

Fabrication And Characterization Of Nickel Nanoparticles Prepared With *Eclipta. Alba* Plant Extract As A Reducing Agent

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ABSTRACT

This study focuses on the fabrication and characterization of Nickel nanoparticles (NiNPs) using an extract derived from the *Eclipta Alba* plant as a reducing agent, along with an exploration of their biological activities. The synthesis process emphasizes green chemistry principles by harnessing the natural reducing capabilities of plant extracts while capitalizing on the inherent properties of *Eclipta Alba*, renowned for its diverse phytochemical profile. Optimization of reaction parameters ensures precise control over NiNP size and morphology. Characterization techniques including UV-Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM) and energy dispersive x-rays(EDX) which confirms the successful synthesis and elucidate the structural and morphological properties of the NiNPs. Furthermore, the biological activities of the synthesized NiNPs are investigated, shedding light on their potential applications in biomedical field such as its antimicrobial potential. This study underscores the significance of utilizing plant extracts for green nanoparticle synthesis and highlights the promising biological activities of the resulting NiNPs.

Introduction

Nanotechnology has emerged as one of the most transformative scientific fields of the twenty-first century, attracting considerable attention due to its wide range of technological and biomedical applications. It involves the manipulation and control of matter at the nanoscale, typically between 1 and 100 nm, where materials exhibit unique physicochemical properties that differ significantly from their bulk counterparts (Pandian et al., 2015). At this scale, variations in surface area, quantum effects, and molecular interactions lead to the development of novel materials with

enhanced mechanical, optical, catalytic, and electrical characteristics. These properties have expanded the applications of nanotechnology in diverse fields including electronics, medicine, environmental science, catalysis, energy production, and materials engineering (Huang et al., 2021).

The conceptual foundation of nanotechnology was first introduced by physicist Richard Feynman in his famous 1959 lecture “There’s Plenty of Room at the Bottom,” where he highlighted the potential of manipulating atoms and molecules to create advanced materials and devices (Ying et al., 2022). Since then, rapid advancements in nanoscience have enabled the development of innovative technologies with significant industrial and economic potential. Nanotechnology is currently applied in areas such as drug delivery systems, information technology, water purification, renewable energy technologies, and the development of lightweight but high-strength materials (Hussain et al., 2016). Owing to these diverse applications, nanotechnology is expected to play a key role in future technological and economic development worldwide (Rafique et al., 2017).

Among the various components of nanotechnology, nanoparticles represent an important class of materials characterized by their extremely small size and large surface-to-volume ratio. These nanoscale particles exhibit distinctive physical, chemical, and biological properties compared with bulk materials, making them valuable for numerous scientific and industrial applications (Mubayi et al., 2012). Nanoparticles have demonstrated promising potential in fields such as catalysis, sensing, biomedical imaging, electronics, and environmental remediation. Their increased surface reactivity and tunable physicochemical characteristics allow them to interact more effectively with surrounding molecules and biological systems (Iravani 2011).

Various physical and chemical techniques have traditionally been employed for the synthesis of nanoparticles. Commonly used methods include sol–gel processing, chemical reduction, microemulsion techniques, laser ablation, pyrolysis, coprecipitation, and hydrothermal synthesis (Srikar et al., 2016). These approaches generally provide precise control over particle size, morphology, and crystallinity. However, many conventional synthesis techniques require high temperatures, high pressure conditions, and the use of toxic chemicals or organic solvents. Such processes may generate hazardous by-products and pose potential risks to both human health and the environment (Hano & Abbasi 2021). Consequently, the development of environmentally friendly and sustainable approaches for nanoparticle synthesis has become a major focus in nanotechnology research.

Green synthesis has emerged as a promising alternative to conventional nanoparticle production methods. This approach utilizes biological systems such as plants, bacteria, fungi, algae, and yeast as reducing and stabilizing agents in the synthesis process (Shreyash et al., 2021). The use of biological materials minimizes the need for toxic chemicals and harsh reaction conditions, making the process safer, cost-effective, and environmentally sustainable. In green synthesis, biomolecules such as proteins, enzymes, polysaccharides, and secondary metabolites play a crucial role in reducing metal ions and stabilizing the formed nanoparticles (Jadoun et al., 2021).

Among the various biological approaches, plant-mediated synthesis has gained particular attention due to its simplicity, rapid reaction rates, and scalability. Plant

extracts contain a wide range of phytochemicals including flavonoids, alkaloids, terpenoids, phenolic compounds, and organic acids, which act as natural reducing and capping agents during nanoparticle formation (Shah et al., 2015). Compared with microbial synthesis, plant-based methods do not require complex culture conditions or expensive growth media, making them more practical for large-scale nanoparticle production. Furthermore, plant-derived nanoparticles often exhibit improved stability and enhanced biological activity due to the presence of bioactive phytochemicals on their surfaces (Kamran et al., 2019).

Nickel nanoparticles (NiNPs) have attracted significant interest because of their unique magnetic, catalytic, and electrical properties. These nanoparticles have been widely investigated for applications in magnetic storage devices, catalysts, sensors, batteries, and biomedical technologies (Dikshit et al., 2021). Nickel nanoparticles exhibit high surface activity and strong catalytic efficiency, making them valuable in chemical reactions and environmental remediation processes. In addition, their magnetic characteristics enable their use in magnetic fluids, memory devices, and magnetic resonance imaging applications (Ghaffari-Moghaddam et al., 2014).

Recent studies have also demonstrated the antimicrobial potential of metal nanoparticles, including nickel nanoparticles. These nanomaterials can inhibit the growth of various pathogenic microorganisms by disrupting cellular membranes, generating reactive oxygen species, and interfering with microbial metabolic pathways (Rauwel et al., 2015). As the global threat of antimicrobial resistance continues to increase, the development of alternative antimicrobial agents has become a critical area of research. Nanoparticles synthesized through eco-friendly methods may provide a promising strategy for combating drug-resistant microbial infections.

Plant-mediated green synthesis of nickel nanoparticles has therefore become an area of growing interest in nanobiotechnology. One plant that has received attention for this purpose is *Eclipta alba* (Bhringraj), a medicinal herb widely distributed in tropical and subtropical regions. This plant is traditionally used in herbal medicine due to its antimicrobial, anti-inflammatory, antioxidant, and hepatoprotective properties (Usman et al., 2019). *E. alba* contains various phytochemical constituents such as flavonoids, terpenoids, and phenolic compounds that can function as natural reducing and stabilizing agents during nanoparticle synthesis (Ghazal et al., 2020).

The use of *Eclipta alba* extract in nanoparticle synthesis offers several advantages, including eco-friendly processing, reduced toxicity, and enhanced biological activity of the synthesized nanomaterials. Plant-derived biomolecules present in the extract facilitate the reduction of nickel ions and contribute to the stabilization of nanoparticles, resulting in biocompatible nanostructures suitable for biomedical applications (Abdullah et al., 2023). Moreover, the integration of green synthesis with medicinal plants provides an innovative approach for producing multifunctional nanoparticles with potential antimicrobial properties.

Therefore, the present study focuses on the green synthesis of nickel nanoparticles using *Eclipta alba* plant extract and the evaluation of their antimicrobial activity. The research aims to develop an environmentally sustainable method for nanoparticle production while exploring the potential biomedical applications of plant-mediated nickel nanoparticles. Such approaches may contribute to the advancement of eco-friendly nanotechnology and provide alternative strategies for addressing emerging

challenges in antimicrobial resistance and biomedical material development.

Materials and Methods

Plant sample collection

A solid example of the *E. Alba* plant will be gathered from Region Peshawar, Khyber Pakhtunkhwa, Pakistan, and masterfully distinguished by a plant taxonomist from the Natural science Division at Islamia college Peshawar, Khyber Pakhtunkhwa, Pakistan. Upon distinguishing proof, the plant leaves were washed with deionized water to eliminate surface residue and then dried in shade for 4 to 5 days. The plant is then grounded into a fine powder utilizing a mortar and pestle and stored for further use (Dhaka 2013, Qureshi & Solanki 2015).



Figure 1: Powder form of *E. Alba* plant.

Preparation of plant extract

A 10g of powdered *E. Alba* plant powdered form was added to 100ml of deionized water, putted on a hot plate set to 60°C and mix consistently for 60 minutes the extract was allowed to cool at room temperature, following vacuum filtration. A 10% solution was made from the stock solution (Kumari et al., 2021).

NiSO₄ salt solution

20 millimolar solution of nickel sulfate was ready by dissolving 0.62g of nickel sulfate salt in 200 ml of deionized water and allow it to stir for 1 hr at 45°C (Madsen et al., 2019).



Figure 2: Preparation of 20mM NiSO₄ salt solution

Preparation of Nickel Nanoparticles with *E.alba* plant extract

NiNPs were synthesized by mixing the aqueous extract (AE) of the plant 2ml with 4ml salt solution in a beaker. The mixture was kept for stirring for 2 hrs at room temperature. The solution in the beaker was covered with aluminium foil, the slight colour change indicated the formation of NiNp. NiNPs solution was washed by utilizing deionized water and ethanol to take out any pollutants or remaining substances and then dried in afurnace to get fine powder (Santhoshkumar et al., 2023).



Figure 3: Nickel Nanoparticles prepared with *E.alba* plant extract

Characterization of NiNPs

The NiNPS solution was then characterized by using following analytical techniques to confirm the formation NiNPs.

UV-Visible spectroscopy

The biosynthesized nickel nanoparticles go through spectrometric investigation utilizing an UV-noticeable spectrophotometer. The decrease of nickel is observed irregularly inside the scope of 350 to 600 nm. A spectrum is produced, where the absorbance is plotted on the y-axis against the wavelength on x- axis (Santhoshkumar et al., 2023).

Fourier-transform infrared radiation (FTIR)

FTIR helps identify the functional groups present on the nanoparticle surface. It provides information about the chemical composition and surface modifications of cobalt nanoparticles. Synthesized cobalt nanoparticles were analyzed using a Fourier-transform infrared spectrophotometer in the range of 600 to 4,000 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans in transmittance mode (Santhoshkumar et al., 2023).

SEM and EDX

SEM (scanning electron microscopy) and EDX (energy-dispersive X-ray spectroscopy) analysis of biosynthesized cobalt nanoparticles can provide valuable information about the morphology, size, structure, and elemental composition of the nanoparticles. For study the morphology of biosynthesized nickle nanoparticles SEM slides were prepared and a thin layer of gold was coated on the smears to make them

conductive and then already biosynthesized sample of NiNPs was observed to determine magnification power and resolution. Then the samples were monitored in SEM at voltage of 20 KV. SEM analysis involves scanning the surface of the nanoparticles with a focused electron beam to generate high-resolution images, allowing for the observation of their shape, size distribution, and surface characteristics. On the other hand, EDX analysis enables the elemental composition of the nanoparticles to be determined by detecting the characteristic X-rays emitted when the sample is irradiated with an electron beam (Santhoshkumar et al., 2023).

Antibacterial activity

The first step is the preparation of media. Nutrient agar is prepared by adding 11g of dehydrated nutrient agar powder using electric balance into 400ml of distilled water. Adjust the PH by electric PH meter and keep it on hot plat with continuously stirring to completely dissolve. The media is sterilized by using automatic autoclave at 121°C for 15 minutes. Media pouring and drying Media was poured in pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media. Four strains of test bacteria such as “*E. coli*” is a Gram-negative, Pathogenic strains can lead to infections, causing symptoms such as diarrhea, abdominal pain. “*Morganella morganii*” is a rod-shaped Gram-negative bacterium cause various infections in humans, including urinary tract infections (UTIs), wound infections, pneumonia, and bacteremia. “*Ralstonia solanacearum*” is a Gram-negative, soil-borne bacterium that is responsible for causing bacterial wilt in a wide range of plants. “*Xanthomonas axonopodis*” is a Gram-negative bacterium and is known to infect a wide range of plants, causing diseases in crops such as citrus, tomato, pepper, and other economically important plants, are used in this acticty. Ciprofloxin is used as a positive control and DMSO as a negative control. After the incubation time, single selective colony of each bacterium from their respective selective agar medium was inoculated into Nutrient agar medium. Sterile wireloop was used for all the bacterial strains. Bacterial strains were picked up by the sterile wireloop and streaked on the Nutrient agar plates inside the streamline horizontal air flow hood. The inoculated plates were kept for 24 hours at 37° C in incubator. After 24 hours the bacterial growth was observed. Prepare the test samples, which may include plant extract of eclipta alba and biosynthesized cobalt nanoparticles being evaluated for their antibacterial activity. Dissolve or suspend the samples in a DMSO and prepare three different concentrations which is 2mg, 4mg and 8mg. Once the bacterial growth is visible on the agar plates, use a sterile cork borer to create wells in the agar. Ensure that the wells are distributed evenly across the agar plate. Carefully add the test samples into the wells using a micropipette, being mindful to not overflow or disrupt the agar surrounding the wells. Incubate the plates at an appropriate temperature for a specified period of time to allow diffusion of the test samples into the agar and interaction with the bacterial colonies. After the incubation period, examine the plates for zones of inhibition, which are clear areas around the wells where bacterial growth has been inhibited by the test samples. Measure the diameter of the zones of inhibition to quantify the antibacterial activity (Wadee et al., 2022).

Antifungal activity

To overview the antifungal action of Nickel nanoparticles and *E. Alba* plant leaf eliminate at groupings of 3mg and 5mg, autonomously, against irresistible strains including *Fusarium oxysporum*, *Alternaria alternata*, and *Aspergillus niger*, the going with show is utilized. Combine Nickel nanoparticles utilizing a green blend technique. Portray the nanoparticles to affirm their size, morphology, and soundness. Autoclave supplement agar and empty it into sterile Petri dishes. Permit the agar to set and afterward mark the parasitic strains onto the agar utilizing sterile methods utilizing a sterile plug drill, make wells in the agar plates. Break down the Nickel nanoparticles and the leaves concentrate of *E. Alba* plant in dimethyl Sulphoxide (DMSO) dissolvable to achieve groupings of 1mg/mL and 2mg/mL. Add the nanoparticle and separate responses for the wells in the agar plates. Incubate the plates at the optimal temperature for the growth of each fungal strain. After the legitimate incubating period, notice the plates for zones of limitation around the wells. Measure and record the broadness of any zones of obstacle (Hendawy et al., 2018).

Results and Discussion

Characterization of NiNPs

The synthesized nanoparticles were characterized by the following analytical techniques to confirm the synthesis of NiNPs.

UV-Vis Spectrophotometer Analysis

UV-Visible spectroscopy is a vital technique frequently used to characterize the structure of nickel nanoparticles (NiNPs). In this study, the synthesis of NiNPs was confirmed by examining the UV-Visible spectra of various ratios of plant extract, within the wavelength range of 200 to 800 nm (Adwin et al., 2023). The Ni^{2+} reduction was detected by the UV-Visible spectra of NiNPs and salt solution as shown in Figure. The peak is attributed to the surface plasmon resonance (SPR), indicating the formation of nickel nanoparticles. UV spectra of synthesized NiNPs shows maximum absorption at 350nm-400nm (Shalichah & Khumaeni 2018).

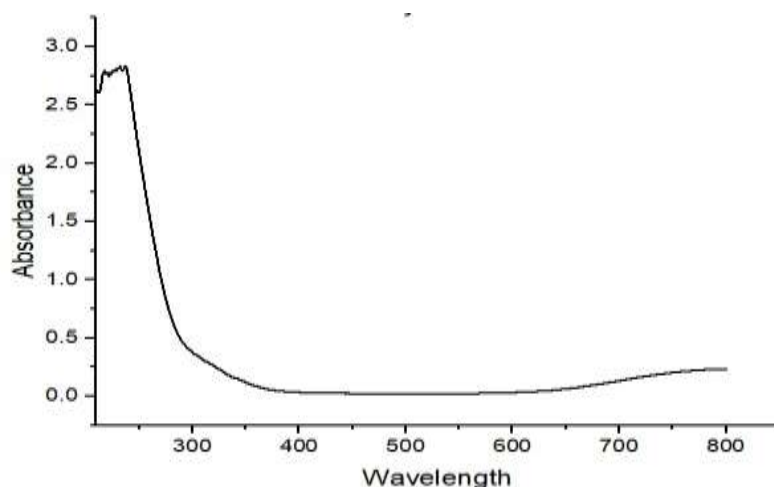


Figure 4: UV-Vis spectra of 0.02M $\text{NiSO}_4 \cdot 5\text{H}_2\text{O}$ salt solution.

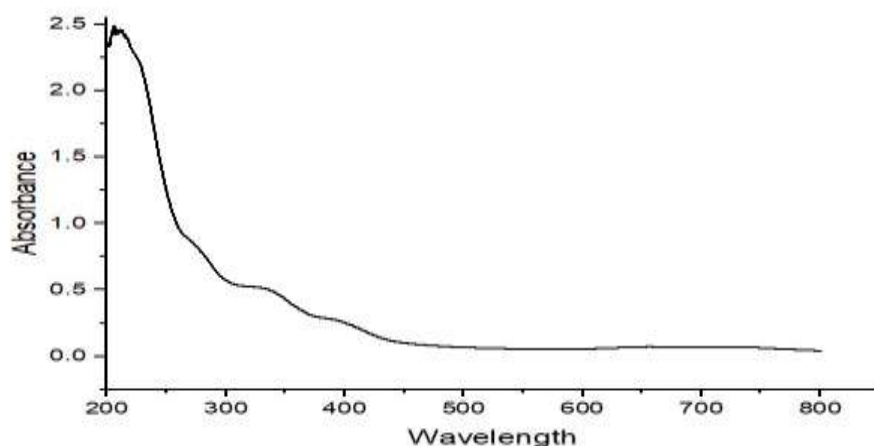


Figure 5: UV-Vis spectra of NiNPs.

Fourier transform infrared (FTIR) analysis

FTIR spectroscopy is an essential tool for identifying functional groups in synthesized Nickel nanoparticles, providing insights into the roles of various phytochemicals as reducing, stabilizing, and capping agents during the transformation of Nickel Sulfate to elemental Nickel. The FTIR spectrum clearly demonstrates the biofabrication process of Nickel nanoparticles mediated by *Eclipta alba* (E. Alba) plant extracts. In the FTIR spectra of *E. Alba* plant extract, major peaks are seen at 3235.32 cm⁻¹ which is typically associated with O-H stretching vibrations, 2907.32 cm⁻¹ indicative of C-H stretching vibrations, 1613.93 cm⁻¹ corresponding to C=O stretching vibrations, 1405 cm⁻¹ related to C=C stretching vibrations, 1323 cm⁻¹ associated with C-H bending vibrations and the peak at 827 cm⁻¹ corresponds to C-H bending vibrations. These peaks suggest the presence of various functional groups that participate in the reduction and stabilization of Nickel nanoparticles, showcasing the effective role of phytochemicals in the biofabrication process. (Egbosiuba et al., 2022, Sasi et al., 2024).

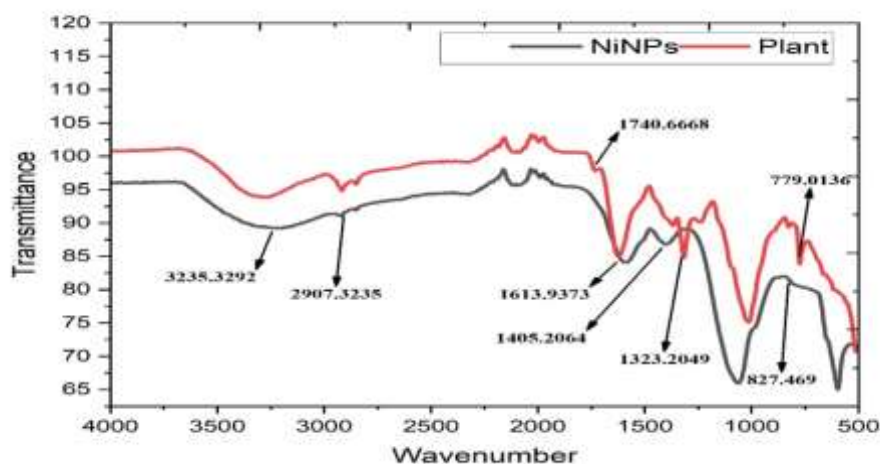


Figure 6: FTIR of nickel nanoparticles and *E.alba* plant.

Energy Dispersive X-ray Spectroscopy (EDX) Analysis

The elemental composition of the synthesized nickel nanoparticles (NiNPs), which were derived from the *Eliptica alba* plant, was analyzed using Energy Dispersive X-ray Spectroscopy (EDX). The EDX spectrum revealed a significant peak corresponding to nickel, confirming the presence of nickel in the nanoparticles. Alongside the nickel peak, there were also noticeable peaks for carbon (C), oxygen (O), and sulfur (S). These additional peaks are likely due to the biomolecules from the plant extract that were utilized during the synthesis process of the nanoparticles. These biomolecules from *Eliptica alba* contribute to the overall composition of the NiNPs, as indicated by the EDX analysis, as shown in the Figure. 7 (Kumar & Dixit 2017).

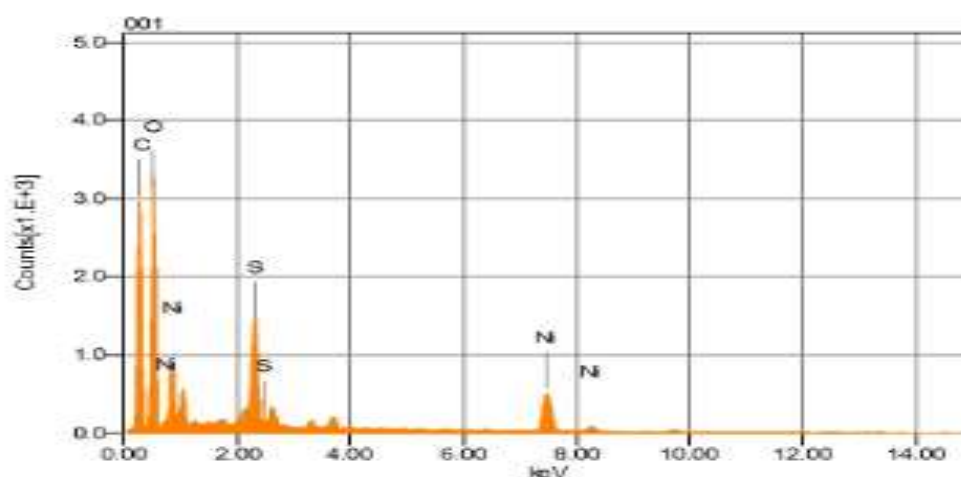
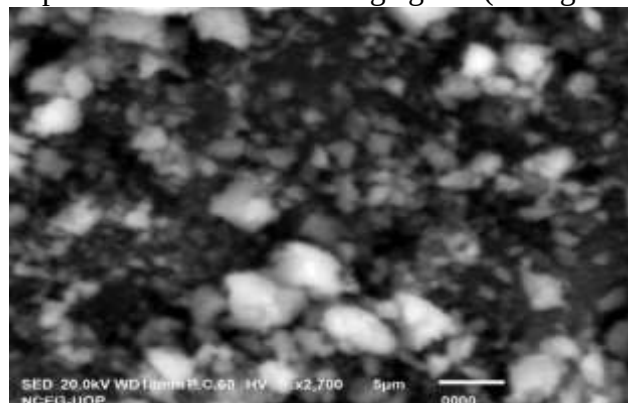


Figure 7: EDX of nickel nanoparticles

SEM Analysis:

The morphology of the Nickel nanoparticles (NiNPs) incorporated from *E. Alba* was inspected utilizing SEM as shown in Figure. The SEM images shows that the NiNPs are in random shapes including including irregular, spherical, pyramidal, rod-shaped, and with sizes between 18 to 72 nm. Particle shape variation is influenced by stabilizing agents used, stirring duration, and the concentration ratio of metal solution to plant extract with reducing agents (Zhang et al., 2020).



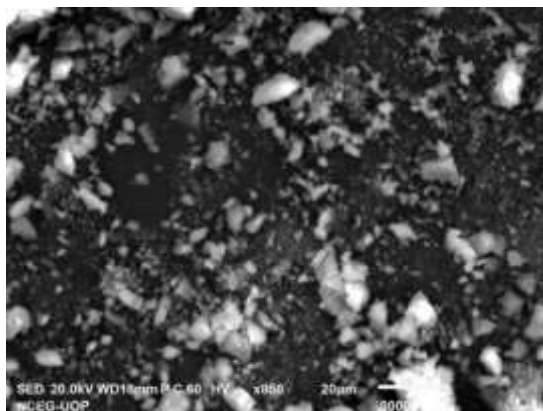


Figure 8: SEM images of nickel nanoparticles

Biological activities

The amalgamation, portrayal, and antimicrobial exercises of Nickel nanoparticles are examined in this section. Nickel nanoparticles were incorporated utilizing the concentrate of *E. Alba* plant and Nickel sulfate salt arrangement. For the green blend, 400ml of 20mM Nickel sulfate and 10ml of *E. Alba* plant extricate were joined with 90ml of refined water. The subsequent Nickel nanoparticles displayed a dull earthy colored tone. The shade of the *E. Alba* plant remove progressed from light orange to dull brown upon the expansion of Nickel sulfate and ensuing warming on a hotplate at 40C for 3 hours with constant blending. The adjustment of variety demonstrates the decrease of Nickel particles to NiNPs, as portrayed (Menezes et al., 2014).

Antibacterial activity

The zone of inhibition (in mm) of Ciprofloxacin, Nickel, and Plant extract against *Xanthomonas*, *E. coli*, and *Solanacearum* bacteria. Ciprofloxacin exhibited inhibition zones of 13 mm against *Xanthomonas*, 40 mm against *E. coli*, and 33 mm against *Solanacearum*. Nickel displayed varying zones of inhibition: 6, 8, and 10 mm against *Xanthomonas*; 9, 10, and 13 mm against *E. coli*; and 11, 12, and 14 mm against *Solanacearum*. Plant extract showed inhibition zones of 6, 9, and 12 mm against *Xanthomonas*; 8, 9.5, and 11.5 mm against *E. coli*; and 10.5, 12.5, and 12.5 mm against *Solanacearum*.

Table 1: Antibacterial activity of NiNPs by using *Eliptica alba* plant extract

Specimen	Conc.	Zone of Inhibition (mm)		
		<i>xanthomonas</i>	<i>E coli</i>	<i>Solanacearum</i>
Ciprofloxacin	13	40	33	34
NiNPs	6	9	11	10
	8	10	12	12
	10	13	14	14
Plant	6	8	11	10.5
	9	9.5	11.5	12.5
	12	11.5	13	12.5

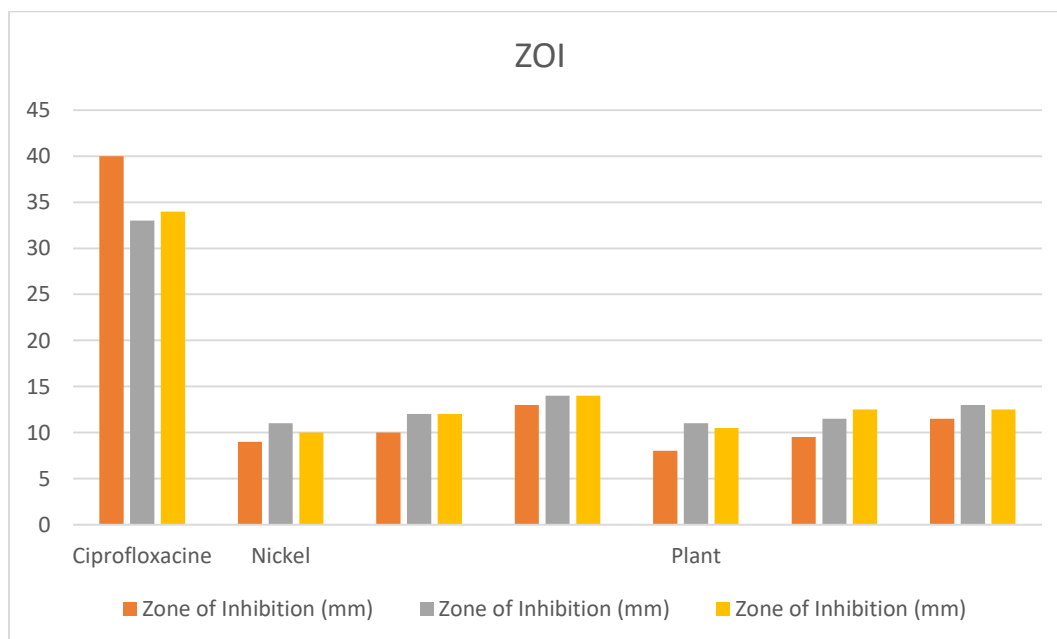


Figure 9: Antibacterial activity of NiNPs by using *Eliptica alba* plant extract
Antifungal activity

The zone of inhibition (in mm) of Nystatin, Nickel, and Plant extract against *Niger*, *Alternata*, and *oxysporium* fungal strain. Nystatin exhibited inhibition zones of 14 mm against *Niger*, 41 mm against *Alternata*, and 43 mm against *oxysporium*. Nickel nanoparticles displayed varying zones of inhibition: 7, 9, and 12 mm against *Niger*; 8.5, 10.5, and 12 mm against *Alternata*; and 9, 11.5, and 12 mm against *oxysporium*. Plant extract showed inhibition zones of 6, 9, and 12 mm against *Niger*; 7, 10.5, and 11.5 mm against *Alternata*; and 8, 7.5, and 10 mm against *oxysporium*.

Table 2: Antifungal activity of NiNPs by using *Eliptica alba* plant extract

Specimen	Conc. (mg/ml)	Zone of Inhibition		
		<i>Niger</i>	<i>Alternata</i>	<i>oxysporium</i>
Nystatine	14	41	43	37
NiNPs	7	8.5	9	11
	9	10.5	11.5	12.5
	12	12	12	16
Plant	6	7	8	8
	9	10.5	7.5	10
	12	11.5	10	11

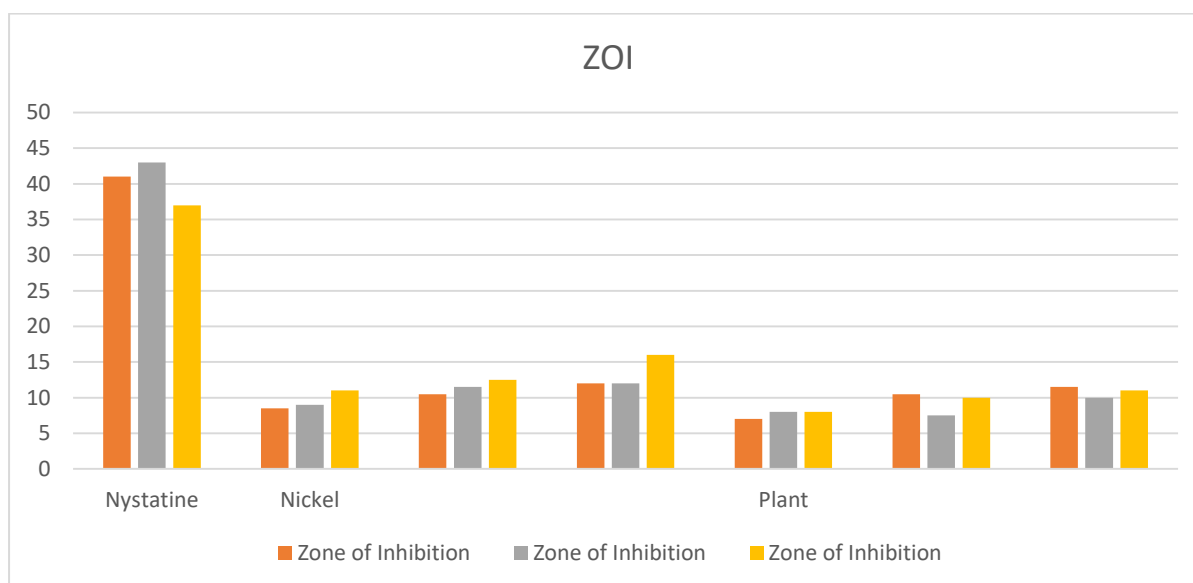


Figure 10: Antifungal activity of NiNPs by using *Eliptica alba* plant extract

CONCLUSION

This study indicates the aqueous extract of *Eliptca alba* plant used in the green production, characterization, and uses of nickel nanoparticles. Various conclusions have been drawn from the synthesis of NiNPs. Using nickel sulphate salt solution salt solution and *Eliptca alba* extract, a green synthesis technique that is both economical and environmentally beneficial was first used to create Co NP. The produced NPs were examined using a variety of methods, such as FTIR, and UV-visible spectroscopy, EDX and SEM. Each technique confirms the effective fabrication

NiNPs. The synthesized NiNPs were evaluated for their antimicrobial properties against different microbial strains which confirms that the synthesized nanoparticles effective antimicrobial agents and could potentially be used as effective antimicrobial agents.

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