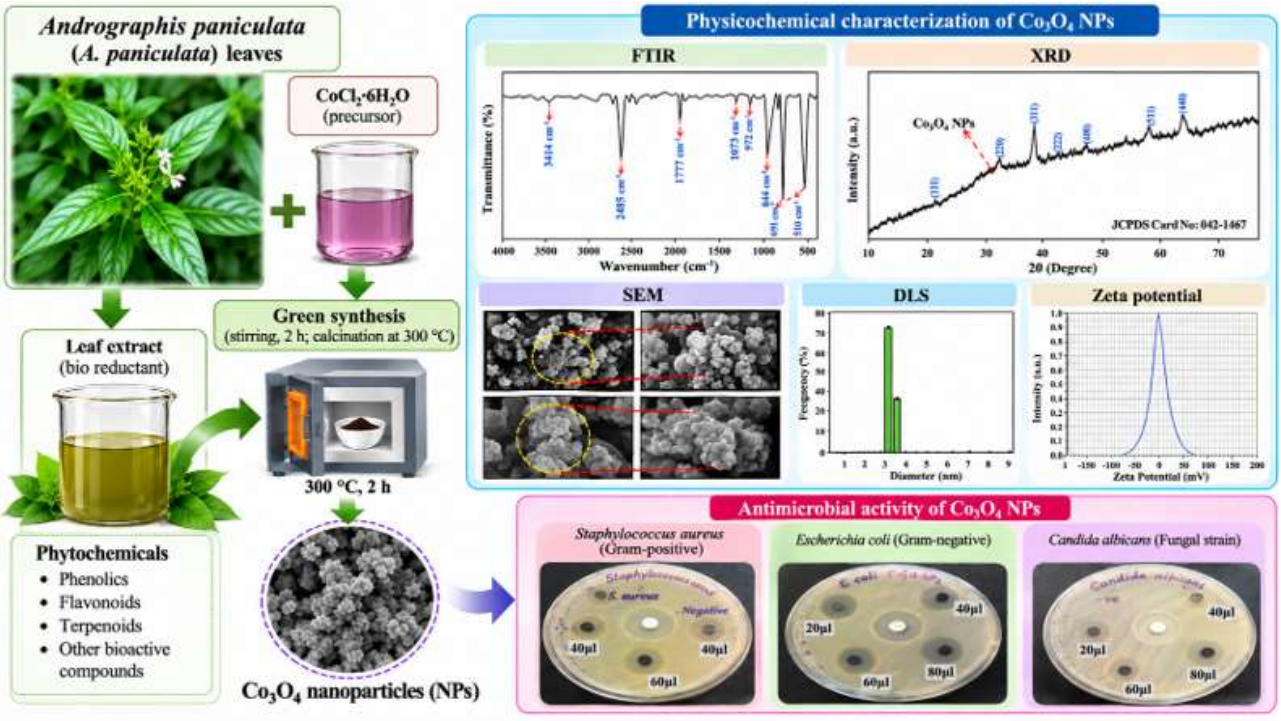
In this study, *Andrographis paniculata (A. paniculata)* leaf extract was employed as a bio reductant for the green synthesis of cobalt oxide (Co_3O_4) nanoparticles (NPs), providing a sustainable alternative to conventional physicochemical synthesis routes that are often costly and environmentally hazardous. The synthesized *A. paniculata*-mediated Co_3O_4 nanoparticles were subjected to comprehensive physicochemical characterization techniques, including Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Dynamic Light Scattering (DLS), and Zeta Potential Analysis. A zeta potential of -3.9 mV indicated weak electrostatic stabilization and a tendency toward partial aggregation. The biologically synthesized Co_3O_4 nanoparticles were further evaluated for their antimicrobial potential. Antibacterial assays demonstrated inhibitory activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial strains. Additionally, antifungal activity was observed against *Candida albicans*, highlighting the enhanced antimicrobial efficacy imparted by the phytochemicals present in the plant extract.

Keywords: Co_3O_4, Phytochemicals, Green synthesis, Sustainable nanotechnology, Zeta potential

Nanoparticles, typically measuring less than 100 nanometers in diameter, exhibit unique physicochemical properties due to their quantum-scale dimensions. Their large surface area to volume ratio enhances surface adsorption and catalytic activity, making them highly suitable for a variety of industrial and biomedical applications[1]. Due to their bio-persistent nature, nanoparticles are extensively explored in the medical and food sectors for targeted drug delivery, food preservation, and diagnostic applications. Nanotechnology, the science of manipulating materials at the nanoscale, has emerged as a transformative technology across diverse fields such as electronics, agriculture, medicine, wastewater treatment, biotechnology, diagnostics, nanomedicine, and nanotheranostics[2,3].

Among transition metal oxides, cobalt oxides have attracted significant interest due to their natural abundance, environmental compatibility, and versatile properties. Cobalt oxide exists in several oxidation states and crystalline forms, namely CoO, Co_2O_3, CoO_2, and Co_3O_4. Of these, Co_3O_4 and CoO are the most thermally stable and commonly studied forms[4]. In the spinel crystal structure of Co_3O_4, Co^{2+} ions occupy tetrahedral (8a) sites, while Co^{3+} ions reside in octahedral (16d) sites, contributing to its p-type semiconducting behavior and enhancing its utility in electronic and energy-related applications. Co_3O_4 nanoparticles (NPs) exhibit a wide range of potential uses, including gas sensors, magnetoresistive devices, electrodes for energy storage systems, supercapacitors, field emission devices, heterogeneous catalysis, lithium-ion batteries, electrochromic films, and solar selective absorbers[5,6]. Their biomedical relevance is equally promising, with applications in antibacterial, antifungal, antioxidant, anticancer, and enzyme inhibition activities, making them viable candidates for therapeutic and diagnostic applications[7]. Conventionally, Co_3O_4 nanoparticles are synthesized using a variety of physical and chemical techniques, including sol-gel methods, hydrothermal processes, combustion synthesis, spray pyrolysis, polyol methods, thermal decomposition, microwave-assisted synthesis, solar synthesis, sonochemical methods, laser ablation, sputtering, ion ejection, electron beam lithography, co-precipitation, reverse micelle formation, electrochemical methods, and ultrasonic

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Graphical abstract

treatments[8-10]. Despite their effectiveness, these methods are often associated with high energy consumption, expensive instrumentation, and the generation of hazardous byproducts, posing significant environmental and economic concerns[11]. To address these limitations, green synthesis has emerged as a sustainable and eco-friendly alternative. This approach utilizes biological resources such as plant extracts, which act as natural reducing and stabilizing agents.

Recent studies have successfully synthesized Co_3O_4 nanoparticles using plant-based extracts from *Piper nigrum*[12], *Vitis rotundifolia* (jumbo muscadine)[13], *Curcuma longa*[14], and *Psidium guajava* [15]. The novelty of the present study lies in the utilization of *Andrographis paniculata* leaf extract as a biological resource for the green synthesis of Co_3O_4 nanoparticles through a simple reduction–calcination approach. The synthesized nanoparticles were systematically investigated using physicochemical characterization techniques, including FTIR, XRD, SEM, DLS, and Zeta potential analysis, to establish their structural, morphological, and surface properties. Furthermore, the antimicrobial potential of the synthesized Co_3O_4 nanoparticles was evaluated against selected Gram-positive, Gram-negative, and fungal strains. The integration of plant-mediated nanoparticle synthesis with physicochemical characterization and comparative antibacterial and antifungal evaluation provides a basis for assessing the potential of the prepared Co_3O_4 nanoparticles for biological applications. We employed *Andrographis paniculata*, a medicinal plant widely distributed across South Asia (India, Pakistan, Indonesia, and Malaysia), known for its anti-inflammatory and antipyretic properties in (Table 1). The plant has been incorporated into over 26 Ayurvedic formulations and was approved by the Government of India for consumption during the COVID-19 pandemic due to its therapeutic potential [16]. Here, *A. paniculata* leaf extract was utilized to synthesize Co_3O_4 nanoparticles through a green route. The synthesized nanoparticles were characterized for their physicochemical properties and evaluated for antimicrobial efficacy.

Materials and Methods

Materials

Fresh leaves of *Andrographis paniculata* were collected from a local botanical garden and thoroughly washed with distilled water to remove surface impurities. Cobalt chloride hexahydrate [$CoCl_2.6H_2O$], used as the precursor for cobalt oxide synthesis and Sodium hydroxide (NaOH) was purchased from Isochem Laboratories (analytical

Table 1 — Scientific Details of *Andrographis paniculata*



Andrographis paniculata

Attribute	Details
Scientific Name	*Andrographis paniculata*
Family	Acanthaceae
Common Names	Green chiretta, King of Bitters, Kalmegh
Plant Type	Herbaceous plant
Geographical Distribution	South and Southeast Asia (India, Sri Lanka, Pakistan, Thailand, Malaysia)
Plant Parts Used	Leaves, stems, roots
Phytochemicals Present	Flavonoids, diterpenoids (andrographolide), alkaloids, polyphenols
Major Bioactive Compound	Andrographolide
Medicinal Properties	Antibacterial, antiviral, anti-inflammatory, antioxidant, hepatoprotective
Therapeutic Applications	Treatment of fever, cold, infections, liver disorders, COVID-19 support

grade) and used without further purification. All reagents and solvents were of analytical grade, and deionized water was used throughout the experiments.

Preparation of *Andrographis paniculata* leaf extract and green synthesis of Co_3O_4 nanoparticles

Fresh *Andrographis paniculata* leaves were collected and thoroughly washed with distilled water to remove surface impurities. Approximately 20 g of the fresh leaves were air-dried at room temperature and finely chopped. The chopped leaves were boiled in 100 mL of deionized water for 6 h. After cooling to room temperature, the extract was filtered using Whatman No. 1 filter paper to obtain a clear green leaf extract. The prepared extract was stored at 4°C until further use.

For the green synthesis of cobalt oxide nanoparticles, 20 mL of the prepared *A. paniculata* leaf extract was mixed with 20 mL of 1 M cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) solution. The pH of the reaction mixture was adjusted to 8.5 using 2 M sodium hydroxide (NaOH) solution. The resulting mixture was stirred for 2 h and subsequently allowed to cool overnight. The cooled reaction mixture was filtered using Whatman filter paper, and the obtained filtered material was calcined at 300°C for 2 h in a muffle furnace. After calcination, the product was ground using a mortar and pestle to obtain fine Co_3O_4 nanoparticles. A schematic representation of the synthesis process is presented in (Fig. 1).

***In vitro* Characterization**

To identify the functional bio-molecules associated with cobalt oxide nanoparticle FTIR (PerkinElmer Spectrum version 10.4.2) analysis was done in the spectrum of 500-4000 cm^{-1}. Material crystallinity and structure phase were evaluated using XRD analysis, using Cu Kα radiation. The structural morphology was observed using SEM (Scanning Electron microscopy). Particle size analysis is done to identify particle size of synthesized nanoparticle.

Antibacterial activity

The anti-bacterial activity of Co_3O_4 nanoparticle was examined two different types of bacterial species: (i) Gram positive (*S. aureus*) (ii) Gram negative (*E. coli*) through the well diffusion method. Nutrient agar medium is sterilized and was poured to petriplates. Diameter of 6 mm well were created then filled with the Co_3O_4 nanoparticle of various concentration from 20 µL to 80 µL and kept for incubation at 37°C for 24 h. Inhibition zone was measured by using standard (Hi-Media) scale.

Antifungal activity

Potato Dextrose agar was used for subculturing *Candida albicans* for four to five days at 25°C. After the growth of the organism's streak plate was done with swab dipped with inoculum suspension. The lid was kept open for 3 min in laminar hood to remove excess moisture content and drug impregnated disk

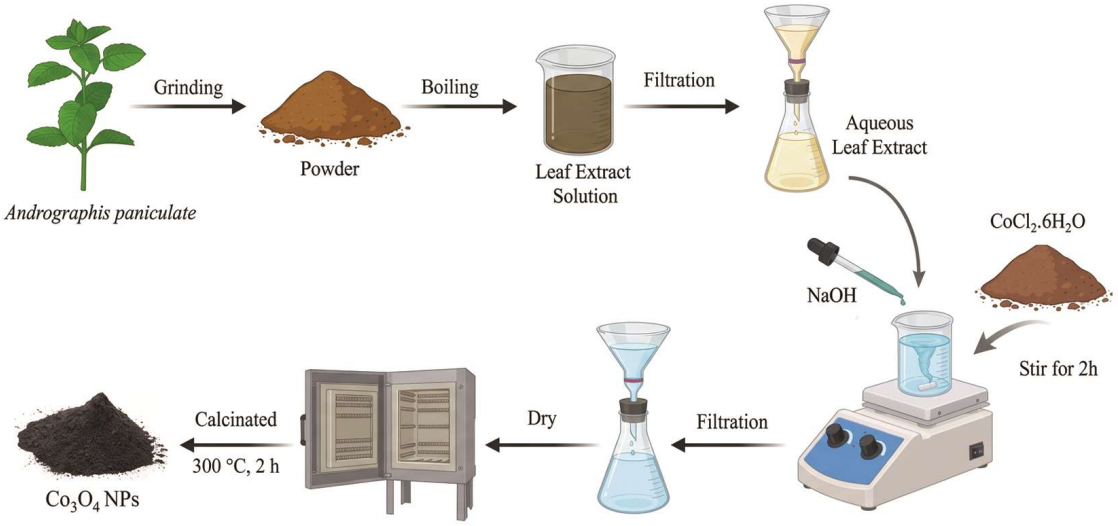


Fig. 1 — Schematic representation of synthesized cobalt oxide nanoparticles using green synthesis method

were placed on incubated plates. Well diffusion assay was done and samples were added in each well at different concentration and kept in incubation at 30°C for 4 to 7 days. Inhibition zone growth was observed which are measured in millimeters.

Result and Discussion

FTIR Analysis

FTIR spectroscopy was used to identify the functional groups present in *A. paniculata*-mediated Co_3O_4 nanoparticles, as shown in (Fig. 2). The extract displayed characteristic bands at 3414, 2485, 1777, 1073, and 844 cm^{-1}, suggesting the presence of primary and secondary amines, alkenes, aromatic moieties, aliphatic amines, and alkyl halides, respectively. The broad absorption band observed at 3414 cm^{-1} is assigned to O-H stretching vibrations originating from hydroxyl-containing phytochemicals, including phenolic compounds, and possibly hydrogen-bonded or adsorbed water molecules. This band suggests the possible presence of phytochemical residues associated with the nanoparticle surface; however, its assignment should be considered tentative because FTIR analysis alone cannot confirm a specific compound such as andrographolide. Following nanoparticle synthesis, two distinct bands were observed at 510 and 691 cm^{-1}, which confirm the formation of cobalt oxide nanoparticles. The band at 510 cm^{-1} is attributed to Co-O stretching, whereas the band at 691 cm^{-1} is assigned to Co(III)-O vibration. These phytochemical constituents, especially amine and carboxyl groups, are believed to have played a key role in the reduction, capping, and stabilization of

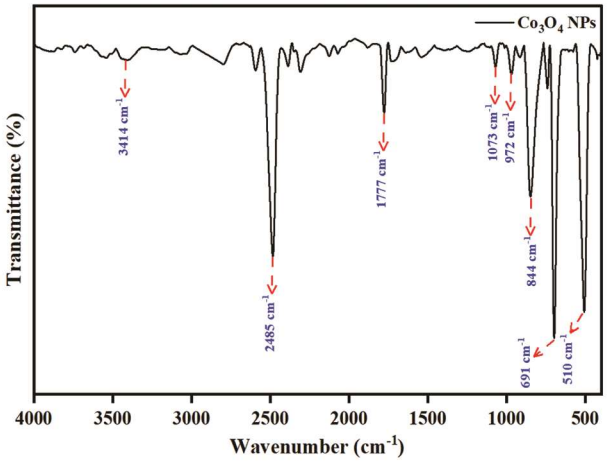


Fig. 2 — FTIR spectra of cobalt oxide nanoparticles

the Co_3O_4 nanoparticles. Additional weak bands at 972, 844 cm^{-1} further support the presence of alkene, alkane, amide, alcohol/carboxylic acid, and aliphatic amine groups derived from *A. paniculata*[17].

The involvement of phytochemicals in the formation and stabilization of Co_3O_4 nanoparticles was proposed based on the FTIR results of the *A. paniculata* leaf extract and the synthesized nanoparticles. The extract exhibited characteristic absorption bands associated with amine, aromatic, and other organic functional groups. After nanoparticle formation, selected organic-related bands remained in the FTIR spectrum, suggesting the possible adsorption or association of plant-derived biomolecules with the nanoparticle surface. These functional groups may contribute to the complexation of cobalt ions, nucleation, and surface stabilization

during synthesis. Andrographolide and other phytochemicals present in *A. paniculata* may participate in these processes.

XRD analysis

The crystalline structure of the synthesized Co_3O_4 nanoparticles was investigated using X-ray diffraction (XRD). The diffraction pattern was compared with the standard reference pattern of cubic spinel Co_3O_4 (JCPDS No. 042-1467). The diffraction peaks observed at 21.72°, 31.2°, 36.8°, 38.5°,44.8°, 59.617°, and 65.473° were indexed to the (111), (220), (311), (222), (400), (511), and (440) crystallographic planes, respectively. The experimental diffraction peaks were evaluated against the reference pattern to confirm the formation of the Co_3O_4 phase. The presence or absence of secondary phases was assessed based on the observed diffraction profile. The crystallographic assignments and peak positions were checked for consistency with the reference standard[15].

The mean crystallite size of the synthesized Co_3O_4 nanoparticles was estimated using the Debye-Scherrer eq.1:

$$D = \frac{k\lambda}{\beta \cos\theta} \quad \ldots (1)$$

where D is the mean crystallite size (nm), K is the dimensionless shape factor (0.98), λ is the wavelength of the Cu Kα radiation (0.15406 nm), β is the full width at half maximum (FWHM) of the selected diffraction peak expressed in radians, and θ is the Bragg diffraction angle. The crystallite size was calculated using the diffraction peak at 2θ = 311°, with an FWHM value of 0.8572, yielding an average crystallite size of approximately 54 nm.

SEM Analysis

Figure 3(a–d) presents the SEM micrographs of the *A. paniculata*-mediated Co_3O_4 nanoparticles. The micrographs reveal a highly agglomerated and clustered morphology consisting of irregular, quasi-spherical particles. The particles are unevenly distributed and appear predominantly as compact aggregates rather than completely isolated individual nanoparticles. Therefore, accurate determination of the primary particle size from the SEM images is limited by the pronounced agglomeration and overlapping particle boundaries.

The observed SEM morphology indicates the formation of nanostructured cobalt oxide, with apparent particle dimensions generally below

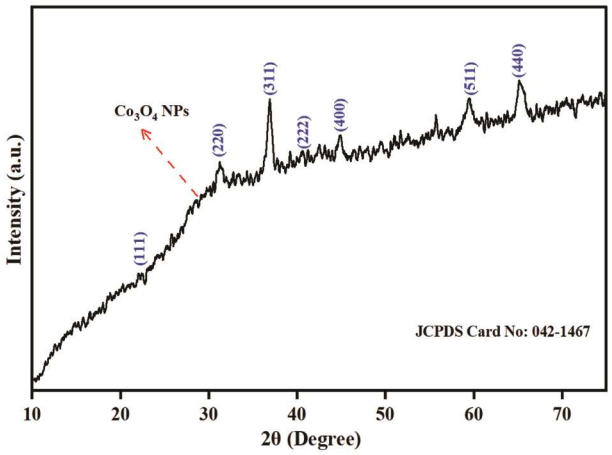


Fig. 3 — XRD patteern of cobalt oxide nanoparticles

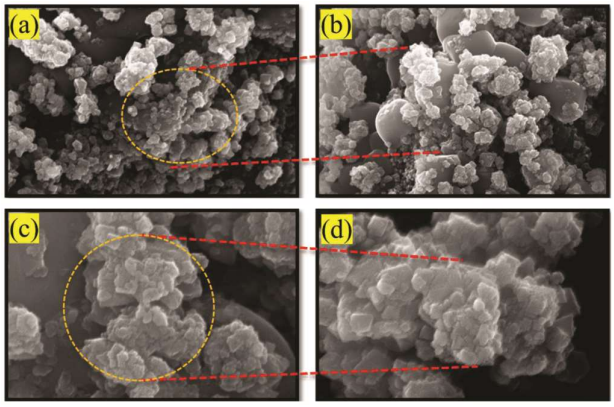


Fig. 4 — (a-d) SEM morphological images of cobalt oxide nanoparticles

approximately 60 nm in distinguishable regions. This value should be considered an approximate visual observation and not a statistically determined average particle diameter. The crystallite size calculated from XRD and the hydrodynamic diameter obtained from DLS are reported independently because they represent different physical parameters. The agglomeration may be associated with interparticle interactions and thermal treatment during calcination, while phytochemicals present in the plant extract may have contributed to partial surface stabilization.

Overall, Figure 4 highlights the formation of nanostructured cobalt oxide with a rough surface texture, non-uniform shape, and dense aggregation, features that may influence the material's catalytic and surface-active properties[15,18].

Dynamic light scattering and Zeta potential

The particle size distribution and colloidal stability of the *A. paniculata*-derived Co_3O_4 nanoparticles were

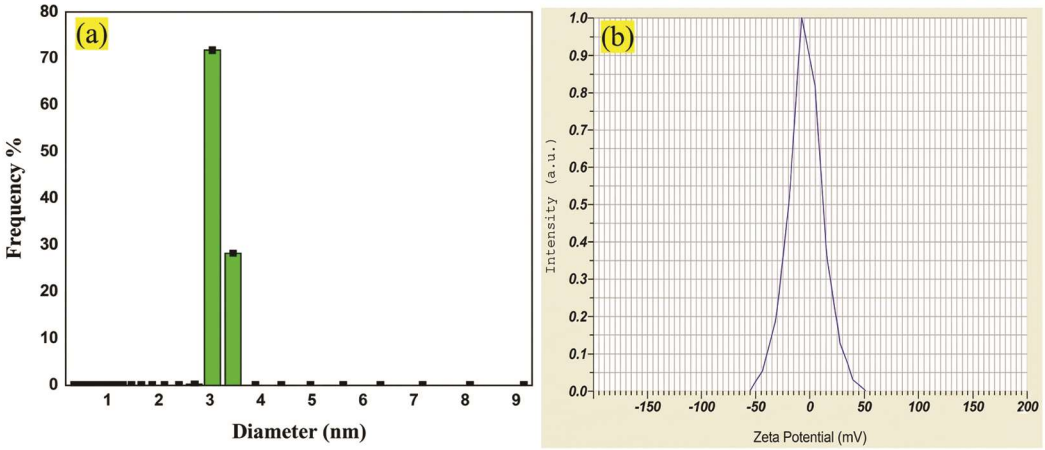


Fig. 5 — (a) particle size distribution; and (b) dynamic light scattering of cobalt oxide nanoparticles

evaluated by dynamic light scattering (DLS), as shown in (Fig. 5a & b).

The DLS analysis indicated an average hydrodynamic particle diameter of approximately 3 nm, suggesting the formation of extremely fine nanosized particles with a narrow size distribution. The zeta potential measurement exhibited a peak at −3.9 mV, indicating a weak negative surface charge on the Co_3O_4 nanoparticle surface. Although this surface charge may provide limited electrostatic repulsion between adjacent particles, the low absolute zeta potential suggests weak electrostatic stabilization rather than strong colloidal stability. Generally, zeta potential values exceedingly approximately +25 mV or lower than −25 mV are commonly associated with enhanced electrostatic stability; however, the stability of nanoparticle dispersions also depends on particle concentration, pH, ionic strength, solvent composition, and the presence of surface-bound phytochemicals. Therefore, the measured −3.9 mV value is interpreted as indicating weak electrostatic stabilization and a tendency toward partial aggregation, which agrees with the agglomerated morphology observed in the SEM analysis[19,20].

The particle-size results obtained from XRD, SEM, and DLS represent different measurement parameters. The average crystallite size calculated from XRD was approximately 54 nm, which corresponds to the coherent crystalline domains within the material. SEM analysis revealed irregular, quasi-spherical, and highly agglomerated structures, with the observed morphological dimensions estimated to be below 60 nm. In contrast, DLS analysis indicated a hydrodynamic diameter of approximately 3 nm under the measurement conditions. The hydrodynamic diameter is associated with the behavior of dispersed species in the liquid medium and is influenced by sample preparation and dispersion characteristics.

The substantial difference between the DLS value and the sizes estimated from XRD and SEM requires cautious interpretation. Since the DLS value is considerably lower than the crystallite size obtained from XRD, it should be verified against the original DLS measurement data, distribution profile, and experimental conditions. Therefore, the reported values are presented as technique-specific measurements rather than direct evidence of a single uniform nanoparticle size.

Biological Activity

Antibacterial activity

A.paniculata contains phytochemicals such as alkaloids, trepenoids, flavonoids[16] which are potential as antibacterial, antiviral, anti-cancer reagent[21]. *A.paniculata* derived Co_3O_4 NP antibacterial activity is tested on *S. aureus* and *E. coli* which are gram-positive and gram-negative bacteria, respectively, by well diffusion method show in (Fig. 6a & b). As the concentration of the nanoparticle increases the zone of inhibition is found to be increasing. Ethanol is used as the negative control and streptomycin is used as the standard.

The zone of inhibition in *S. aureus* is 7 mm, 10 mm, 15 mm and 16 mm for the 20 µL, 40 µL, 60 µL and 80 µL of sample, respectively. The zone of inhibition in *E. coli* is 14 mm, 17 mm, 18 mm and 20 mm for the 20 µL, 40 µL, 60 µL, and 80 µL of the sample, respectively, in (Fig. 7). Usually, green synthesis of Co_3O_4 NP has higher efficiency towards

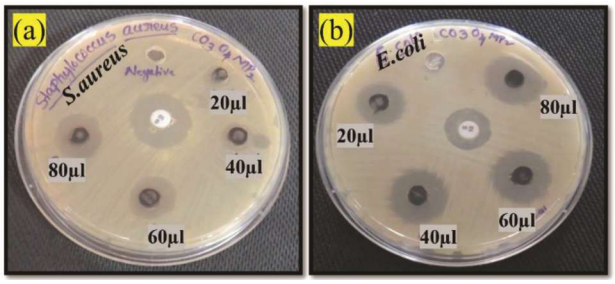


Fig. 6 — Anti-bacterial activity of Co_3O_4 NPs with various concentration against (a) *S. aureus*; and (b) *E. coli*

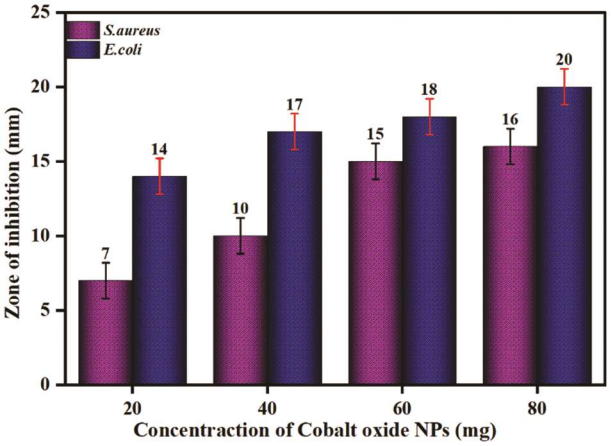


Fig. 7 — Anti-bacterial activity of Co_3O_4 NPs against *S. aureus* and *E. coli*

gram positive bacteria compared to gram negative bacteria as in previous literature works[14,15]. In *A. paniculata* derived Co_3O_4 NP shows higher sensitivity towards the gram-negative bacteria as *A. paniculata* antibacterial activity is high in gram negative bacteria in (Table 2)[21,22].

Antifungal activity

A. paniculata contains several bioactive phytochemicals with pronounced antifungal potential. Of these, 3-O-β-D-glycosyl-14-deoxyandrographiside, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide are considered important active constituents, as they have demonstrated notable efficacy against a range of fungal pathogens (Table 3)[23].

Ethanol is used as negative control and Amphotericin is used as positive control. At 20 µL and 40 µL of *A.paniculata* derived Co_3O_4 NPs zone of inhibition was not observed at 60 µL and 80 µL concentration of NP zone of inhibition was 12 mm and 16 mm, respectively, which increases as the concentration increases in shown in (Fig. 8). Surface area of the particle with small size influences the microbial growth inhibition. The

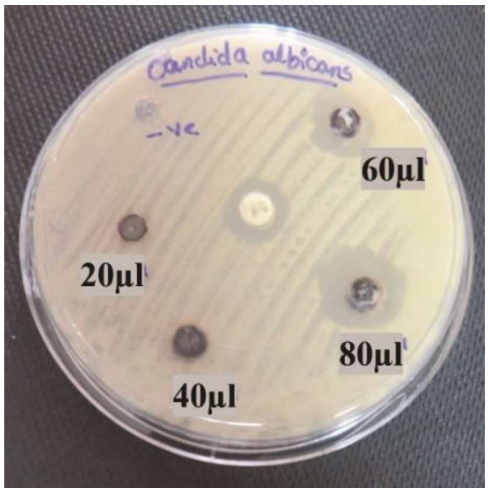


Fig. 8 — Antifungal activity of activity of Co_3O_4 nanoparticles of *Candida albicans*

Table 2 — Anti-bacterial activity details of Co_3O_4 NPs and their zone of inhibition

Sample	Zone of Inhibition (mm)	
	S. aureus	*E. coli*
Standard: Streptomycin	19 mm	15 mm
Negative Control: Ethanol	0 mm	0 mm
20 µL	7 mm	14 mm
40 µL	10 mm	17 mm
60 µL	15 mm	18 mm
80 µL	16 mm	20 mm

Table 3 — Antifungal activity of *Candida albicans* and its zone of inhibition

Sample	Inhibition Zone diameter (mm)
	Candida albicans
Positive control- Amphotericin	12 mm
Negative control- Ethanol	0 mm
20 µL	0 mm
40 µL	0 mm
60 µL	12 mm
80 µL	16 mm

small sized NPs encourage the penetration of microbial cell wall, cytoplasmic membrane rupture and leads to the microbial death in (Fig. 9). NPs show antimicrobial activity which depends upon the particle size[24].

Comparative analysis

The comparative analysis demonstrates that the antimicrobial activity of plant-mediated nanoparticles depends on several factors, including the plant extract composition, nanoparticle synthesis conditions,

concentration, particle characteristics, and microbial species. The present *A. paniculata*-mediated Co_3O_4 nanoparticles showed inhibition zones of 16 and 20 mm against *S. aureus* and *E. coli*, respectively, at 100 µg/mL.

The results confirm the antimicrobial potential of the synthesized nanoparticles and provide a comparative reference with previously reported plant-mediated nanoparticle systems in (Table 4).

The synthesized Co_3O_4 nanoparticles demonstrated concentration-dependent antimicrobial activity. The maximum inhibition zones were 16 mm against *Staphylococcus aureus*, 20 mm against *Escherichia coli*, and 16 mm against *Candida albicans* under the experimental conditions used in this study. The higher inhibition zone observed for *E. coli* compared with *S. aureus* indicates a difference in the response of the tested microorganisms; however, this observation should not be generalized to all Gram-negative or Gram-positive bacteria because antimicrobial activity can be influenced by the microbial strain, nanoparticle concentration, particle characteristics, and assay conditions.

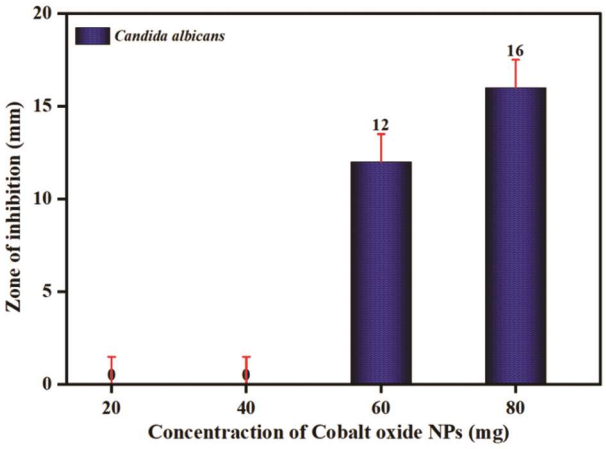


Fig. 9 — Antifungal activity of Co_3O_4 nanoparticles of *Candida albicans*

The significance of the present work lies in the integration of an aqueous plant-extract-mediated synthesis route with structural, morphological, particle-size, and surface-charge characterization, followed by antibacterial and antifungal evaluation. The use of a reduction–calcination process at 300 °C provides a straightforward preparation route, while the observed concentration-dependent antimicrobial response supports the potential of the synthesized nanoparticles for further investigation in antimicrobial material applications. Nevertheless, additional studies involving quantitative phytochemical analysis, detailed mechanistic investigations, cytotoxicity assessment, reproducibility studies, and evaluation under application-specific conditions are necessary before practical biomedical or antimicrobial applications can be established.

Conclusion

In the present study, Co_3O_4 nanoparticles were synthesized using *Andrographis paniculata* leaf extract through a green reduction–calcination

Table 4 — Comparative analysis of antimicrobial activity for plant-mediated metal oxide nanoparticles

S. No	Source	Organism	Nanoparticle Conc.	Zone of Inhibition (mm)	Ref.
1.	*Andrographis paniculata*	*S. aureus*	80 µg/mL	16	This study
		E. coli		20	
2.	*Curcuma longa*	*S. aureus*	100 µg/mL	14	14
		E. coli		11	
3.	*Ziziphus Oxyphylla Edgew*	*E. coli*	16 mg/mL	23.1	25
		S. aureus		14.8	
4.	*Psidium guajava*	*E. coli*	200 µg/mL	18	26
				15	
5.	*Allium sativum and Allium cepa*	*E. coli*	200 µg/mL	21.31	27
		Proteus		20.13	
		S. aureus		22.12	
		S. cohnii		23.23	
		K. pneumonia		21.66	
6.	*Phytolacca dodecandra*	*E. coli*	50 µg/mL	12.5	11
		S. aureus		11.6	
7.	*Croton macrostachyus*	*S. aureus*	20 mg/mL	12.33	28

**Escherichia coli- E. coli; Klebsiella pneumoniae- K. pneumonia; Proteus- Proteus species; Staphylococcus aureus- S. aureus; Staphylococcus cohnii- S. cohnii*

approach. The synthesized nanoparticles were characterized using FTIR, XRD, SEM, DLS, and zeta potential analysis to investigate their structural, morphological, particle-size, and surface-charge properties. The XRD analysis confirmed the crystalline nature of the synthesized cobalt oxide nanoparticles, while SEM revealed an agglomerated morphology. FTIR analysis indicated the presence of metal-oxygen vibrations and organic functional groups that may be associated with phytochemical components from the plant extract. The synthesized Co_3O_4 nanoparticles exhibited concentration-dependent antimicrobial activity against the tested microorganisms. The maximum inhibition zones were 20 mm against *Escherichia coli*, 16 mm against *Staphylococcus aureus*, and 16 mm against *Candida albicans* under the experimental conditions used in this study. These results indicate the potential antimicrobial activity of the synthesized nanoparticles. However, further investigations involving quantitative phytochemical analysis, antimicrobial mechanism studies, cytotoxicity evaluation, and reproducibility assessment are required to establish their suitability for practical biomedical and antimicrobial applications. Overall, the present study demonstrates the feasibility of using *A. paniculata* leaf extract for the green synthesis of Co_3O_4 nanoparticles and provides preliminary evidence of their antimicrobial potential. The findings contribute to the development of plant-mediated cobalt oxide nanoparticle synthesis and provide a basis for future investigations into their application in antimicrobial materials.

Acknowledgement

The authors have no acknowledgements to declare.

Conflicts of interest

All authors declare no conflicts of interest.

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