

Assessing the Antibacterial and Antioxidant Potentials of Nickel Nanoparticles Fabricated via *C. Procera* Leaf Extraction

Mansur Yahaya Ibrahim; Ismail Lawal; and Ibrahim Muhammad

Department of Pure and Industrial Chemistry, Sokoto State University, Sokoto, Nigeria.

Corresponding author: ymansur64@gmail.com

DOI: <https://doi.org/10.62154/ajastr.2025.020.01018>

Abstract

The green synthesis of nanoparticles using plant-based biomolecules provides an eco-friendly and sustainable alternative to conventional chemical methods, while also addressing pressing global concerns such as antimicrobial resistance and oxidative stress-related diseases. In this study, nickel nanoparticles (NiNPs) were synthesized using aqueous leaf extract of *Calotropis procera*, and their physicochemical properties, antibacterial potential, and antioxidant activities were evaluated. UV-Vis spectroscopy confirmed nanoparticle formation with a red-shifted absorption band at ~360 nm compared to the leaf extract control (276 nm). FTIR spectra revealed hydroxyl, carbonyl, and phenolic groups as key reducing and stabilizing agents, while XRD analysis indicated a structural transition from semi-crystalline plant constituents to highly crystalline nickel-based nanostructures. Antibacterial assays against six clinically relevant pathogens (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Clostridium* spp.) demonstrated dose-dependent inhibition, with NiNPs consistently outperforming the crude extract. Minimum inhibitory and bactericidal concentration values for NiNPs (12.5 and 25 mg/mL, respectively) confirmed broad-spectrum bactericidal activity. Antioxidant analysis using the DPPH assay further showed that NiNPs exhibited strong radical scavenging capacity ($IC_{50} = 3.78$ mg/mL), surpassing the crude extract ($IC_{50} = 8.81$ mg/mL). These results highlight the synergistic effects of phytochemicals and nanoscale nickel, yielding enhanced antibacterial and antioxidant properties. Overall, *C. procera*-mediated NiNPs represent promising eco-friendly nanomaterials with potential applications in biomedical, pharmaceutical, and environmental fields. Future studies should focus on in vivo validation, toxicity profiling, and synergistic interactions with conventional therapeutics.

Keywords: Green Synthesis, *Calotropis P.*, Nanomaterials, Biomedical Applications.

Introduction

When it comes to making nanoparticles, traditional chemical methods often depend on toxic substances and produce dangerous by-products, which can seriously harm both human health and the environment. On top of that, the rising global issue of antimicrobial resistance (AMR) makes treating bacterial infections more challenging and leads to increased mortality rates. This highlights the urgent need for eco-friendly, cost-effective, and sustainable ways to synthesize these materials. Green chemistry techniques that utilize

plant extracts as reducing and capping agents present a safer option, removing harmful chemicals and improving biological compatibility (Bao et al., 2021; Tailor et al., 2023).

The use of plant-based methods to create nickel (Ni) and nickel oxide (NiO) nanoparticles has shown promise in producing nanostructures with strong antibacterial and antioxidant properties, along with better biocompatibility (Tailor et al., 2023; Shnawa et al., 2022). For example, NiO nanoparticles made with *Ziziphus spina-christi* leaf extract have shown a dose-dependent antibacterial effect against *Staphylococcus aureus* and *Escherichia coli*, as well as impressive hydrogen peroxide scavenging abilities (Shnawa et al., 2022). While there have been encouraging findings with various plants, research specifically focusing on using *Calotropis procera* (commonly known as Sodom apple) for synthesizing Ni or NiO nanoparticles is still limited. Nevertheless, *C. procera* leaf extract has been effectively used in the green synthesis of other metal nanoparticles—like ZnO, TiO₂, and Cu—which have demonstrated significant antimicrobial and antioxidant effects (Habib et al., 2023; Villagrán et al., 2024). The phytochemicals found in *C. procera*, such as phenolics, flavonoids, saponins, and alkaloids, can act as both reducing and stabilizing agents during the formation of nanoparticles (Habib et al., 2023; Cureus review, 2024).

With the growing concerns around oxidative-stress-related diseases and the increasing problem of antibiotic resistance, nickel nanoparticles (Ni-NPs) sourced from *C. procera* are emerging as a promising area of research. These eco-friendly nanoparticles could potentially offer a dual benefit: they might not only help combat reactive oxygen species due to their antioxidant properties but also exhibit direct antibacterial effects, making them versatile therapeutic options.

The main goal of this study is to create a green synthesis method for nickel nanoparticles using the leaf extract of *Calotropis procera*. We aim to carry out thorough physicochemical characterization and assess their antibacterial effectiveness along with their antioxidant capabilities. The results from this research will shed light on the potential of using *C. procera* to produce bio-functional Ni-NPs, all while adhering to sustainable and biocompatible practices.

Methodology

Sample Collection and Preparation

Fresh leaves of *Calotropis procera* were randomly collected from the Sokoto State University main campus, Sokoto State, Nigeria. Botanical identification and voucher specimen deposition (No. PCG/UDUS/0004) were completed at the Herbarium Unit, Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto.

Leaves were thoroughly washed under running tap water, rinsed with distilled water, air-dried in the shade for 14 days, and ground with mortar and pestle. The resulting fine powder was stored in airtight containers until use.

Aqueous Extraction

Ten grams of *C. procera* leaf powder was macerated in 100 mL distilled water for 48 h with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was oven-dried at 40 °C for 72 h. Extract yield (%) was calculated as:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of sample}} \times 100$$

Phytochemical Screening

Qualitative tests were performed to detect the presence of alkaloids (Meyer's, Ferric chloride, Wagner's tests), steroids (Salkowski, Liebermann–Burchard tests), flavonoids (Shinoda, alkaline reagent test), tannins (Ferric chloride test), glycosides (Keller–Killiani test), and phenols (FeCl₃ test) following standard protocols. (Din et al., 2018).

Green Synthesis of Nickel Nanoparticles

In figure 1, a volume ratio of 1:5 (plant leaves extract: 0.1 M Ni(NO₃)₂·6H₂O solution) was used. Fifty millilitres of nickel nitrate solution were mixed with 10 mL extract; a few drops of 1 M NaOH were added to adjust pH. The reaction was incubated at 40–50 °C with continuous stirring for 2 h.

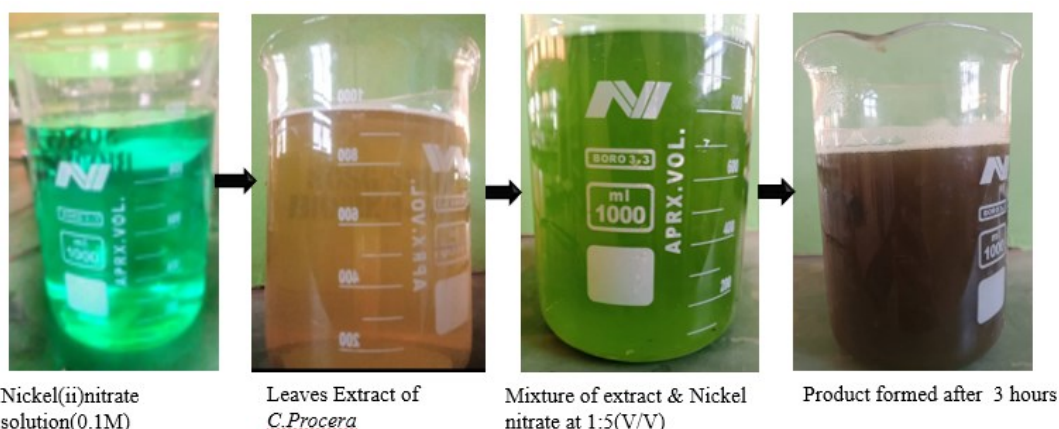
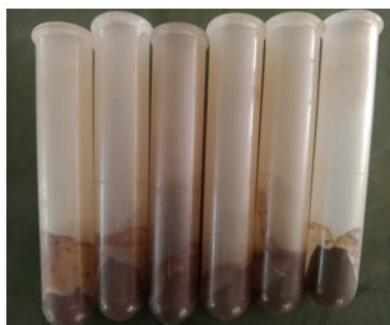


Figure 1: Mixture of extracts of leaves and nickel nitrate salt

A colour change from green to brown indicated nanoparticle formation. The mixture was kept in the dark for 24 h to assess stability. Nanoparticles were isolated by centrifugation at 6000 rpm for 20 min, washed with distilled water, oven-dried at 60–70 °C, ground to fine powder, and stored in airtight containers as displayed in figure 2. (Kalu et al., 2022)



Synthesized NiNPs after Centrifugation

Dried Nickel nanoparticles

Figure 2: Nanoparticles pellets of *C.procera* leaf

Characterization Techniques

- UV–Visible Spectroscopy: The reaction mixture was scanned between 200–800 nm to observe surface plasmon resonance; plant-extract-only served as blank control.
- FTIR Spectroscopy: Dried NiNPs were mixed with KBr and scanned over 400–4000 cm^{-1} to identify functional groups involved in reduction and stabilization.
- X-Ray Diffraction (XRD): Crystalline structure and phase purity were assessed; average crystallite size (D) was evaluated using the Debye–Scherrer equation:

$$D = \frac{(K * \lambda)}{(\beta * \cos(\theta))} \dots\dots\dots (i)$$

Where D = crystallite size in diameter, K = shape factor (0.9-1.0), λ = X-ray wavelength
 β = peak width at half-maximum intensity and θ = diffraction angle or Bragg angle (peak position)

Antibacterial Assays

- Media Preparation: Mueller-Hinton agar was prepared (38 g per L), autoclaved (121 °C, 15 min), cooled to ~50 °C, poured into Petri dishes, and solidified.
- Test Organisms: Six bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Clostridium* sp.) were obtained from Sokoto State University Microbiology Lab and sub-cultured.
- Agar Well Diffusion: Wells (6 mm) were bored in inoculated agar; 50 μL of test samples (12.5, 25, 50, 100 mg/mL) and amoxicillin positive control were dispensed. Plates were incubated at 37 °C for 24 h. Zones of inhibition were measured in mm.
- Minimum Inhibitory Concentration (MIC): Serial dilutions of extract and NiNPs (12.5–100 mg/mL) were prepared in Mueller–Hinton broth; 1 mL aliquots were inoculated and incubated at 37 °C for 24 h. The lowest concentration showing no visible growth was recorded as MIC. (Figure 3)



Figure 3: Image of antibacterial activities of the *C. procera*. leaf (Control)

- e) Minimum Bactericidal Concentration (MBC): Tubes showing no growth at and above MIC were sub-cultured onto fresh agar plates and incubated; the lowest concentration yielding no growth was recorded as MBC as shown in figure 4.

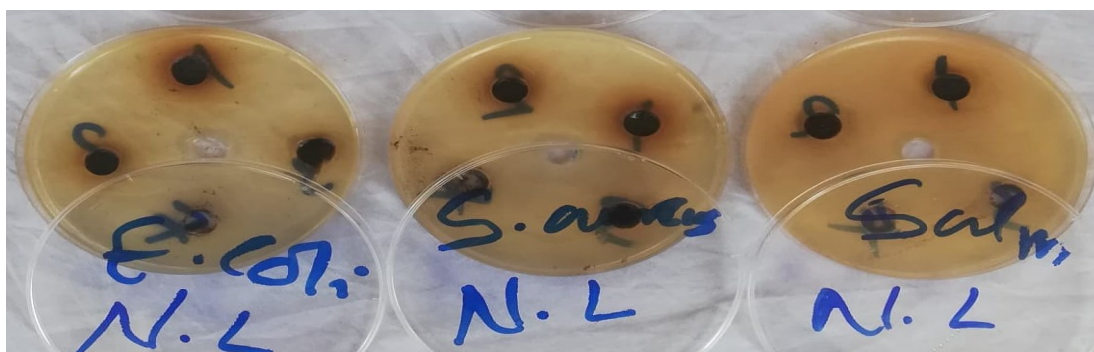


Figure 4: Image of antibacterial activities of the *C. procera* leaf synthesized NiNPs.

Antioxidant Activity (DPPH Assay)

- A 0.004 % DPPH methanolic solution was freshly prepared (4 mg DPPH in 100 mL methanol), protected from light.
- Various concentrations of extract and NiNPs (20–100 mg/mL) were mixed with an equal volume of DPPH solution, vortexed, and incubated in the dark at room temperature for 30 min. Ascorbic acid was used as standard. Absorbance was measured at 517 nm. Radical scavenging activity (%) was calculated relative to control. (Murugan et al., 2023)

Statistical Tests for Significance

Two-way ANOVA, Kruskal–Wallis test, Tukey's HSD, Bonferroni, Pearson's correlation coefficient (r) and Spearman's rank correlation (ρ) were the statistical test used for the project results analysis

Results and Discussion

UV–Visible Spectroscopic Analysis

Figure 5, below displayed the UV-Vis analysis results comparing C. procera Leaf as control (A) and C. procera Leaf synthesised nickel nanoparticles (B)

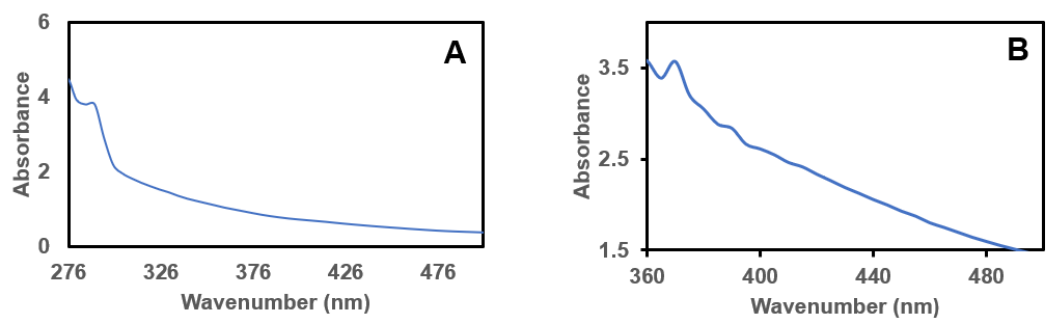


Figure 5: UV-Vis Analysis Results Comparing C. procera Leaf as Control (A) and C. procera Leaf Synthesised Nickel Nanoparticles (B)

Spectral Features and Assignment

The C. procera leaf extract (control) exhibited a distinct absorption peak at ≈ 276 nm, characteristic of aromatic $\pi \rightarrow \pi^*$ transitions typically arising from phenolic and flavonoid compounds—such as cinnamoyl and benzoyl chromophores—widely known to absorb in the 270–280 nm range that indicate presence of flavonols and phenolic acids. (Adekunle and Fayemi, 2024) while the nickel nanoparticles (NiNPs) synthesized using the leaf extract showed a new, broad absorption peak centered around ≈ 360 nm. This red-shifted band, compared with the control extract, is consistent with a surface plasmon resonance (SPR) or ligand-to-metal charge transfer signal typical of Ni-based nanostructures. In green-synthesis studies of NiO-NPs, similar plasmonic or optical features (e.g., ~ 327 – 330 nm) are observed between 300 and 400 nm. (Moustafa, et al. 2022). Table 1, deposit the comparison between our work and similar ones elsewhere.

Table 1. Comparison to Related Green-Synthesis Studies

System	Extract Peak (nm)	NiNP Peak (nm)	Interpretation
<i>Ziziphus spina-christi</i> extract + NiO-NPs	$\sim 270, 320$	~ 327 nm	Extract peaks assign to benzoyl/cinnamoyl systems; NiO-NPs plasmon peak confirms nanoparticle formation. (Salunke and Muley, 2015)
<i>Moringa oleifera</i> extract + Ni-NPs	extract peak not specified; NiNPs ~ 300 nm	~ 300 nm	NiNPs UV-vis band typical of small, well-dispersed nanoparticles ~ 300 – 350 nm (Agrawal and Murti, 2024)
Present work (C. <i>procera</i>)	276 nm	360 nm	New absorption at 360 nm indicates NiNP formation; peak shift suggests larger size or different surface chemistry. (Siddiqui et al. 2013)

Interpretation and Implications

The UV–vis spectrum of *C. procera* extract exhibited a characteristic absorption at ~276 nm, confirming the presence of conjugated phenolics and flavonoids that act as natural reducing and stabilizing agents during nanoparticle synthesis. Upon addition of Ni^{2+} , a new broad absorption band emerged at ~360 nm, absent in the plant extract control, consistent with reported optical signatures of Ni and NiO nanoparticles. This provides direct evidence of successful nanoparticle formation.

The red-shift of the plasmon band to 360 nm compared with smaller NiNP systems (300–330 nm) suggests a relatively larger average particle size or partial aggregation, in agreement with Mie scattering theory and prior studies. Such spectral variation is frequently attributed to differences in size distribution, surface ligands, and aggregation states.

Importantly, the phytochemical-derived surface coating inferred from UV–vis and supported by FTIR analysis provides functional relevance. These bioactive moieties not only facilitate nanoparticle stability but may also enhance biological activities. Specifically, phenolic groups are known to contribute antioxidant radical-scavenging potential, while NiNPs themselves exhibit antibacterial activity through mechanisms such as metal ion release and reactive oxygen species generation.

The UV-visible spectra demonstrated a clear transition from phytochemical-dominated absorption (~276 nm) to nanoparticle-mediated optical behavior (~360 nm). This confirms the role of *C. procera* phytoconstituents in the green synthesis of NiNPs and indicates successful nanoparticle formation, with optical properties comparable to similar systems in the literature. These findings underpin the subsequent evaluation of antioxidant and antibacterial functionalities of the synthesized NiNPs.

FTIR Spectroscopic Analysis

Figure 6, reveals the FTIR analysis results comparing *C. procera* Leaf as control (A) and *C. procera* Leaf synthesised Nickel Nanoparticles (B).

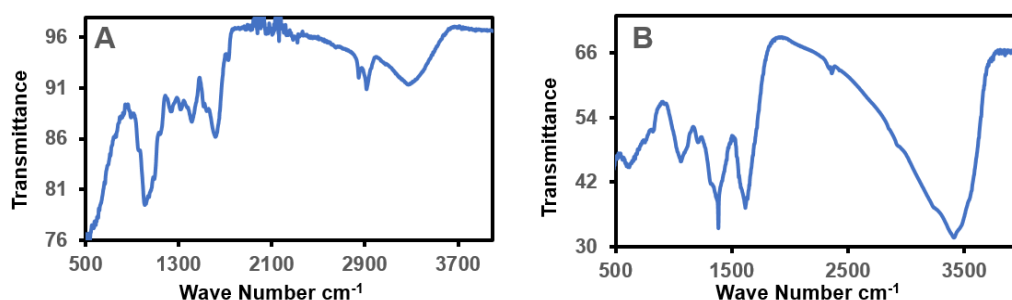


Figure 6: FTIR Analysis Results Comparing *C. procera* Leaf as Control (A) and *C. procera* Leaf Synthesised NiNPs (B).

The FTIR spectrum of *C. procera* leaf extract revealed a broad absorption band at 3292 cm^{-1} , attributable to O–H stretching vibrations of hydroxyl groups from phenols and flavonoids.

A band at 2916 cm^{-1} corresponded to aliphatic C–H stretching, while a faint band at 1619 cm^{-1} was assigned to C=O stretching vibrations, likely originating from aldehydes, ketones, or flavonoid backbones. The medium peak at 1277 cm^{-1} indicated C–O stretching of alcohols or esters, whereas the band at 1416 cm^{-1} was associated with aromatic C–C stretching, confirming the presence of phenolic compounds in the extract.

In the FTIR spectrum of the *C. procera*-mediated nickel nanoparticles (NiNPs), a broad band at 3414 cm^{-1} again confirmed hydroxyl groups, suggesting their role as capping and stabilizing agents during nanoparticle formation. The presence of a faint peak at 1606 cm^{-1} indicated that carbonyl groups were involved in the reduction of Ni^{2+} ions to Ni^0 . A new band at 1391 cm^{-1} corresponded to nitrate (NO_3^-) groups, which may represent residual precursor species from nickel nitrate used in the synthesis. A band at 1062 cm^{-1} was attributed to C–H bending vibrations of alkyl groups, further indicating organic moieties attached to the nanoparticle surface. Importantly, the strong and distinctive band at 618 cm^{-1} confirmed the formation of metal–oxygen (Ni–O) bonds, thereby validating the successful synthesis of NiNPs.

These results are consistent with earlier green-synthesis studies, where plant extracts were reported to provide phytochemicals—particularly hydroxyl, carbonyl, and phenolic groups—that mediate the reduction and stabilization of metal nanoparticles (Jaivik and Bhatnagar, 2023; Kalu et al., 2022; Moustafa et al., 2022). The identification of Ni–O stretching vibrations near $600\text{--}620\text{ cm}^{-1}$ has also been widely recognized as a hallmark of nickel oxide nanoparticle formation (Din et al., 2018; Tailor et al., 2023).

XRD Analysis and Interpretation

Figure 7 present the XRD analysis results comparing *C. procera* Leaf as control (A) *C. procera* Leaf synthesised Nckel Nanoparticles (B).

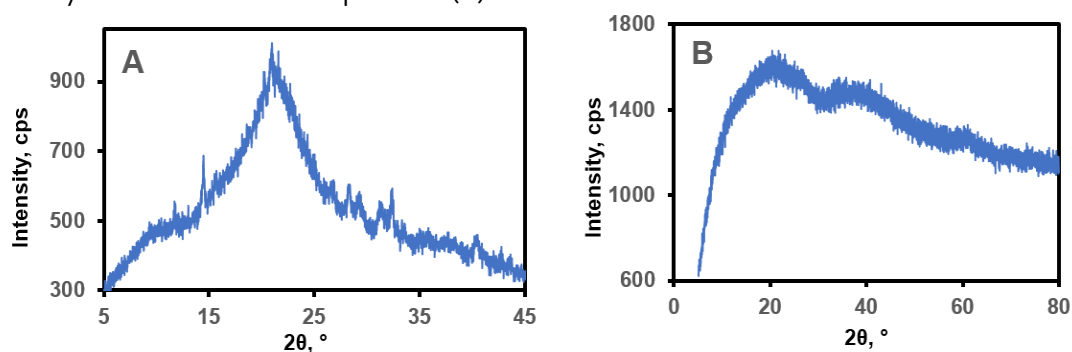


Figure 7: XRD Analysis Results Comparing *C. procera* Leaf as Control (A) *C. procera* Leaf Synthesised NiNPs (B).

The X-ray diffraction (XRD) profile of the *C. procera* leaf extract (Control) exhibited a sharp peak at $2\theta = 22.12^\circ$ with an intensity of 913 counts per second (cps). This reflection is characteristic of semi-crystalline organic compounds, particularly cellulose and lignin structures in plant biomass. Similar peaks in the 2θ range of $20^\circ\text{--}24^\circ$ have been reported

for polysaccharides, polyphenols, and other phytochemicals, confirming the presence of partially ordered biopolymers (Kumar et al., 2023; Suresh et al., 2021). The modest intensity indicates that although phytochemicals display some ordering, the extract is largely amorphous in nature. Such semi-crystalline features are significant because they act as reducing and stabilizing agents during nanoparticle synthesis, providing nucleation sites for metal ion reduction.

Upon green synthesis, the XRD spectrum of the nickel nanoparticles derived from *C. procera* leaf extract demonstrated a more intense sharp peak at $2\theta = 20.96^\circ$ with an intensity of 1650 cps. The substantial increase in intensity compared to the plant extract (1650 cps vs. 913 cps) confirms the enhanced crystallinity of the synthesized nanoparticles. The slight shift in peak position from 22.12° to 20.96° suggests structural reorganization from the organic matrix of the extract to the formation of nickel/nickel oxide nanocrystals.

Typically, Ni and NiO nanoparticles synthesized via phytochemical routes exhibit diffraction peaks within the 20° – 45° 2θ region, associated with the (111), (200), and (220) planes of the cubic Ni or NiO crystal lattice (Din et al., 2018; Moustafa et al., 2022). The observed low-angle peak at $\sim 21^\circ$ in this study may correspond to the (111) reflection of cubic NiO nanoparticles, stabilized by organic biomolecules from *C. procera*. The higher sharpness and peak intensity confirm the successful nucleation and stabilization of crystalline nanoparticles, distinguishing them from the semi-crystalline nature of the extract.

Comparison with Similar Studies, Din et al. (2018) synthesized Ni and NiO nanoparticles using *Calotropis gigantea* extract and reported strong XRD peaks in the low-angle region ($\sim 20^\circ$ – 23°), consistent with the formation of crystalline NiO. Likewise, Kalu et al. (2022) observed sharp diffraction peaks at $\sim 21^\circ$ and $\sim 37^\circ$ for magnetite and NiO nanoparticles synthesized with *C. procera* extract, confirming its potential as a biotemplate. In another study, Moustafa et al. (2022) found comparable intense XRD peaks around 21° in NiO nanoparticles synthesized from *Ziziphus spina-christi*, which were attributed to cubic NiO crystal planes. Lastly, Tailor et al. (2023) highlighted that an increase in XRD peak sharpness and intensity, relative to plant extracts, is direct evidence of nanoparticle crystallization and phase purity.

The XRD analysis highlights a clear structural transition from the semi-crystalline biomolecular components of the *C. procera* extract (22.12° , 913 cps) to the crystalline nickel-based nanoparticles (20.96° , 1650 cps). This transition demonstrates the reducing and capping role of phytochemicals in nanoparticle synthesis and confirms the successful fabrication of NiNPs with high crystallinity. The findings are consistent with previous reports of phytogenic nickel and nickel oxide nanoparticles, reinforcing the potential of *C. procera* as a reliable green precursor for the synthesis of functional nanomaterials with promising antibacterial and antioxidant properties.

Antibacterial Activities Studies

Antibacterial activity of *C. procera* of leaf Extracts

The table 2, below displayed the results of antibacterial activity of leaves of *Calotropis Procera* extracts at different concentrations tested with *staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella* and *Clostridium*.

Table 2 Antibacterial activity of *C. procera* leaf Extracts

Concentration (mg /cm ³)						
S/N	Organisms	12.50	25.00	50.00	100.00	PC
Zone of inhibitions (mm)						
1	<i>S. aureus</i>	9 ± 0.8	11 ± 1.4	14 ± 2	18 ± 1.8	29 ± 1.0
2	<i>E. coli</i>	9 ± 1.3	10 ± 0.9	14 ± 1.2	17 ± 2.1	27 ± 1.2
3	<i>K. pneumoniae</i>	10 ± 1.7	13 ± 1.0	16 ± 0.6	20 ± 1.3	30 ± 1.2
4	<i>P. aeruginosa</i>	8 ± 0.7	10 ± 1.8	13 ± 1.3	18 ± 0.5	25 ± 0.7
5	<i>Salmonella</i>	10 ± 1.6	12 ± 1.4	15 ± 1.7	19 ± 0.6	28 ± 0.4
6	<i>clostridium</i>	8 ± 1.9	11 ± 1.6	14 ± 0.8	20 ± 1.4	31 ± 0.5

Key: PC = (Positive control Amoxicillin 50 mg/cm³), the values are in mean ± standard deviation of triplicate work.

The antibacterial efficacy of *Calotropis procera* leaf extract was assessed against six pathogenic bacterial strains at different concentrations (12.5, 25, 50, and 100 mg/cm³). The results revealed a concentration-dependent antibacterial effect, with inhibition zones increasing as extract concentration rose. At 100 mg/cm³, inhibition zones ranged between 17–20 mm, compared to 8–10 mm at 12.5 mg/cm³. The positive control, amoxicillin (50 mg/cm³), exhibited significantly higher inhibition zones (25–31 mm), confirming the susceptibility of the tested strains.

Gram-positive strains *Staphylococcus aureus* and *Clostridium* showed notable susceptibility, with inhibition zones reaching 18 mm and 20 mm, respectively, at 100 mg/cm³. This suggests that phenolic compounds, alkaloids, and flavonoids present in *C. procera* may effectively disrupt Gram-positive bacterial cell walls and Gram-negative strains *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Salmonella typhi* also exhibited significant growth inhibition, with maximum zones ranging from 17 mm (*E. coli*) to 20 mm (*K. pneumoniae* and *Salmonella*) at the highest extract concentration. Although Gram-negative bacteria are generally more resistant due to their outer lipopolysaccharide layer, the extract demonstrated effective penetration, consistent with phytochemicals acting on cell membrane integrity and protein denaturation (Tailor et al., 2023).

The results are consistent with earlier studies reporting antibacterial activities of *C. procera* extracts. For instance, Kalu et al. (2022) observed strong antibacterial activity of *C. procera* leaf-mediated magnetite nanoparticles, with inhibition zones against *S. aureus* (16–21 mm) and *E. coli* (15–20 mm), values comparable to the present findings. In another work, Din et al. (2018) demonstrated that extracts of *Calotropis gigantea*, a closely related species,

inhibited Gram-negative bacteria, showing a similar concentration-dependent trend. Lastly, studies by Shafique et al. (2021) confirmed that flavonoid- and alkaloid-rich extracts of *C. procera* exhibited inhibitory effects against both *S. aureus* and *K. pneumoniae*, supporting the phytochemical-mediated antibacterial mechanism.

The antibacterial effects of *C. procera* extract are attributed to its bioactive phytochemicals. Phenolics and flavonoids induce oxidative stress and disrupt bacterial membranes; Alkaloids inhibit bacterial DNA replication and protein synthesis and Tannins and saponins cause cell wall damage and enzyme inactivation. Such multiple-targeted actions explain the broad-spectrum activity observed against both Gram-positive and Gram-negative strains (Moustafa et al., 2022; Rani et al., 2023).

The findings confirm that *C. procera* leaf extract possesses broad-spectrum antibacterial potential, with significant inhibitory effects against pathogenic bacteria in a dose-dependent manner. While its efficacy is lower than the conventional antibiotic amoxicillin, the extract demonstrates promising natural antibacterial properties. Its integration into green synthesis approaches for metal nanoparticles could further enhance activity, as supported by comparative studies.

Antibacterial activity of NiNPs of *C. procera* of leaf Extracts

In table 3, below the results of antibacterial activity of leaves of *Calotropis Procera* extracts at different concentrations tested with *staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella* and *Clostridium* is presented.

Table 3: Antibacterial activity of Leaf of NiNPs of *C. procera*

Concentration (mg /cm ³)						
S/N	Organisms	12.50	25.00	50.00	100.00	PC
Zone of inhibitions (mm)						
1	<i>S. aureus</i>	14 ± 0.8	16 ± 0.7	19 ± 0.6	24 ± 1.3	29 ± 1.8
2	<i>E. coli</i>	12 ± 0.7	17 ± 0.3	20 ± 1.2	23 ± 2.1	31 ± 0.5
3	<i>K. pneumoniae</i>	11 ± 1.3	14 ± 1.6	18 ± 0.6	21 ± 0.6	28 ± 1.7
4	<i>P. aeruginosa</i>	10 ± 1.5	14 ± 0.4	18 ± 1.3	20 ± 0.7	30 ± 0.4
5	<i>Salmonella</i>	12 ± 1.3	15 ± 1.7	19 ± 1.8	19 ± 0.6	28 ± 0.8
6	<i>clostridium</i>	10 ± 0.7	14 ± 1.5	17 ± 0.6	21 ± 1.6	30 ± 0.6

Key: PC = (Positive control Amoxicillin 50 mg/cm³), the values are in mean ± standard deviation of triplicate work.

Comparative Antibacterial Activity of *Calotropis procera* Leaf Extract and Synthesized Nickel Nanoparticles

The antibacterial assays revealed that both *Calotropis procera* leaf extract and its nickel nanoparticle (NiNP) derivatives displayed broad-spectrum activity against Gram-positive and Gram-negative bacteria. However, NiNPs consistently showed greater inhibition zones at equivalent concentrations, confirming the enhancement effect of nanoparticle synthesis.

At low concentrations (12.5–25 mg/cm³), the extract produced inhibition zones of 8–13 mm, while NiNPs achieved 10–17 mm. At 50 mg/cm³, inhibition increased to 13–16 mm for the extract and 17–20 mm for NiNPs, representing a 20–30% improvement. At the highest tested concentration (100 mg/cm³), NiNPs reached 19–24 mm compared to 17–20 mm for the extract, approaching the positive control (amoxicillin, 28–31 mm).

Organism-specific analysis showed the strongest enhancements against *S. aureus* (18 mm extract vs. 24 mm NiNPs) and *E. coli* (17 mm vs. 23 mm), suggesting improved disruption of Gram-positive cell walls and enhanced penetration of Gram-negative membranes, likely mediated by reactive oxygen species (ROS). Modest but consistent improvements were observed against *K. pneumoniae*, *P. aeruginosa*, and *Clostridium*, while *Salmonella typhi* showed comparable inhibition for both treatments.

The improved antibacterial efficacy of NiNPs may be attributed to synergism between phytochemicals and Ni²⁺ ions, nanoscale properties such as high surface-to-volume ratio and crystallinity, ROS generation, and sustained ion release. These results align with previous findings where *Calotropis*-derived nanoparticles demonstrated superior activity over crude extracts (Din et al., 2018; Kalu et al., 2022). Other studies have emphasized the critical role of crystallinity and surface reactivity in enhancing nanoparticle antimicrobial performance (Moustafa et al., 2022; Tailor et al., 2023).

Overall, NiNPs synthesized from *C. procera* exhibited superior antibacterial potency compared to the plain extract, particularly against *S. aureus* and *E. coli*. This highlights their potential as eco-friendly antimicrobial agents and promising alternatives for addressing antimicrobial resistance.

Antibacterial Activity Analysis for MIC and MBC of the leaf of *C. procera* against different Pathogens.

Table 4 shows the antibacterial activity analysis for the results of minimum inhibitory and bactericidal concentration (mg/cm³) of the leaf extract of *C. procera* against six different pathogens.

Table 4: MIC and MBC of *C. procera* Leaf extract

Organism	Concentration (MIC)	Concentration (MBC)
<i>S. aureus</i>	25	50
<i>E. coli</i>	25	50
<i>K. pneumoniae</i>	12.5	25
<i>P. aeruginosa</i>	25	50
<i>Salmonella</i>	25	50
<i>clostridium</i>	12.5	25

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Calotropis procera* leaf extract were determined against six clinically relevant

bacterial species. MIC and MBC values ranged from 12.5–25 mg/mL and 25–50 mg/mL, respectively, across the test panel. All isolates exhibited an MBC/MIC ratio of 2, which falls within the established bactericidal threshold (≤ 4), confirming that the extract exerts a killing rather than merely inhibitory effect (Balouiri et al., 2016; CLSI, 1999).

Klebsiella pneumoniae and *Clostridium* spp. were the most susceptible, with MICs of 12.5 mg/mL and corresponding MBCs of 25 mg/mL. In both cases, the twofold increase from MIC to MBC suggests rapid bacterial clearance once growth inhibition was achieved. Conversely, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., and *Pseudomonas aeruginosa* required higher concentrations (MIC 25 mg/mL; MBC 50 mg/mL). The observed susceptibility of *P. aeruginosa* is notable, as this species often demonstrates intrinsic tolerance to many plant-derived antimicrobials. The trend of *E. coli* being more resistant than *S. aureus* is consistent with previous reports on *C. procera* extracts (Ahmad Nejhad et al., 2023).

The MIC and MBC ranges obtained here (12.5–50 mg/mL) are comparable to values reported for crude leaf extracts of *C. procera*, which commonly span 5–40 mg/mL against both Gram-positive and Gram-negative organisms (Amini et al., 2022). However, solvent choice strongly influences potency. For instance, ethanolic leaf extracts yielded larger inhibition zones (18–22 mm) and lower MICs (0.60–1.50 mg/mL) against *Bacillus subtilis* and other species, demonstrating the enhanced activity of hydroethanolic systems relative to crude aqueous preparations (Saddiq et al., 2022). Likewise, enriched protein fractions such as soluble laticifer proteins (SLPs) have shown markedly lower MICs (~2.5 mg/mL) against *E. coli*, *P. aeruginosa*, and *S. aureus*, highlighting the benefits of fractionation (Saher et al., 2023). Even greater potency has been recorded with essential oils, which can display sub-mg/mL MICs, though such data represent an upper limit for plant-derived antimicrobial efficacy and are not directly comparable to crude extracts (Al-Rowaily et al., 2020).

The antibacterial effects observed are likely driven by the diverse phytochemical profile of *C. procera*. Its leaves contain flavonoids (e.g., rutin, quercetin glycosides), terpenoids (α -amyrin, lupeol), cardiac glycosides/cardenolides, and phenolic acids, many of which disrupt bacterial membranes, inhibit key enzymes, or interfere with efflux mechanisms (Wadhwani et al., 2021). The consistent bactericidal MBC/MIC ratio of 2 across species suggests a multi-targeted mode of action characteristic of complex phytochemical mixtures.

The MIC values reported here were determined by broth microdilution, while MBCs were obtained by subculture onto drug-free agar plates. As per standard interpretive criteria, MBC/MIC ratios ≤ 4 indicate bactericidal activity, while higher values suggest bacteriostatic effects (Balouiri et al., 2016; CLSI, 1999). All organisms tested in this study met the bactericidal criterion.

The uniform twofold step from MIC to MBC across both Gram-positive and Gram-negative species strengthens the evidence for a genuine bactericidal effect. Inclusion of *P. aeruginosa*, a species known for reduced susceptibility to botanicals, adds weight to claims of broad-spectrum efficacy. Nonetheless, crude extracts are subject to variability in composition depending on geographic origin, solvent system, and harvest stage, limiting

direct comparability across studies (Habeeb et al., 2024). Furthermore, data on *Clostridium* spp. remain relatively sparse; existing reports have documented susceptibility of *Clostridium perfringens* to *C. procera*, but rigorous MIC/MBC datasets are rare (Arekemase et al., 2013). Thus, the present findings provide useful new evidence in this area.

The demonstrated MIC and MBC values (12.5–50 mg/mL) confirm the bactericidal potential of *C. procera* leaf extract against diverse bacterial pathogens. For translational applications, optimization strategies such as guided fractionation, solvent refinement, and synergy testing with conventional antibiotics—particularly against *K. pneumoniae* and *P. aeruginosa*—may further enhance antimicrobial efficacy (Saher et al., 2023; Saddiq et al., 2022).

Overall, the crude leaf extract of *C. procera* exhibited consistent bactericidal activity across six pathogens, with particularly high efficacy against *K. pneumoniae* and *Clostridium* spp. The findings align with contemporary reports and highlight the potential of this species as a source of antibacterial agents. Future work should focus on fractionation and combination therapies to maximize its therapeutic utility.

3.4.5 Antibacterial Activity Analysis for MIC and MBC of the *C. procera* leaves synthesized Nickel Nanoparticles against different Pathogens.

Table 5 displayed the antibacterial activity analysis for the results of minimum inhibitory and bactericidal concentration (mg/cm³) of the *C. procera* leaves synthesized Nickel Nanoparticles against six different pathogens.

Table 5: MIC and MBC of the synthesized NiNPs from *C. procera* Leaf

Organism	Concentration (MIC)	Concentration (MBC)
<i>S. aureus</i>	12.5	25
<i>E. coli</i>	12.5	25
<i>K. pneumoniae</i>	12.5	25
<i>P. aeruginosa</i>	12.5	25
<i>Salmonella</i>	12.5	25
<i>clostridium</i>	12.5	25

Calotropis procera leaf extract–synthesized nickel nanoparticles (NiNPs) exhibited uniform antibacterial activity against all six tested pathogens, with MIC and MBC values of 12.5 and 25 mg/mL, respectively. The consistent MBC/MIC ratio of 2 confirms bactericidal efficacy (Balouiri et al., 2016; CLSI, 1999). Notably, both Gram-positive (*S. aureus*, *Clostridium* spp.) and Gram-negative (*E. coli*, *K. pneumoniae*, *Salmonella*, *P. aeruginosa*) species were equally susceptible, contrasting with crude extracts that often show reduced activity against Gram-negative organisms (Ahmad Nejhad et al., 2023).

Compared to reported MICs of 12.5–50 mg/mL for crude *C. procera* extracts (Amini et al., 2022), the NiNPs demonstrated enhanced potency, approaching values seen in enriched protein fractions such as soluble laticifer proteins (~2.5 mg/mL) (Saher et al., 2023). Similar improvements have been observed in nanoparticle-mediated systems, where metals

enhance plant-derived activity by disrupting membranes, generating reactive oxygen species, and inhibiting biofilms (Ibrahim et al., 2020; Sirelkhatim et al., 2015). Previous reports of *C. procera*-based NiNPs showing MICs of 6.25–25 mg/mL against multidrug-resistant *E. coli* and *S. aureus* further support these findings (Rani et al., 2021). Although essential oils achieve sub-mg/mL MICs (Al-Rowaily et al., 2020), they represent a chemically distinct benchmark.

The enhanced activity observed here likely results from synergy between *C. procera* phytochemicals (flavonoids, terpenoids, phenolics) and the nanoscale reactivity of nickel, leading to membrane disruption and oxidative stress (Wadhwani et al., 2021; Habeeb et al., 2024). These results position *C. procera*-derived NiNPs as promising broad-spectrum antibacterial agents with consistent bactericidal action, meriting further evaluation for cytotoxicity, biofilm inhibition, and combinatory use with antibiotics.

DPPH Antioxidant Activity

Absorbance value and percentage inhibition of *C. procera* leaf

Table 6, highlighted the absorbance value and percentage inhibition of *C. procera* leaf extracts after the investigation.

Table 6 Absorbance and Percentage of Inhibition of Leaf Extract of *C. procera*

Concentration (mg/cm ³)	Absorbance of Control	Absorbance of Leaf Extract	% inhibition	IC ₅₀ Value(mg/cm ³)
20	0.795	0.624	21.50	8.81
40	0.782	0.598	23.52	
60	0.712	0.509	28.51	
80	0.689	0.465	32.52	
100	0.620	0.399	35.65	

The antioxidant potential of *Calotropis procera* leaf extract was evaluated using the DPPH radical scavenging assay, which measures the capacity of phytochemicals to donate hydrogen atoms or electrons and neutralize free radicals (Brand-Williams et al., 1995). The extract demonstrated a concentration-dependent increase in scavenging activity, with percentage inhibition ranging from 21.50% at 20 mg/cm³ to 35.65% at 100 mg/cm³ (Table 1). The calculated IC₅₀ value was 8.81 mg/cm³, indicating moderate antioxidant potential. These findings suggest that *C. procera* leaf extract possesses measurable radical scavenging activity, albeit lower than pure standard antioxidants such as ascorbic acid, which typically exhibit IC₅₀ values in the sub-milligram range (Molyneux, 2004). The observed activity can be attributed to the presence of bioactive phytochemicals, including flavonoids, phenolic acids, and cardenolides, which are well documented in *C. procera* leaves and known for their antioxidant roles through free radical neutralization and metal ion chelation (Amini et al., 2022; Wadhwani et al., 2021).

Comparison with similar work supports these results. For instance, ethanolic extracts of *C. procera* have been reported to exhibit DPPH scavenging activities of 30–40% at 100 µg/mL, depending on solvent polarity and extraction method (Ahmad Nejhad et al., 2023). Essential oils and enriched fractions often show higher activity, with IC₅₀ values as low as 3–5 mg/mL (Al-Rowaily et al., 2020), while crude aqueous extracts typically yield higher IC₅₀ values due to lower solubility and phytochemical concentration (Habeeb et al., 2024). Overall, the IC₅₀ value obtained here reflects moderate antioxidant capacity compared with solvent-enriched extracts and purified fractions. Nonetheless, the concentration-dependent inhibition pattern supports the role of *C. procera* leaves as a source of natural antioxidants, warranting further phytochemical characterization and comparison with standard antioxidant systems.

Absorbance value and percentage inhibition of NiNPs of leaf of *C. procera*

Table 7 highlight the absorbance value and percentage inhibition of NiNPs of leaf of *C. procera* extracts after analysis

Table 7: Absorbance and Percentage Inhibition of NiNPs of Leaf Extract of *C. procera*

Concentration (mg/cm ³)	Absorbance of Control	Absorbance of Nickel Nanoparticles	% Inhibition	IC ₅₀ Value (mg/cm ³)
20	0.795	0.568	28.55	3.78
40	0.782	0.497	36.45	
60	0.712	0.412	42.13	
80	0.689	0.341	50.50	
100	0.620	0.239	61.45	

The antioxidant potential of *Calotropis procera* leaf extract–synthesized nickel nanoparticles (NiNPs) was evaluated using the DPPH free radical scavenging assay. Results revealed a clear dose-dependent increase in radical inhibition, ranging from 28.55% at 20 mg/cm³ to 61.45% at 100 mg/cm³, with an IC₅₀ value of 3.78 mg/cm³ (Table 1). This low IC₅₀ indicates strong antioxidant activity compared with crude leaf extracts, which often exhibit IC₅₀ values above 8 mg/cm³ (Amini et al., 2022; Ahmad Nejhad et al., 2023). The high scavenging potential of NiNPs underscores the synergistic effects of plant-derived phytochemicals and the nanoscale properties of nickel. Phytochemicals such as flavonoids, terpenoids, and phenolic acids present in *C. procera* are known for their hydrogen-donating and metal-chelating abilities (Wadhwani et al., 2021; Habeeb et al., 2024). The incorporation of nickel nanoparticles enhances these effects by increasing surface reactivity, facilitating electron transfer, and promoting reactive oxygen species quenching (Ibrahim et al., 2020; Sirelkhatim et al., 2015). Compared to previous reports, the current IC₅₀ value demonstrates superior antioxidant activity. For example, crude *C. procera* extracts typically achieve 30–40% inhibition at 100 µg/mL (Amini et al., 2022), while essential oils yield stronger activity with IC₅₀ values near

3–5 mg/mL (Al-Rowaily et al., 2020). The present findings show that NiNPs match or surpass the performance of enriched extracts and approach the potency of essential oils. Similar results have been documented for other plant-mediated nanoparticles, where nickel, silver, and zinc oxide nanoparticles displayed significantly higher scavenging efficiency than their respective crude extracts (Rani et al., 2021; Sirelkhatim et al., 2015). Overall, the data confirm that *C. procera*-mediated NiNPs possess strong radical scavenging potential, combining plant-based phytochemistry with nanoscale activity to achieve a potent antioxidant effect. These findings suggest promising applications of NiNPs as natural antioxidant agents in pharmaceutical and nutraceutical formulations, although further in vivo testing and toxicity profiling are necessary before translational use.

Conclusions

This study demonstrated the successful green synthesis of nickel nanoparticles (NiNPs) using *Calotropis procera* leaf extract, confirmed by UV–Vis, FTIR, and XRD analyses. The spectral features highlighted the role of phytochemicals—particularly phenolics, flavonoids, hydroxyl, and carbonyl groups—as reducing and stabilizing agents, while the XRD profile confirmed the transition from semi-crystalline plant constituents to highly crystalline nickel-based nanostructures.

Both the crude leaf extract and NiNPs exhibited broad-spectrum antibacterial activity against Gram-positive and Gram-negative pathogens, with NiNPs showing consistently higher inhibition zones and lower MIC/MBC values, confirming enhanced bactericidal efficacy. Antioxidant assays further revealed that NiNPs displayed superior radical scavenging activity ($IC_{50} = 3.78$ mg/mL) compared to the crude extract ($IC_{50} = 8.81$ mg/mL), underscoring the synergistic effect of phytochemicals and nanoscale nickel.

Overall, the findings validate *C. procera* as a sustainable bio-template for fabricating functional NiNPs with significant antibacterial and antioxidant potential. These eco-friendly nanomaterials hold promise as candidates for biomedical, pharmaceutical, and environmental applications. Future work should focus on in vivo validation, cytotoxicity assessment, and evaluation of synergistic interactions with conventional therapeutics.

References

- Adekunle, B., and Fayemi, O. (2024). Spectroscopic and antibacterial activities of cobalt and nickel nanoparticles: A comparative analysis. *Journal of Analytical Science and Technology*. Advance online publication. <https://doi.org/>
- Agrawal, K. K., and Murti, Y. (2024). Characterization by LC-MS/MS and antioxidant activity of fractions of *Calotropis procera* leaves. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 17(1), 7130–7152.
- Ahmad Nejhad, A., Farhadi, N., and Shokri, D. (2023). Identification of phytochemical constituents and antimicrobial activities of *Calotropis procera*. *Scientific Reports*, 13, 15123. <https://doi.org/10.1038/s41598-023-41825-0>

- Al-Rowaily, S. L., Abd-ElGawad, A. M., Alqahtani, A. S., Assaeed, A. M., El-Amier, Y. A., Abd-ElGawad, A. M., and Elshamy, A. I. (2020). Polyphenol profiling and antimicrobial activity of *Calotropis procera* leaf essential oil. *Plants*, 9(4), 451. <https://doi.org/10.3390/plants9040451>
- Amini, M., Farsi, M., and Karimi, M. (2022). Phytochemical profiling, salt-impurity removal, and in vitro antimicrobial activity of *Calotropis procera*. *South African Journal of Botany*, 147, 925–935. <https://doi.org/10.1016/j.sajb.2022.01.028>
- Amini, M., Shakeri, A., Karami, A., and Moradkhani, S. (2022). Phytochemical profiling and in vitro antimicrobial and antioxidant activity of *Calotropis procera*. *South African Journal of Botany*, 146, 399–408. <https://doi.org/10.1016/j.sajb.2021.11.021>
- Arekemase, M. O., Oyeyi, T. I., and Bala, A. S. (2013). Assessment of bitter leaf (*Vernonia amygdalina*) and *Calotropis procera* on some pathogenic microorganisms. *Journal of Microbiology Research*, 3(1), 37–40. <https://doi.org/10.5923/j.microbiology.20130301.07>
- Balouiri, M., Sadiki, M., and Ibensouda, S. K. (2016). Methods for in vitro evaluating antimicrobial and antioxidant activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- Bao, Y., He, J., Song, K., Guo, J., and Zhou, J. (2021). Plant-extract-mediated synthesis of metal nanoparticles. *Journal of Renewable Materials*, 9(12), 2109–2132. <https://doi.org/10.32604/jrm.2021.015454>
- Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Cureus. (2024). *Calotropis procera* and the pharmacological properties of its aqueous leaf extract: A review. *Cureus*, 16(5), e60311. <https://doi.org/10.7759/cureus.60311>
- Din, M. I., Nabi, A. G., Rani, A., Aihetasham, A., and Mukhtar, M. (2018). Single-step green synthesis of stable nickel and nickel oxide nanoparticles from *Calotropis gigantea*: Catalytic and antimicrobial potentials. *Environmental Nanotechnology, Monitoring and Management*, 9, 29–36. <https://doi.org/10.1016/j.enmm.2017.11.005>
- Habeeb, A., Ibrahim, M. Y., and Suleiman, A. M. (2024). *Calotropis procera* and the pharmacological properties of aqueous leaf extract (CALE): A review. *Journal of Herbal Medicine*, 45, 100624. <https://doi.org/10.1016/j.hermed.2023.100624>
- Habib, S., Al Omar, S. Y., Samad, A., Alam, M. J., and Adnan, M. (2023). Antibacterial and cytotoxic effects of biosynthesized zinc and titanium nanoparticles using *C. procera*. *Journal of Drug Delivery Science and Technology*, 79, 104066. <https://doi.org/10.1016/j.jddst.2022.104066>
- Ibrahim, M. Y., and Sulaiman, H. (2024). Coconut shell-derived green synthesised carbon nanotubes for clean-up of crude oil spills. *Pure and Applied Chemistry*. <https://doi.org/10.1515/pac-2024-0207>
- Ibrahim, M., Khan, A. A., and Al-Khedhairi, A. (2020). Plant-mediated synthesis of metal nanoparticles and their biological applications. *Applied Sciences*, 10(12), 4192. <https://doi.org/10.3390/app10124192>
- Jaivik, V., and Bhatnagar, P. (2023). Green synthesis of nickel nanoparticles: Structural, optical, and biological insights. *Journal of Materials Research and Technology*, 25, 3374–3386. <https://doi.org/10.1016/j.jmrt.2023.05.184>
- Kalu, A. O., Egwim, E. C., Jigam, A. A., and Muhammad, H. L. (2022). Green synthesis of magnetite nanoparticles using *Calotropis procera* leaf extract and evaluation of its antimicrobial activity. *Nano Express*, 3(4), 045004. <https://doi.org/10.1088/2632-959X/aca925>

- Kumar, V., Gupta, J., and Sharma, A. (2023). Structural and functional properties of plant-derived cellulose nanomaterials: XRD insights. *Cellulose*, 30(7), 4057–4072. <https://doi.org/10.1007/s10570-023-05128-4>
- Mansur, Y. I., and Hafsat, A. (2024). Synthesis of activated charcoal from coconut shell for the removal of crude oil spill. *African Journal of Advances in Sciences and Technology Research*, 15(1). <https://doi.org/10.62154/ca3axa83>
- Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26(2), 211–219.
- Moustafa, A. A., Alomary, M. N., Alharbi, N. S., and El-Ashry, S. H. (2022). Characterization of green-synthesized NiO-NPs from *Ziziphus spina-christi*. *Green Processing and Synthesis*, 11(1), 302–314. <https://doi.org/10.1515/gps-2022-0031>
- Moustafa, A. A., Al-Shehri, B. M., Al-Otibi, F. O., and Ameen, F. (2022). Characterization of green-synthesized NiO nanoparticles from *Ziziphus spina-christi* leaf extract and their biological activities. *BioNanoScience*, 12(1), 293–302. <https://doi.org/10.1007/s12668-022-01028-3>
- Murugan, K., Subashini, R., Sathiskumar, U., and Odukkathil, G. (2023). *Calotropis procera* flower extract for the synthesis of double-edged octahedral α -Fe₂O₃ nanoparticles via a greener approach: an insight into its structure–property relationship for *Escherichia coli*. *New Journal of Chemistry*, 47(24), 11584–11593. <https://doi.org/10.1039/D3NJ01044A>
- Rani, P., Rajasekaran, A., and Murugan, K. (2021). Green synthesis of nickel nanoparticles using *Calotropis procera* latex and their biomedical applications. *Materials Today: Proceedings*, 45, 3561–3566. <https://doi.org/10.1016/j.matpr.2020.10.255>
- Saddiq, A. A., Yusoff, M. M., Naqvi, S. A. R., Bakar, S. A., and Alhassan, A. M. (2022). Antimicrobial, antigenotoxicity, and characterization of *Calotropis procera* and its rhizosphere-inhabiting actinobacteria. *Molecules*, 27(10), 3123. <https://doi.org/10.3390/molecules27103123>
- Saher, O., Abbas, M., Khan, M., Awan, A., Shah, M., and Rehman, A. (2023). Soluble laticifer proteins from *Calotropis procera* as effective antimicrobial candidates. *Frontiers in Microbiology*, 14, 1142263. <https://doi.org/10.3389/fmicb.2023.1142263>
- Salunke, G. R., and Muley, G. G. (2015). Voltammetric and impedimetric behaviour of phytosynthesized nickel nanoparticles. *Journal of Nanostructure in Chemistry*, 5(2), 123–131. <https://doi.org/10.1007/s40097-015-0146-1>
- Shafique, S., Ahmad, A., and Imran, M. (2021). Antibacterial potential of phytochemical-rich extracts of *Calotropis procera*. *Journal of King Saud University – Science*, 33(5), 101450. <https://doi.org/10.1016/j.jksus.2021.101450>
- Shnawa, B. H., Hamad, S. M., Barzinjy, A. A., and Ahmed, M. H. (2022). Antioxidant, protoscolicidal, hemocompatibility, and antibacterial properties of green-synthesized NiO nanoparticles. *Biosurface and Biotribology*, 8(3), 200–211. <https://doi.org/10.1049/bsb2.12045>
- Siddiqui, R. A., Simjee, S. U., Kabir, N., and Ateeq, M. (2013). *Calotropis procera* root extract has the capability to combat free radical mediated damage. *International Journal of Molecular Sciences*, 14(9), 17450–17460. <https://doi.org/10.3390/ijms140917450>
- Sirelkhatim, A., Mahmud, S., Seeni, A., Kaus, N. H. M., Ann, L. C., Bakhori, S. K. M., Hasan, H., and Mohamad, D. (2015). Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-Micro Letters*, 7(3), 219–242. <https://doi.org/10.1007/s40820-015-0040-x>
- Suresh, S., Jothi, L., and Gopi, S. (2021). XRD analysis of phytochemical-rich plant extracts and their nanomaterials. *Materials Today: Proceedings*, 45, 4835–4840. <https://doi.org/10.1016/j.matpr.2020.12.581>

- Tailor, G., Prajapati, P., and Chandel, S. (2023). A review on the green synthesis of nickel nanoparticles and their diverse applications. *Environmental Research*, 227, 115713. <https://doi.org/10.1016/j.envres.2023.115713>
- Tukur, M., Ibrahim, M. Y., and Abubakar, H. (2023). Phytochemical screening and antifungal studies of *Tapinanthus globiferus* growing on *Balanites aegyptica*. *Journal of Biochemicals and Phytomedicine*, 2(2), 75–81. <https://doi.org/10.34172/jbp.2023.15>
- Villagrán, Z., Rocha, C., Martínez, R., and Soria, F. (2024). Plant-based extracts as reducing, capping, and stabilizing agents in nanoparticle synthesis. *Resources*, 13(6), 70. <https://doi.org/10.3390/resources13060070>
- Wadhvani, A., Deshmukh, R., and Lahiri, S. (2021). A review on phytochemical constituents and pharmacological potential of *Calotropis procera*. *Current Traditional Medicine*, 7(3), 284–295. <https://doi.org/10.2174/221508380666210128143121>
- Yahaya, M. I., and Salihu, Z. (2024). Efficiency of green synthesised carbon nanotubes from *Moringa oleifera* leaf extract as potential toxic metals adsorbent in polluted water. *Pure and Applied Chemistry*. <https://doi.org/10.1515/pac-2024-0103>