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Biogenic synthesis of copper oxide nanoparticles using medicinal plant extracts and their antibacterial and antioxidant potential with characterization

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Abstract
The development of sustainable method for nanoparticle synthesis has garnered significant interest in recent years. In the present study, biogenic fabrication of copper oxide (CuO) nanoparticles using extracts from selected medicinal plants as reducing and stabilizing agents. Antibacterial activity was examined using the agar well diffusion method, revealing significant inhibition zones, particularly against Gram-negative bacteria. Additionally, the antioxidant efficacy of the nanoparticles was assessed via DPPH free radical scavenging assay and ABTS cation radical scavenging, demonstrating remarkable concentration-dependent antioxidant activity. The synthesized nanoparticles were characterized using UV-Vis spectroscopy and zeta potential analysis. The findings highlight the potential of plant-mediated CuO nanoparticles as effective agents in biomedical applications due to their notable antibacterial and antioxidant properties.

Keywords: Copper oxide nanoparticles, medicinal plants, antibacterial, antioxidant, characterization

Introduction
In recent times, nanotechnology has garnered significant attention because of its potential applications in diverse areas such as food science, medical, farming, environment and cosmetics (Kaneria and Rakholiya, 2023; Pratibha *et al*., 2024; Parveen and Riazunnisa, 2025) [8, 14, 13]. Among the different types of nanomaterials, copper oxide nanoparticles (CuONPs) stand out due to their unique physicochemical characteristics, which include a high surface area, catalytic abilities, and antimicrobial as well as antioxidant features (Gawande *et al*., 2016) [6]. Traditional chemical and physical methods for producing CuONPs often involve toxic substances and require a lot of energy, raising concerns for the environment. However, biogenic fabrication using plant extracts offers a sustainable and eco-friendly alternative, utilizing bioactive compounds such as flavonoids, alkaloids, and polyphenols to aid in the formation of nanoparticles (Zeebaree *et al*., 2020; Paganotti *et al*., 2025) [23, 12].

Secondary metabolites presents in high amounts especially in medicinal plants that not only assist in the production of nanoparticles but also improve their biological effectiveness. Recent research has found that CuONPs produced using plant-based methods demonstrate enhanced antibacterial and antioxidant properties, making them advantageous in food preservation, pharmaceuticals, and biomedicine (Devaraji *et al*., 2024; Dejene 2025) [4, 3]. CuONPs obtained from medicinal plant extracts also show notable antioxidant properties contributed to the synergistic effect of copper ions alongside bioactive phytochemicals. Antioxidants are crucial in mitigating free radicals, responsible to induce oxidative stress, contributing to cellular aging and various health issues. CuONPs influence key biomarkers associated with oxidative stress, including antioxidant enzymes, which are vital for enhancing the body’s natural defenses against oxidative stress and damage (Do *et al*., 2024) [5]. The green synthesis of CuONPs using plant extracts boosts their antioxidant capabilities, rendering them increasingly beneficial for use in the biomedical, food, and pharmaceutical industries. In this research, we detail the biogenic synthesis of CuONPs utilizing extracts from selected medicinal plants and evaluate their antibacterial and antioxidant properties. The resulting nanoparticles were further characterized through UV-visible spectroscopy and zeta potential analysis.

Materials and Methods

Fresh leaves of four different plants, i.e., *Diplocyclos palmatus*, *Gymnosporia montana*, *Heliotropium indicum*, *Sapindus mukorossi* were collected from the Saurashtra University campus, Rajkot, Gujarat, India. The plant extract for the synthesis of copper oxide (CuO) nanoparticles was prepared by the decoction extraction method (Li *et al*., 2007) [10]. Synthesis and characterization of CuONPs was followed as described earlier (Tramta *et al*., 2024) [20]. The antibacterial activity of CuONPs was determined by agar well diffusion technique as described earlier (Rakholiya *et al*., 2015a) [16]. Antioxidant activity of CuONPs was determined by DPPH free radical scavenging method (McCune and Johns, 2002) [11] and ABTS radical cation ($ABTS^{\bullet+}$) scavenging method (Re *et al*., 1999) [19] by the modified method as earlier in details (Rakholiya *et al*., 2015b) [17].

Results and Discussion

As the plant extract was introduced into the copper sulfate solution, a color change occurred, indicating the development of CuONPs within the solution. At first, when extract was combined with the copper sulfate solution, the solution remained light brown; however, after an incubation period, it transformed to a dark brown hue, signaling the successful creation of copper oxide nanoparticles. The fabrication of CuONPs involves the reduction of Cu^{2+} to CuONPs through a reducing agent derived from the plant extract (Tramta *et al*., 2024) [20]. A similar color transition was observed in *Cissus vitiginea* (Wu *et al*., 2020) [22], *Cardiospermum halicacabum* (Punniyakotti *et al*., 2020) [15] and *Pongamia pinnata* (Tramta *et al*., 2024) [20].

CuONPs have been extensively researched for their ability to combat microbes due to their interactions with microbial cells through various pathways. These nanoparticles possess broad-spectrum antimicrobial properties. The primary mechanisms behind their antimicrobial effects include the interference with bacterial metabolism by binding to essential enzymes and proteins, which inhibits processes like respiration, DNA replication, and ATP synthesis, ultimately hindering microbial growth (Amoon *et al*., 2024) [1]. Tetracycline (T) served as the standard for comparison with CuONPs. In general, CuONPs exhibited greater antibacterial effectiveness against Gram-negative bacteria compared to Gram-positive ones (Fig. 1). The results from the antibacterial assay indicated that CuONPs synthesized from four different medicinal plants inhibited all tested bacteria, with the exception of Staphylococcus aureus and Bacillus cereus by *Diplocyclos palmatus*, *Bacillus cereus* and *Corynebacterium rubrum* by *Gymnosporia montana*, *Bacillus cereus* by *Heliotropium indicum*, and *Bacillus cereus* and *Corynebacterium rubrum* by *Sapindus mukorossi* (Fig. 1). Notably, the highest antibacterial action was noted against *Pseudomonas aeruginosa*, followed by *Klebsiella pneumoniae* and *Escherichia coli* by *Diplocyclos palmatus*, *Escherichia coli* followed by *Staphylococcus aureus* and *Salmonella typhimurium* by *Gymnosporia montana*, and *Escherichia coli* followed by *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* by *Heliotropium indicum*. Lastly, *Staphylococcus aureus* was inhibited, followed by *Salmonella typhimurium*, *Escherichia coli*, and *Klebsiella pneumoniae* by *Sapindus mukorossi* (Fig. 1). The antibacterial potency of CuONPs is influenced by their size, shape, concentration, duration of exposure, and charge. The surface charge of the nanoparticles plays a significant role in their antibacterial properties, as bacterial membranes carry a negative charge due to components like lipopolysaccharides, peptidoglycan, and various groups such as carboxyl, amino, and phosphate. Conversely, the positive charges on nanoparticles can facilitate their adhesion to bacterial membranes through electrostatic attraction. Thus, altering the surface charges of nanoparticles could enhance their antibacterial activity (Kaneria and Rakholiya 2023; Khan *et al*., 2024) [8, 9].

The antioxidant efficacy of CuONPs were evaluated using DPPH and ABTS assays, employing four different medicinal plants. Overall, the antioxidant capacity increased as the concentration of CuONPs. The DPPH assay is known for its stability, simplicity, and relatively greater feasibility. The scavenging activity against DPPH radicals increased with higher concentrations of CuONPs. The DPPH solution's color transitioned gradually from deep violet to pale yellow in the presence of CuONPs. The CuONPs concentration (50 - 350 µg/ml) synthesized from *Diplocyclos palmatus* exhibited inhibition levels between 12% and 56%, while *Gymnosporia montana* showed inhibition from 16% to 70%. *Heliotropium indicum* displayed inhibition ranging from 12% to 55%, and *Sapindus mukorossi* had inhibition rates between 14% and 91% (Fig. 2). Variability in DPPH was noted with higher CuONPs concentrations. The ABTS cation radical is generated by the reaction of ABTS salt with potassium persulphate, a potent oxidizing agent. Hydrogen-donating antioxidants reduce the bluish-green ABTS radical, which is evaluated by its bleaching and the decrease in its typical long-wave absorption spectrum. The extent of color change is dependent on the total concentration and quality of the sample (Chandraker *et al*., 2022; Vala *et al*., 2024) [2, 21]. The ABTS results of CuONPs at different concentrations are depicted in Fig. 3. A consistent increase in ABTS activity was observed with rising concentrations of CuONPs; specifically, the concentration ranges of 10-70 µg/ml showed inhibition of 11% - 48%, 13% - 62%, 15% - 82%, and 9% - 70% for CuONPs synthesized from *Diplocyclos palmatus*, *Gymnosporia montana*, *Heliotropium indicum* and *Sapindus mukorossi*, respectively (Fig. 3).

UV-Vis spectroscopy is an essential tool for examining the creation of metal NPs. The UV-Visible absorption spectrum was employed to analyze the optical characteristics of the fabricated CuONPs. Initially, the CuONPs were assessed using UV-Vis absorption spectroscopy to investigate their surface plasmon resonance (SPR). Additionally, the size and intensity of the peaks provide clear evidence regarding both the quantity and dimensions of the NPs that were produced; a broader peak signifies greater particle formation, while a narrower peak suggests smaller particle sizes. The intensity of the peak is directly related to the number of NPs created (Ramesh *et al*., 2015) [18]. The UV-Vis spectrum of the reaction mixture was obtained in the range of 200-800 nm (Fig. 4). The reduction of the extract was validated by the absorptive peaks observed at 275 nm for *Diplocyclos palmatus*, 236 nm for *Gymnosporia montana*, 248 nm for *Heliotropium indicum*, and 283 nm for *Sapindus mukorossi* in the UV-Vis spectroscopy (Fig. 4). These peaks can be associated with the CuONPs created using the plant extracts. A broadened SPR peak in the UV-Vis spectrum confirmed the formation of poly-dispersed nano-sized particles (Wu *et al*., 2020; Tramta *et al*., 2024) [22, 20]. In the green synthesis process, the natural plant extract serves as a reducing agent to produce CuO nanoparticles. Zeta potential is a tool used to characterize and measure the electric charge present on the surface of nanoparticles. The zeta potential value reflects the stability of the nanoparticles. It is regarded as a significant factor for assessing colloidal stability. The particle sizes and zeta potentials of CuONPs synthesized from *Diplocyclos palmatus*, *Gymnosporia montana*, *Heliotropium indicum* and *Sapindus mukorossi* were recorded as -0.31 mV, -0.38 mV, -0.24 mV, and -0.33 mV, respectively (Fig. 5). The negative charge on the nanoparticles' surfaces generates considerable repulsion between the particles, thus enhancing their stability (Gengan *et al*., 2013) [7].

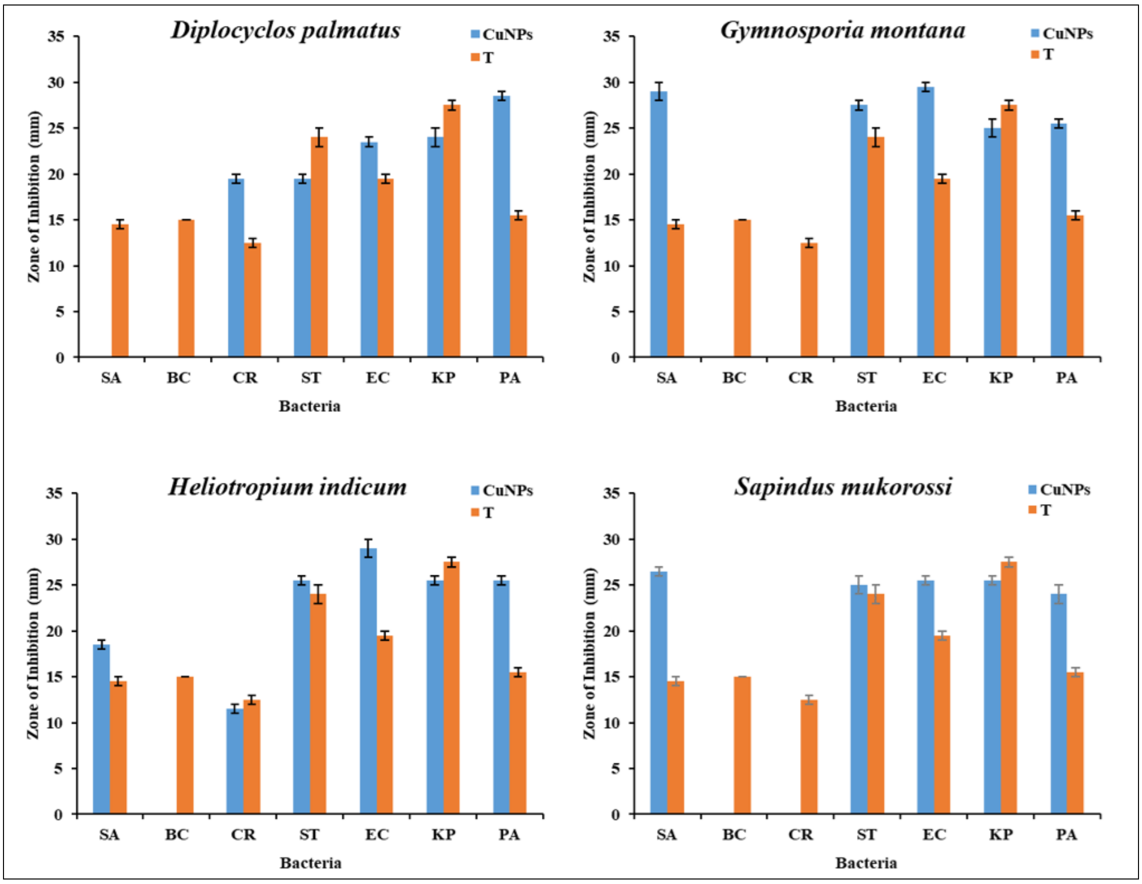

Fig 1: Antibacterial activity of CuONPs against Gram-positive and Gram-negative bacteria.

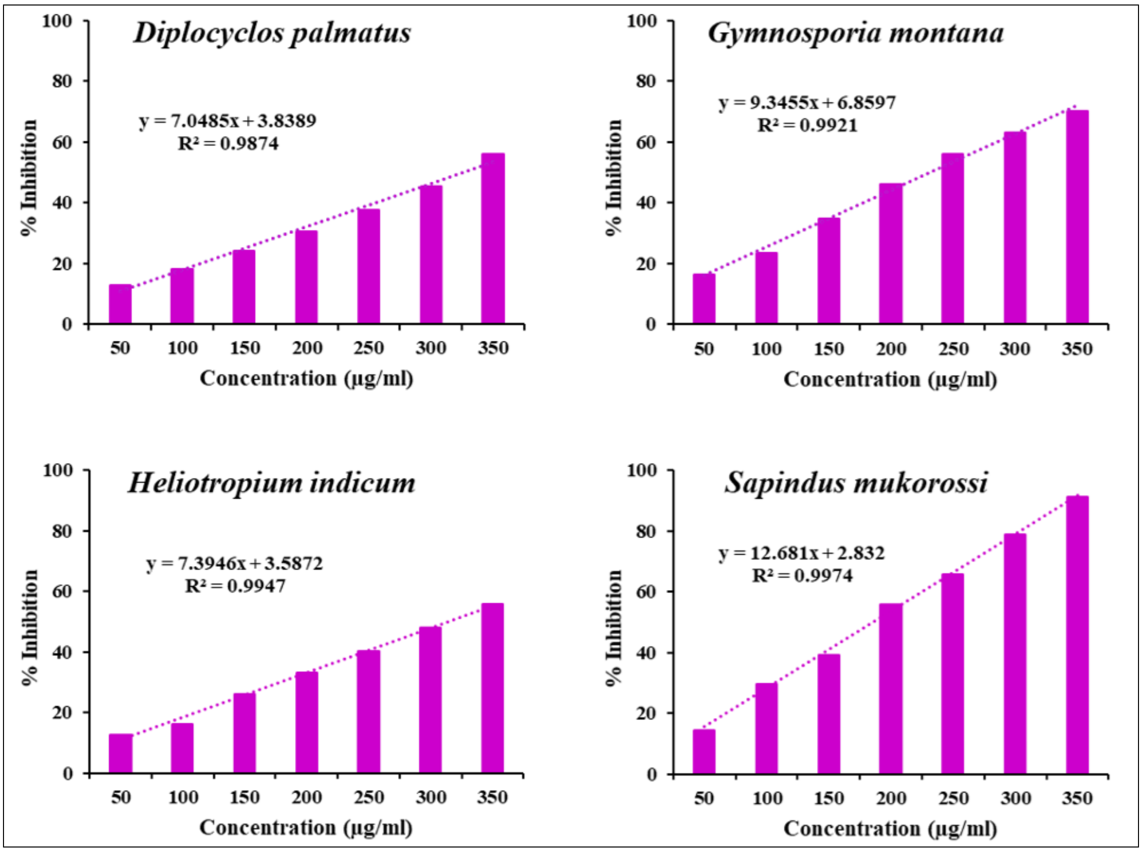

Fig 2: DPPH free radical scavenging activity of CuONPs.

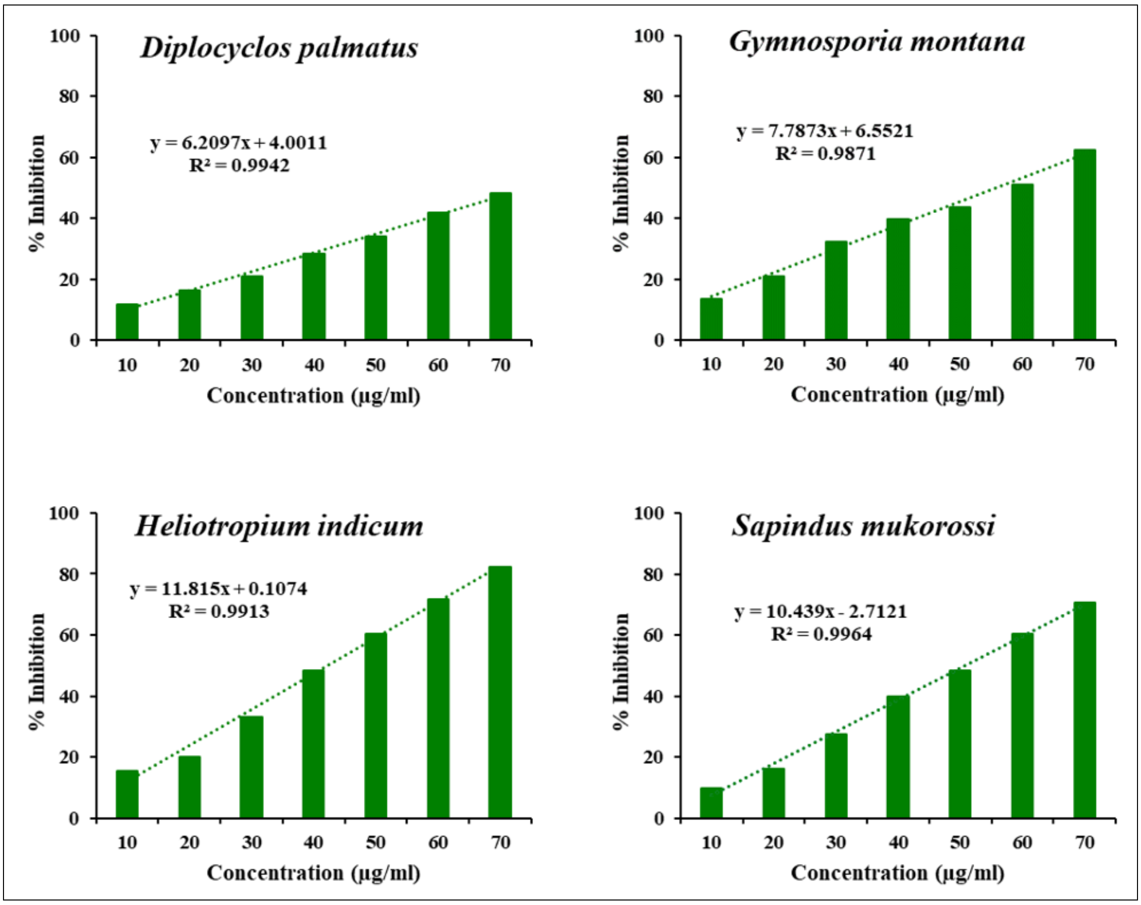

Fig 3. ABTS radical cation scavenging activity of CuONPs.

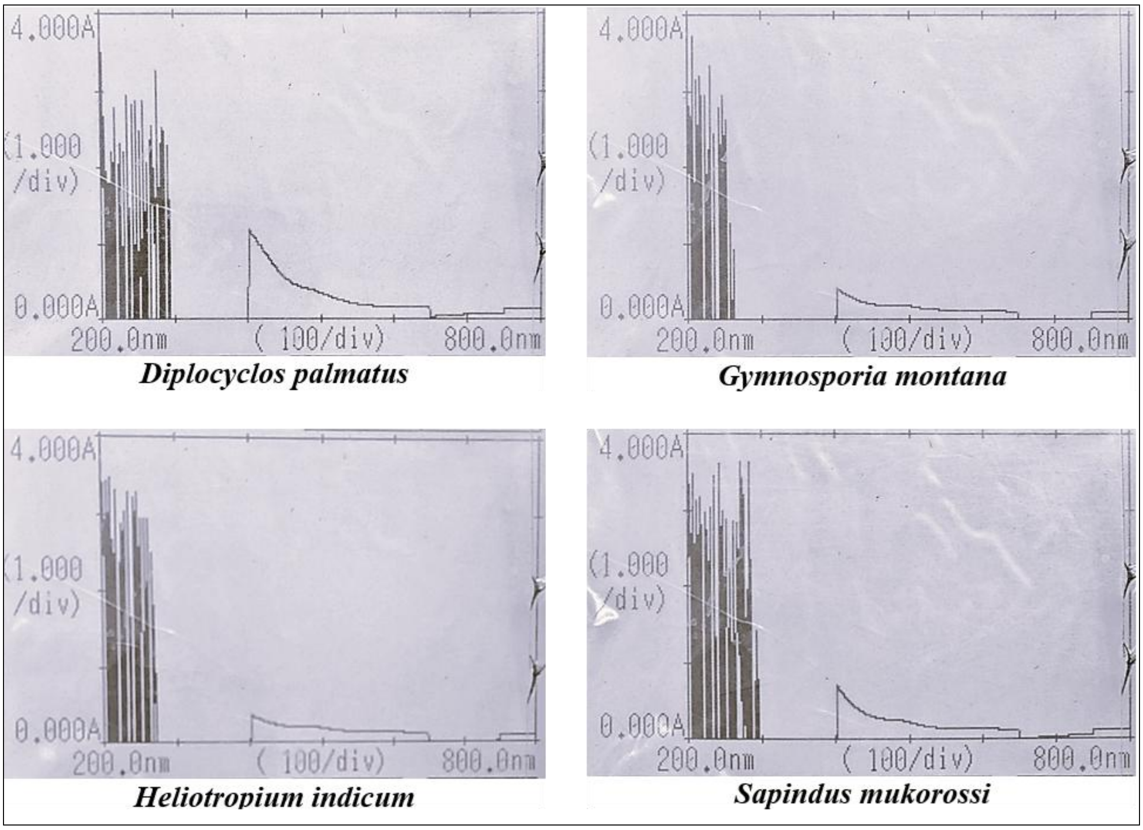

Fig 4: UV-Vis spectrum of green synthesized CuONPs.

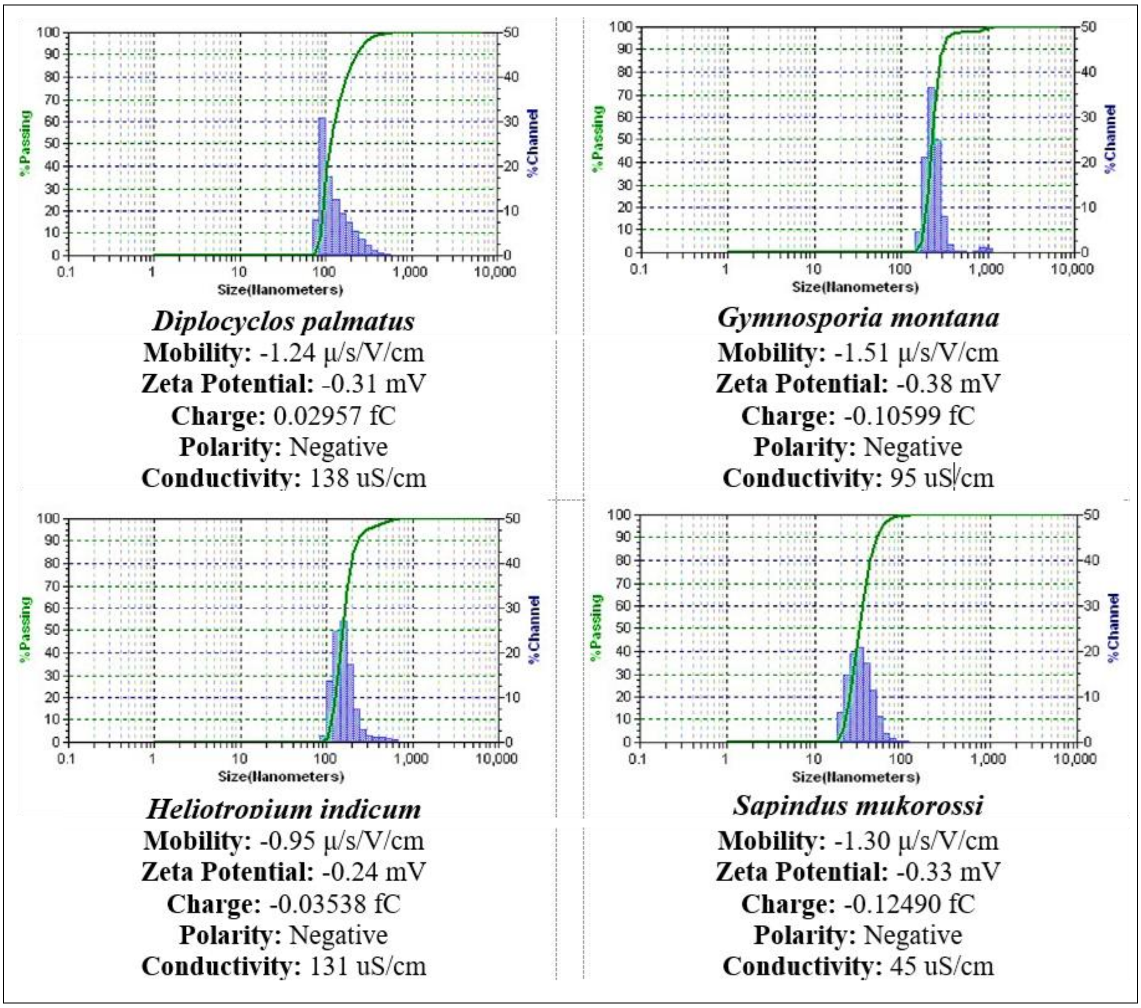

Fig 5: Zeta potential graph of green synthesized CuONPs.

Conclusion

CuONPs were produced from a copper sulfate precursor via an environmentally friendly method utilizing the leaf extracts of *Diplocyclos palmatus*, *Gymnosporia montana*, *Heliotropium indicum* and *Sapindus mukorossi* as both a reducing and capping agent. CuONPs showed significant antibacterial and antioxidant properties in a concentration-dependent manner. Various characterization techniques confirmed the formation of CuONPs. This research illustrates an eco-friendly approach for the synthesis of effective bioactive NPs that are safe and non-toxic. The results from this investigation may assist in the advancement of green nanotechnology-based antimicrobial and antioxidant agents, which could have potential uses in biomedical sciences, as well as in food safety and preservation to enhance shelf life.

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