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Antibacterial Activity of Nickel Nanoparticle from Calotropis Procera and Its Green Synthesis

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Abstract: *Antimicrobial drugs are a necessary element of daily living, but they are frequently misused by consumers. This, combined with microbes' innate ability to build resistance mechanisms quickly, has resulted in the creation and spread of multidrug-resistant pathogenic diseases. As a result, there is a critical need for innovative antimicrobial scaffolding to overcome these microorganisms' resistance. Nanotechnology has opened up new possibilities for the development of novel or enhanced antibacterial chemicals. The antibacterial activity of nickel nanoparticles synthesized from Calotropis procera is investigated in this study. The leaves of the Calotropis procera (Sodom apple) were collected in Agric Oke Osun, Osogbo, Osun State, Nigeria. Calotropis procera (Sodom apple) leaves were cleaned well and incised into small pieces with distilled-deionized water. Using a conventional process, a nickel nanoparticle was made from Sodom apple leaf extract. Scanning Electron Microscopy (SEM) was used to characterize the nanoparticle, which was made using a drop casting approach. The antibacterial properties of nickel nanoparticles and Sodom apple leaf extract were tested using Agar well diffusion methods against Staphylococcus aureus and Pseudomonas aeruginosa. The characterization of produced nanoparticles revealed that some particles have a rod shape and others have a hexagonal shape, which is the ideal morphology for nickel nanoparticles. For nickel nanoparticles, the maximum zone of inhibition was discovered against Pseudomonas aeruginosa (6mm) and the lowest degree of antibacterial activity was recorded against Staphylococcus aureus (2mm). The zone of inhibition of Calotropis procera leaf extract against Staphylococcus aureus and Pseudomonas aeruginosa was measured at 2 mm and 4 mm. The produced nanoparticles exhibited a broad antimicrobial susceptibility range, indicating that they might be used as promising antimicrobial agents in the pharmaceutical industry to control pathogenic germs.*

Keywords: Nickel, Nanoparticles, antibacterial agent, Sodom apple leaves, *Staphylococcus aureus* and *Pseudomonas aeruginosa*

1. INTRODUCTION

There is an increasing need to develop simple, cost-effective, dependable, biocompatible, and environmentally acceptable methods of nanomaterial fabrication that do not involve the use of hazardous chemicals. Green synthesis mediated by microbes has lately been regarded as a possible source for mining metallic nanoparticles (Gurunathan *et al*., 2009). Nanotechnology, which deals with the production, design, and manipulation of particles with approximate sizes ranging from 1 to 100 nanometers, has emerged as a new topic in the realm of biotechnology. Biomedical sciences, healthcare, drug–gene delivery, space industries, cosmetics, chemical industries, optoelectronics, and other fields all use nanoparticles (Khan *et al*., 2019).

Green nanoparticle synthesis is a cost-effective and ecologically friendly technology that has advantages over traditional processes that use potentially harmful chemicals. The solvent medium as well as the selection of environmentally safe reducing and stabilizing agents is the most essential concerns in green NP synthesis (Badr *et al*., 2008). Nanoparticles can be found in biomedical, catalysis, chemical industries, cosmetics, drug delivery, electronics, environment, energy science, food and feed, health care, mechanics, optics, space industries, non-linear optical devices, single-electron transistors, and photo-electrochemical applications (Zhang *et al*., 2020).

Nanoparticles have unique capabilities that are influenced by their size, shape, and morphology, allowing them to interact with plants, animals, and microbes (Siddiqi and Husen, 2017; Siddiqi and Husen, 2016). In addition to enzymes, a variety of plant components, such as flowers, leaves, and fruits (Husen and Siddiqi, 2014), have recently been employed for nanoparticle manufacturing. The technique of preparation, nature of solvent, concentration, strength of reducing agent, and temperature all affect the size, shape, and stability of nanoparticles (Siddiqi and Husen, 2016).

The top-down approach, in which a larger structure is broken down into smaller pieces using chemical, physical, and biological energy; and the bottom-up approach, in which material is synthesized at the atomic level using various chemical, physical, and biological reactions to produce a large nanostructure (Das *et al*., 2017). For the creation of nanoparticles, both physical and chemical methods are employed. Carcinogenicity, toxicity, and environmental toxicity are all possible risks associated with the use of harmful compounds (Gupta and Xie, 2018). The usage of hazardous compounds like reducing agents, organic solvents, and stabilizers has resulted in a significant increase in toxicity. Colloidal agglomeration is prevented by these substances. The use of harmful solvents and chemical contamination prevents nanoparticles from being used in a variety of clinical and biological applications (Hua *et al*.,

2018). To produce nanoparticles, a dependable, clean, physiologically acceptable, and environmentally benign approach is required (Kulkarni and Muddapur, 2014).

Numerous infections in hospitals are caused by gram-negative and gram-positive bacteria. Due to the continual growth in the number of multidrug resistant bacteria and virus strains due to mutation, pollution, and changing environmental circumstances, many researchers are interested in discovering novel and effective antimicrobial drugs (Fayaz *et al*., 2010). To get around this problem, scientists are working on developing medications to treat microbial infections (Siddiqi *et al*., 2018). Many metal salts and metal nanoparticles have been discovered to prevent the growth of a variety of pathogenic bacteria. Mutation, pollution, and changing environmental circumstances have all contributed to an increase in the number of multidrug resistant bacterial and viral strains (Siddiqi *et al*., 2018). Many metal salts and metal nanoparticles have been discovered to prevent the growth of a variety of pathogenic bacteria.

Plants are believed to be more suitable for green nanoparticle manufacturing than microorganisms because they are non-pathogenic and several pathways have been thoroughly investigated. Various plants have been used to manufacture a wide range of metal nanoparticles (Das *et al*., 2017). Plants and their parts contain carbohydrates, lipids, proteins, nucleic acids, pigments, and a variety of secondary metabolites that work as reducing agents to make nanoparticles from metal salts with no hazardous by-products (Siddiqi *et al*., 2018). Similarly, biomolecules found in microbes, such as enzymes, proteins, and biosurfactants, operate as reducing agents, allowing their nanoparticle formation to be verified. Bio-surfactants are utilized as capping and/or stabilizing agents in many bacterial strains, for example (Siddiqi *et al*., 2018).

Pseudomonas aeruginosa is a member of the Proteobacteria phylum (Madigan *et al*., 2006). *Pseudomonas aeruginosa* is one of mankind's most hazardous opportunistic infectious organisms (Engel, 2007). The bacteria *Pseudomonas aeruginosa* is mostly seen in hospitals. According to the Centers for Disease Control and Prevention (CDC), the overall rate of *P. aeruginosa* infection in US hospitals is around 0.4 percent (4 out of 1000 discharges), and the bacterium is the fourth most commonly isolated nosocomial pathogen, accounting for 10.1 percent of all hospital-acquired infections. *P. aeruginosa* is thought to be responsible for around 10% of all hospital-acquired infections, with fatality rates ranging from 20% to 70% in immune-compromised patients (Parsons *et al*., 2007).

Nickel has a powerful antibacterial property that has been used since antiquity. However, once antibiotics became more widely available, nickel's medical applications as an antibacterial decreased (Majeed and Wadee, 2019). Nickel's antimicrobial action can be enhanced by altering its size at the nanoscale. Nickel nanoparticles have emerged as antimicrobial agents due to changes in physiochemical properties, as well as their high surface-area-to-volume ratio and unique chemical and physical properties (Majeed and Wadee, 2019). Nickel nanoparticles with sizes ranging from 10 to 100 nm have been shown to be effective against gram-positive and gram-negative bacteria (Morones *et al*., 2005).

Many scientists have studied the bactericidal action of nickel nanoparticles against pathogenic, MDR, and multidrug-susceptible bacteria, and it has been proven that nickel nanoparticles are effective weapons against MDR bacteria like *Pseudomonas aeruginosa*, ampicillin-resistant *Escherichia coli*, erythromycin-resistant *Streptococcus pyogenes*, and methicillin-resistant *Staphylococcus aureus* (Majeed and Wadee, 2019).

The efficiency of antibiotics in the treatment of several diseases connected with infections from microbial sources has been hampered by the prevalence of multi-drug resistance. In order to put a stop to the problem, new treatments for microbial diseases must be found rather than relying on unreliable medications. As a result, the current study focuses on the antibacterial properties of nickel nanoparticles made from Calotropis procera.

2. METHODOLOGY

2.1 Preparation of Leaf Extract of Sodom apple (*Calotropis procera)*

The leaves of the *Calotropis procera* (Sodom apple) were collected at Agric Oke Osun, Osogbo, Osun State. All reagents were analytical grade from Sigma-Aldrich in the United States, and all experiments were conducted with distilled-deionized water. *Calotropis procera* (Sodom apple) leaves were collected and incised into small pieces after being properly cleansed with distilled-deionized water. About 10g of finely cut Calotropis procera (Sodom apple) leaves were weighed and placed in a 250 cm3 beaker with 100 cm3 of deionized water, which was thoroughly mixed and heated for 5 minutes. The obtained extract was filtered using Whatman No.1 filter paper, and the filtrate was collected in a 250 cm3 Erlenmeyer flask to be used later (Cristina *et al*., 2007).

2.2 Synthesis of Nickel Nanoparticles using the Plant Extracts of Sodom apple (*Calotropis procera*)

At a ratio of 1: 10 (v/v), 10 mL of each plant extract was mixed with 100 mL of aqueous nickel nitrate solution (0.5 - 2.0 mM). The resulting mixture was constantly stirred and gradually heated until the reaction solution took on a different color. Using a UV-Vis spectrophotometer, samples were taken at 5, 10, 15, 20, 30, 45, and 60 minutes of reaction time to evaluate the bioreduction of Ni+ ions to Ni0 (double beam thermo scientific GENESYS 10S model). To determine the absorbance peak, the sample was placed in a quartz cuvette and run at a resolution of 1 nm. The reducing, capping, and stabilizing agents were plant extracts (Ahmad & Sharma, 2012).

2.3 Isolation of the Biosynthesized Nickelnanoparticles

Centrifugation and multiple washings with distilled deionized water were used to separate nanoparticles from the reaction mixture. The biosynthsized nanoparticles were collected using a centrifuge model 0508-1 that was run at 5000 rpm for 30 minutes to collect the biosynthsized nanoparticles. To purify the nanoparticle suspension, it was redispersed in distilled deionized water to eliminate unbound organics, and then centrifuged for 10 minutes at 5,000 rpm. The suspension was dried in the oven and stored for later analysis. The supernatant was also maintained in a refrigerator at 4°C for toxicity testing and nanoparticle recovery. Isolation of nickel and nickel hybrid nanoparticles from other plant extracts was accomplished using this method (Shankar *et al*., 2004).

2.4 Characterization of Nickel nanoparticles synthesized by Sodom apple (*Calotropis procera*)

A drop casting approach was used to examine the size and shape of nickel nanoparticles using a scanning electron microscope (SEM). A Scanning electron microscope (SEM) model NOVA NanoSEM, operating at a working distance of 6–13 mm, 5–20 keV accelerating voltage, and 75–80 A emission current, was used to record the surface morphology of the nanoclusters. A small amount of black colloidal solution of l-cysteine capped nickel nanoparticles produced in ethylene glycol was applied to a cleaned glass cover slip as the SEM sample. The cover slip was carefully placed on a temperature-controlled electric hot plate that was already set at 100 °C to remove the solvent.

2.5 Antimicrobial Activity

2.5.1 Turbidity Standard for Inocula Preparation

Standardization of organisms for susceptibility testing followed the McFarland standard on laboratory guidelines. A modified approach developed by the British Society for Antimicrobial Chemotherapy was used to standardize inocula density for susceptibility testing (BSAC, 1990). The optical equivalent of $BaSO_4$ turbidity standards, comparable to 0.5 McFarland standards, was employed. To maintain a suspension, 0.5 mL of 0.048 M BaCl2 (1.175 percent w/v BaCl2 in 2H2O) was added to 99.5 mL of 0.18 M H2SO4 with constant stirring.A pg instrument UV-Vis spectrophotometer model T90+ with a 1 cm light path and matched cuvette was used to verify the right density of the turbidity standard at a wavelength of 625 nm. For a 0.5 McFarland standard, which is equivalent to 1.5 x 108 bacterial cells per mL, the acceptable range is 0.08–0.13. The standard was divided into screw cap tubes of the same size and volume, identical to how the bacterial inocula were grown or diluted. To prevent evaporation, the tubes were firmly sealed. They were then kept at room temperature in the dark. Before use, the turbidity standard was vigorously agitated in a vortex mixer. The standard is effective for six months; the appearance of large particles in the standard indicates that it is about to expire (BSAC, 1998).

2.5.2 Preparation of Inocula

The bacteria used in the study (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) were isolated from a clinical source (hospital samples). The strains were grown in Mueller Hinton broth, which was made by dispersing 5 mL of the prepared broth medium into each screw-capped test tube and sterilizing at 121 degrees for 15 minutes. In order to determine sterility, the test tubes were cooled and placed in an incubator for 24 hours at 37°C. The isolates were injected into sterilized test-tubes containing the medium and incubated at 37 degrees Celsius overnight. Turbidity in broth cultures was corrected to McFarland standards of 0.5. This was done in order to achieve a uniform suspension. To achieve turbidity optically comparable to the 0.5 McFarland standards or against a white background with a contrasting black line, sterile normal saline was added. The turbidity of the McFarland 0.5 standard was comparable to that of a bacterial culture containing 1.5 108 cfu/mL. The inocula resulted in semi-confluent colony growth after an overnight incubation period, indicating that denser inoculums result in a smaller zone of inhibition while lighter inoculums have the reverse effect (NCCLS, 1993).

Note: The suspension was used within 5 minutes so as to avoid population increase.

2.5.3 Sensitivity of Test Organisms

Using a modified version of Aida's method, the antimicrobial properties of biosynthesized nanoparticles were investigated via sensitivity testing (Aida, Rosa, Blamea, Thomas & Salvador, 2001). The test organisms were collected on a sterile agar slant and cultured for 24 hours at 37 degrees Celsius. The stock culture was then stored on a slant in the refrigerator at 4°C. The procedure followed the National Committee for Clinical Laboratory Standards' suggested guidelines (NCCLS).

2.5.4 Agar Well Diffusion Method

The antibacterial activity of produced nanoparticles was determined using the Aida modified well plate agar diffusion method (Aida et al., 2001). The microbial cultures were adjusted to 0.5 McFarland turbidity standards before being inoculated on a 9 cm Mueller Hinton Agar plate. Each of the standardized test organisms (1 mL) was flooded into the plate, which was then swirled. Excess inoculums was decanted with care. On the agar plates, sterile cork borers were used to make wells (6 mm in diameter). Aliquots of the nanoparticle dilutions (0.1 mL) were reconstituted in 50% DMSO at 100 mg/mL and applied to each well of the culture plates that had already been inoculated with the test organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). However, each extract was tested in duplicate with 0.1 mL of 5 g/mL ciprofloxacin as a positive control for bacteria, and then left on the bench for

1 hour to allow the nanoparticles to diffuse properly (NCCLS, 1993). After that, the plates were cultured for microorganisms for 24 hours at 37°C. The zone of inhibition around each well (except the diameter of the well) for each nanoparticle made from the plant extract was measured to determine antimicrobial activity. Each organism was subjected to two separate tests.

3. RESULTS AND DISCUSSION

3.1 SEM analysis of Nickel Nanoparticles

SEM was used to characterize the morphology of the produced nanoparticles. Figure 1 illustrates a SEM picture of NiO NP. The results demonstrate that some particles have a rod shape while others have a hexagonal shape, which is the ideal morphology for nickel nanoparticles. The particles are found to be aggregated and have an uneven shape with a diameter of 1 m. The mechanisms of $Ni(OH)_2$ and the end product NiO are influenced by the reaction system and factors.Based on reaction systems and characteristics, the urea acts as a hydrolysis agent (Ramar, 2015), generating OH ions along with Ni2+ to form Ni $(OH)_2$. By raising the temperature to 400°C, the Ni (OH) 2 is degraded into NiO.

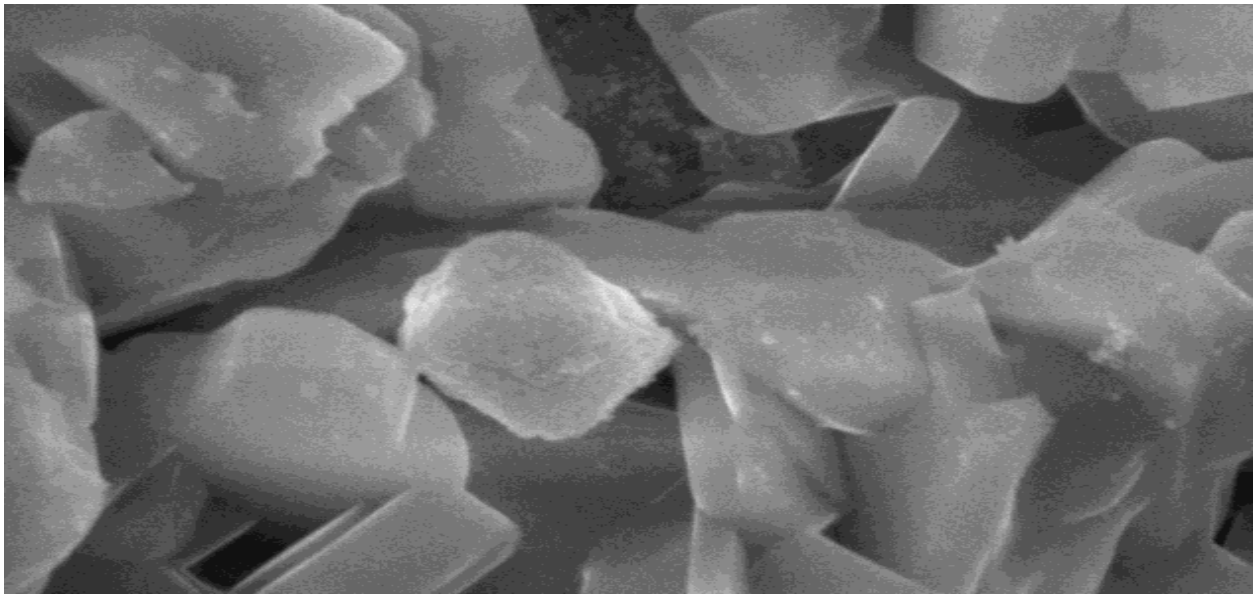

Figure 1: Morphological analysis of Nickel Nanoparticle by Scanning Electron Microscope (SEM)

3.2 Antimicrobial activity

The antibacterial activity of various doses of green produced nickel nanoparticles was tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in this work, and the results are shown in figure 1 and plate 1. At all concentrations, nickel nanoparticles demonstrated good efficacy against all species tested (Table 1). For nickel nanoparticles, the maximum zone of inhibition was discovered against *Pseudomonas aeruginosa* (6mm) and the lowest degree of antibacterial activity was recorded against *Staphylococcus aureus* (2mm) (Table 3). The zone of inhibition of leaves extract of *Calotropis procera* against Staphylococcus aureus and *Pseudomonas aeruginosa* was measured at 2 mm and 4 mm. The results above clearly showed that the produced nickel nanoparticles have bacterial inhibitory capabilities.

Plate 1: Zone of inhibition of antimicrobial activity of *Calotropis procera* and *Nickel* nanoparticle against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Table 3: Antibacterial activity of *Calotropis procera* and Nickel nanoparticle against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

ORGANISMS	**Nanoparticle**	*Calotropis procera* Extract
Pseudomonas aeruginosa	**6mm**	**2mm**
Staphylococcus aureus	**2mm**	**4mm**

4. CONCLUSION AND RECOMMENDATION

Conclusion

Due to the increasing demand for innovative, biocompatible antimicrobial agents, the development of greener options for nickel nanoparticle manufacturing has arisen as a major branch of nanotechnology. In the current investigation, nickel nanoparticles with appropriate physical properties were equally well synthesized from *Calotropis procera* leaf extract samples in the current investigation. In terms of yield, shape, and bioactivities, the nickel nanoparticles generated were comparable, implying that freeze-drying the leaf material could be effective in preserving it for future nanoparticle synthesis. Importantly, nickel nanoparticles exhibited a broad antibacterial susceptibility range, indicating that they could be used to control harmful germs in the pharmaceutical industry.

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