

**Synthesis and Characterization of Cobalt Nanoparticles Using Neem (*Azadirachta indica*)****Leaf Extract and Evaluation of Its Antibacterial Efficacy**

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**ABSTRACT**

The study presented the green synthesis of cobalt nanoparticles (Co-NPs) using aqueous leaf extract of *Azadirachta indica* (neem) and the evaluation of their antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli*. Ten grams of dried neem leaf powder were boiled in 100 mL of distilled water to obtain the extract, which was used as both a reducing and stabilizing agent. A 0.032 mol/dm<sup>3</sup> cobalt chloride solution was prepared and reacted with the extract in a 3:1 ratio under continuous stirring at room temperature for one hour, resulting in a visible colour change from wine red to dark brown, confirming nanoparticle formation. Characterization was performed using UV-visible (UV-Vis) spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD). The UV-vis analysis showed a characteristic surface Plasmon resonance (SPR) peak at 318 nm, indicating successful nanoparticle formation. FTIR revealed active functional groups (–OH, –NH, C=O) involved in reduction and stabilization, while XRD confirmed crystalline face-centered cubic (FCC) cobalt with an average crystalline size of 13 nm. Antibacterial activity assessed via agar well diffusion revealed concentration-dependent inhibition zones, with maximum activity against *S. aureus* (22 ± 0.6 mm) and moderate activity against *E. coli* (17 ± 0.6 mm) at 100 µg/mL. The findings validate neem as an effective biogenic medium for eco-friendly synthesis of cobalt nanoparticles with promising antibacterial potential.

**Keywords:** Antibacterial activity, Cobalt nanoparticles, FTIR, green synthesis, neem extract, XRD.

## INTRODUCTION

Nanotechnology has emerged as a promising field in biomedical research for developing antimicrobial agents against drug-resistant pathogens. Among various metal nanoparticles, Co-NPs exhibit excellent magnetic and catalytic properties, making them suitable for drug delivery, imaging, and antibacterial applications [1]. Green synthesis offers an eco-friendly alternative to chemical synthesis by utilizing plant phytochemicals as reducing and stabilizing agents. *Azadirachta indica*, a plant native to the Indian subcontinent, is known for its medicinal and antimicrobial properties, attributed to its rich phytochemical composition of flavonoids, alkaloids, and terpenoids [2]. Neem extract acts as both a reducing and stabilizing agent during nanoparticle synthesis, minimizing environmental impact and improving biocompatibility [3]

Nano-chemistry a subfield of nanoscience, focuses on synthesizing and applying nanoscale materials. Metal-based nanoparticles, particularly those of cobalt, silver, and copper, have demonstrated antimicrobial activity through mechanisms like reactive oxygen species (ROS) generation and cell wall disruption [4, 5]. Green synthesis using neem extract has been successfully employed for producing metal nanoparticles, attributed to phytochemical-mediated reduction and stabilization [6, 7]. Cobalt nanoparticles synthesized this way have demonstrated activity against both gram-positive and gram-negative bacteria [8].

Nanoparticles are materials with at least one dimension in the range of 1–100 nm [9]. They exhibit unique properties such as high surface area-to-volume ratio, quantum confinement effects, and enhanced reactivity compared to bulk materials [9]. These properties have made nanoparticles valuable in fields such as catalysis, drug delivery, and antimicrobial treatments. The increasing resistance of pathogenic bacteria to conventional antibiotics is a pressing global concern. Traditional chemical synthesis of nanoparticles often involves toxic reagents and environmentally unfriendly, limiting their biomedical application. Utilizing neem leaf extract for nanoparticle synthesis addresses these challenges by offering a green, sustainable method that aligns with the principles of sustainability and opens avenues to develop biocompatible antibacterial agents.

Although several studies have reported the green synthesis of metal nanoparticles using plant extracts, including *Azadirachta indica* (neem), most of these investigations have centered on silver, gold, or copper nanoparticles, while research on cobalt nanoparticles (Co-NPs) remained limited. Furthermore, existing syntheses often relied on elevated temperatures and long reaction times,

which contradicted the principles of green chemistry. The mechanistic role of specific neem phytochemicals in reducing and stabilizing cobalt ions has not been clearly elucidated, and there is an absence of detailed spectral evidence linking these functional groups to nanoparticle formation and crystallinity. Similarly, few studies have established a clear correlation between the structural features of neem-mediated Co-NPs and their antibacterial efficacy against both Gram-positive and Gram-negative bacteria.

This research seeks to bridge these gaps by developing a simple, low-temperature and one-hour eco-friendly green synthesis route of cobalt nanoparticles using *Azadirachta indica* leaf extract as both a reducing and stabilizing agent at ambient conditions, avoiding toxic reagents and energy-intensive processes. The study further provides detailed spectroscopic and diffraction analyses (UV–Vis, FTIR, and XRD) to identify the phytochemical groups involved in nanoparticle formation and to relate the structural characteristics of the synthesized Co-NPs to their antibacterial performance, thereby contributing to the understanding and advancement of eco-friendly nanomaterial synthesis for biomedical applications.

This study aims to synthesize and characterize cobalt nanoparticles using neem leaf extract as a reducing and stabilizing agent and to evaluate its antibacterial efficacy of the synthesized Co-NPs against *Staphylococcus aureus* and *Escherichia coli*.

## **MATERIALS AND METHOD**

### ***Materials***

Fresh neem (*Azadirachta indica*) leaves were collected from Orji Housing Estate Gombe, Gombe state, Nigeria and identified at the Department of Plant Science and Ecology by a Botanist in Gombe State University, Nigeria. Cobalt (II) chloride (Sigma-Aldrich, UK), distilled water, Mueller–Hinton agar (MHA), ethanol (Merck, Germany) and all other reagents used in this study were of analytical grade and were used without further purification.

### **Green Synthesis of Cobalt Nanoparticles**

Freshly neem leaves were cleaned, air-dried for 6 days, and ground into powder. About 10 grams of dried neem leaf powder were boiled in 100 mL distilled water for 1 hour at 80 °C, filtered, and stored at 4 °C. A 0.032 mol/dm<sup>3</sup> cobalt chloride solution was prepared by dissolving 4.2 g CoCl<sub>2</sub> in 50 mL distilled water. The synthesis involved the dropwise addition of 10 mL neem leaf extract

into 30 mL cobalt chloride solution under magnetic stirring for one hour at room temperature. The mixture's colour changed from wine red to dark brown, indicating nanoparticle formation. The resulting product was centrifuged (Thermo-Electron corporation Germany, Labofuge 300) at 3000 rpm for 10 minutes, washed with ethanol and distilled water, and air-dried to obtain fine Co-NP powder and this dried powder was used for further characterization [10].

### **Characterization**

The presence of the synthesized cobalt nanoparticles was confirmed by sampling the aqueous component using a UV-visible spectrophotometer (PEC Medical, 752N UV-Vis spectrophotometer). Absorption spectra were recorded in the range of 300–800 nm at a scan interval of 5 nm using distilled water as a blank to observe surface Plasmon resonance (SPR) bands. The Functional groups responsible for the reduction and stabilization of nanoparticles were identified using Fourier Transform Infrared (FTIR) spectroscopy (Agilent Cary 630 FTIR). The Crystalline size of the prepared Co-NPs was determined using X-ray diffraction (Rigaku Corporation Japan, Rigaku MiniFlex Benchtop X-ray diffractometer, Cu K $\alpha$ ,  $\lambda = 1.5406 \text{ \AA}$ ).

### **Antibacterial Efficacy**

Antibacterial efficacy was tested using the agar well diffusion method against *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). Co-NPs were tested at four different concentrations of 25%, 50%, 75%, and 100%. Ciprofloxacin served as the positive control. Zones of inhibition were measured after 24 hours' incubation at 37 °C.

## **RESULTS AND DISCUSSION**

UV-visible spectroscopy was employed to ascertaining the formation and stability of metal nanoparticles in aqueous solution. Metallic nanoparticles display characteristic optical absorption spectra in the UV-visible region called SPR which is due to the physical absorption of light by metallic nanoparticles. The visual stages in the synthesis of Co-NPs using neem leaf extract is that, the neem leaf extract exhibited a characteristic brown colour due to the presence of various phytochemicals such as flavonoids, terpenoids, and polyphenols, which act as reducing and stabilizing agents [10].

The wine red colour of the aqueous 0.05 mol/dm<sup>3</sup> cobalt chloride (CoCl<sub>2</sub>) solution, indicated the presence of Co<sup>2+</sup> ions. Upon the addition of 10 mL of the neem extract to the cobalt chloride solution with continuous stirring for one hour, the mixture turned dark brown. This colour change indicates the reduction of Co<sup>2+</sup> ions to zero-valent cobalt nanoparticles, confirming nanoparticle formation [11]. This observation aligns closely with earlier studies by Prabhu *et al* [8], who reported a similar colour shift as an initial visual marker for Co-NPs formation using *Azadirachta indica* extract. Colour transition as a primary qualitative indicator is consistently reported in green synthesis studies using neem [7, 8]. This study used a lower temperature (room temp) and shorter reaction time (1 hour) compared to higher thermal-assisted protocols (60–90 °C for 2–3 h) in other works reported by Bhattacharya and Raj [6].

The synthesis of metal nanoparticles using neem leaf extract involves the reduction of metal salts through phytochemicals present in the extract, leading to the formation of nanoparticles. This green synthesis method was employed to reduce cobalt chloride to its metal nanoparticles. The formation and confirmation of these nanoparticles were carried out using UV-visible spectroscopy, which detects the surface Plasmon resonance, a characteristic optical property of metal nanoparticles due to the collective oscillation of electrons. The reduction of Co<sup>2+</sup> ions was confirmed by a colour change from wine red to dark brown, signifying the formation of Co-NPs. The UV-visible spectrum (Figure 1) showed a distinct absorption peak at 318 nm corresponding to surface Plasmon resonance, confirming nanoparticle formation.

This observation agrees with previous studies reporting SPR peaks between 300–330 nm for Co-NPs [8–12]. Also previous works [3, 8] report Co-NP SPR peaks between 300–330 nm, reinforcing the result obtained in this study, [4], noted slight red-shifted SPR bands (~330–350 nm), possibly due to aggregation or particle size variation from different synthesis protocols. Figure 2 is the FTIR spectrum of the synthesized Co-NPs using neem leaf extract. The FTIR spectrum of the neem leaf extract exhibited prominent absorption bands at 3257.7 cm<sup>-1</sup> and 1636.3 cm<sup>-1</sup>.

The band at 3257.7 cm<sup>-1</sup> corresponds to the stretching vibrations of –OH or –NH functional groups, commonly associated with alcohols, phenols, or amines. The 1636.3 cm<sup>-1</sup> band is typically attributed to C=O stretching (carbonyl group) or C=C stretching in aromatic compounds.

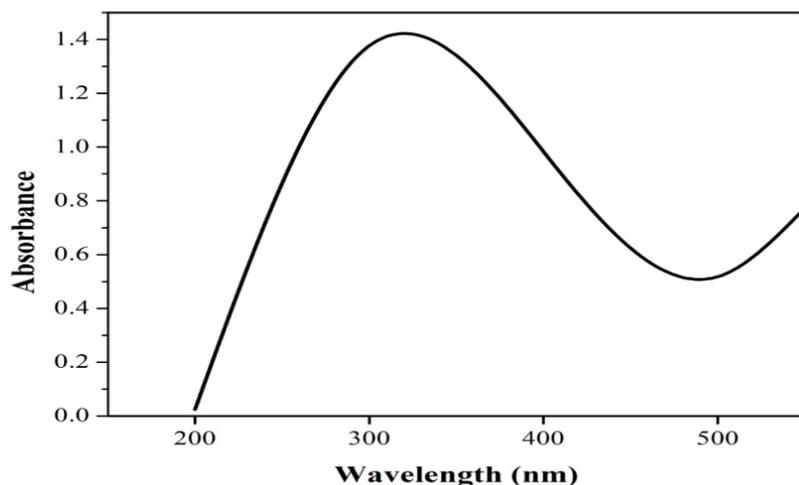


Figure 1: UV-visible absorption spectrum of Co-NPs

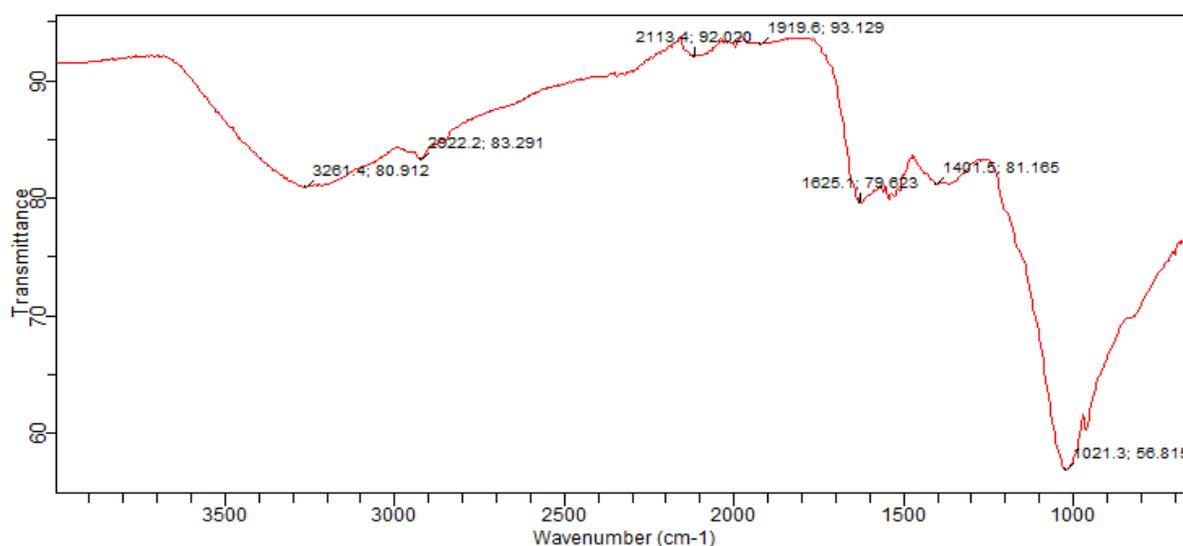


Figure 2: FTIR Analysis of Co-NPs

These functional groups are indicative of bioactive phytochemicals such as flavonoids, terpenoids, or polyphenols present in the neem extract. Upon analysis of the cobalt nanoparticles, the IR spectrum in Figure 2 revealed peaks at 3261.4, 2922.2, 2113.4, 1919.6, 1625.1, 1401.5, and 1021.3 cm<sup>-1</sup>. Notably, the bands observed at 3257.7 and 1636.3 cm<sup>-1</sup> in the neem extract were absent in

the cobalt nanoparticles, suggesting the functional groups corresponding to these bands were involved in the reduction and stabilization of the nanoparticles [13,14]. These biomolecules may have acted as reducing agents, converting  $\text{Co}^{2+}$  ions into  $\text{Co}^0$  nanoparticles, and possibly capping/stabilizing them post-synthesis. These shifts are consistent with findings from [2,6], where phenolics, flavonoids, and terpenoids participate in nanoparticle formation. The FTIR profile of study showed additional peaks at peaks at  $2113.4$  and  $1401.5 \text{ cm}^{-1}$  indicate unique stabilization interactions [14].

The X-ray diffraction analysis was conducted to evaluate the crystalline structure of the synthesized nanoparticles. The XRD pattern in Figure 3 revealed intense diffraction peaks at  $2\theta$  values of  $21.03^\circ$ ,  $26.85^\circ$ ,  $27.84^\circ$ ,  $29.84^\circ$ , and  $43.86^\circ$ , corresponding to the (100), (110), (200), (210), and (111) planes of face-centered cubic (FCC) cobalt. The sharp and narrow peaks indicated high crystallinity. The average crystallite size, calculated using Scherrer's equation, was approximately 13 nm. Minor peaks at  $26.85^\circ$ ,  $27.84^\circ$ , and  $29.84^\circ$  possibly due to cobalt oxide phases and it is a common feature in biosynthesized metal nanoparticles [15,16].

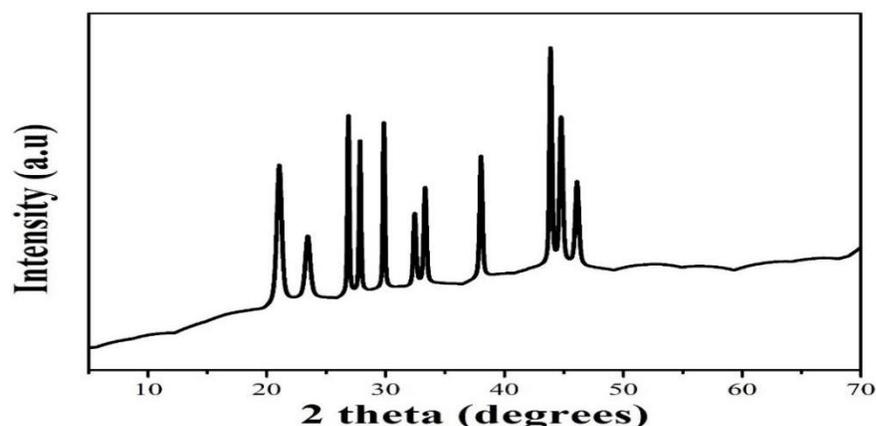


Figure 3: Powdered XRD Patterns of the synthesized Co-NPS

The XRD data presented in Table 1, revealed distinct peaks at  $43.86^\circ$  ( $2\theta$ ), matching the (111) plane of face-centered cubic (FCC) cobalt.

Table 1: XRD Peak Data for Co-NPs

2θ (°)	d-spacing (Å)	Rel. Intensity (%)	FWHM (°)
21.03	4.224	53.00	0.4723
26.85	3.321	76.95	0.1574
27.84	3.205	65.80	0.1574
29.84	2.994	73.20	0.1574
43.86	2.064	100.00 (max)	0.1968

This matches well with reported crystallographic data for biosynthesized cobalt nanoparticles as previously reported [1,12]. The crystallite size (~13 nm) is comparable to sizes reported where average particle sizes ranged from 10–20 nm depending on synthesis parameters [5,8]. The partial oxidation is a phenomenon commonly reported in green synthesis when ambient oxygen interacts with metal precursors during or after synthesis [7].

The agar well diffusion method was used to clinically assess the antibacterial activity of synthesized nanoparticles (Co-NPs) [17]. Anti-bacterial effect of Co-NPs was visualized against *Staphylococcus aureus* and *Escherichia coli* while Ciprofloxacin was used as a control. The results of the study are presented in Table 2. The nanoparticles showed antibacterial effect in a dose-dependent manner. From Table 2, the antibacterial activity of Co-NPs demonstrated a dose-dependent effect against both bacterial strains tested. The inhibition zones at 100 µg/mL were significantly higher than those at 25 µg/mL and 50 µg/mL. Against *S. aureus*, the maximum zone of inhibition was 22 ± 0.6 mm at 100 µg/mL, which approaches the CLSI/EUCAST [16,18] clinical breakpoint for ciprofloxacin (≥ 21 mm).

Table 2: Antibacterial Activity of Synthesized Co-NPs (ZOI in mm)

Nanoparticles (µg/mL)	<i>Staphylococcus aureus</i> (Gram +)	<i>Escherichia coli</i> (Gram -)
Co-NPs 25	0.0 ± 0.0	9.0 ± 0.6
50	17.0 ± 0.6	10.0 ± 0.6
75	19.0 ± 0.6	15.0 ± 0.6
100	22.0 ± 0.6	17.0 ± 0.6

The highest inhibition against *E. coli* was  $17 \pm 0.6$  mm, which falls below the ciprofloxacin standard, reflecting lower efficacy, which indicate the synthesized Co-NPs are more effective against gram-positive bacteria, which is in consistent with previous reports [5,7]. Compared to report by Prabhu *et al* [11], who observed inhibition zones of  $\sim 18$  mm against *S. aureus* at similar concentrations, the results of the study presented in Table 2 recorded a larger inhibition zone (22 mm), possibly due to smaller particle size or enhanced reactivity from neem-mediated synthesis. The reduced sensitivity of *E. coli* may be attributed to its outer lipopolysaccharide layer, which restricts nanoparticle penetration as reported by Raghunandan *et al* [15]. These findings indicate that Co-NPs synthesized via neem extract have clinical relevance, especially against Gram-positive pathogens, and may serve as a potential alternative to conventional antibiotics in treating multidrug-resistant bacterial infections [6,19].

## CONCLUSION

Cobalt nanoparticles were successfully synthesized using neem leaf extract as an eco-friendly reducing and stabilizing agent. Characterization by UV-vis, FTIR, and XRD confirmed nanoparticle formation, functional group involvement, and crystalline FCC structure. The Co-NPs exhibited significant antibacterial activity, particularly against *S. aureus*, demonstrating their potential as sustainable antibacterial agents in biomedical and environmental applications. The research work reported the desired approach for the synthesis of cobalt nanoparticles by adopting an eco-friendly method. This study does not only reaffirm the validity of neem-mediated nanoparticle synthesis but also adds valuable new data to the growing body of literature.

The methodological consistencies were evident, such as phytochemical involvement and FCC crystal phases. The improved antibacterial results and precise crystallinity emphasize the unique contribution of the work. The research substantiates that green-synthesized Co-NPs via *Azadirachta indica* offer a biocompatible, sustainable, and clinically promising solution to combat antibiotic-resistant microbes, supporting global efforts toward eco-friendly antibacterial strategies.

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