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Nikel Nanoparticle with Sunflower Plant Leaf Extract and Antimicrobial Potential against Clinical Bacterial Isolates

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Abstract: *Despite antimicrobial drugs' widespread efficacy against diseases, a major source of concern is that certain bacteria have acquired resistance to them as a result of their overuse. The current study focuses on the manufacture of nanoparticles from sunflower leaf extract and the evaluation of their potency against bacteria from a clinical source. Sunflower leaves were collected in open spaces in Akoda, Osun State, Nigeria. Using a conventional approach, a nickel nanoparticle was produced from a sunflower plant extract. Standard UV-visible spectroscopy techniques were used to characterize the nanoparticle. Using agar well diffusion methods, the antibacterial properties of nickel nanoparticles and sunflower leaf extract were investigated against E. coli and Proteus sp. According to the results of their characterization, the spectra of produced nanoparticles can be detected as two distinct peaks. The first peak, at 245 nm, relates to the formation of nickel nanoparticles, whereas the second peak, at 675 nm, corresponds to the formation of nickel nanoparticles. Leaves extract (sunflower) and manufactured nickel nanoparticles inhibited E. coli in zones of 5 mm and 2 mm, respectively. Leaf extract (sunflower) and manufactured nickel nanoparticles had no zone of inhibition against Proteus sp. Nickel nanoparticles made from plants were found to have antibacterial action against E. coli. They were cheap, eco-friendly, and cost-effective. As a result, the nickel nanoparticles that have been manufactured have been proven to be effective against clinical isolates.*

Keywords: Nickel, Nanoparticles, antibacterial agent, sunflower leaves, *E coli*, Proteus species

1. INTRODUCTION

Nanoparticles are categorized as nanocubes, nanoflowers, nanotubes, and nanowires, among other things, based on their morphology (Khatami *et al*., 2018e; Khatami *et al*., 2018a) Nanoparticles are classified as clusters, core shells, or bimetallic based on their structure (Karthik *et al*., 2018b; Khatami *et al*., 2018a). In a variety of industries, including food, cosmetics, agriculture, medicine delivery, cancer detection and diagnosis, cancer therapy, and many more (Iqbal *et al*., 2018b). Silver, platinum, gold, copper, magnesium, cobalt, cesium oxide, and zinc oxide are just a few of the metal nanoparticles and metal oxide nanoparticles found in nature (Iqbal *et al*., 2018b; Yang *et al*., 2018).
Due to their unique and interesting features (magnetic, optical, chemical, electronic, mechanical, and sensing properties), nanoparticles have a wide range of uses. Many features of metallic nanoparticles (MNPs) differ greatly from those of their bulk counterparts, including size, shape, surface effect, electrical, and magnetic properties (Han *et al*., 2017; Mayedwa *et al*., 2018). Nanotechnology has extended the possibilities for researchers, manufacturers, and consumers in practically every area over the last decade by allowing the engineering of functioning systems at the nanoscale level, primarily in the form of nanoparticles (Paramo *et al*., 2020). Nanoparticles are the building blocks of nanotechnology.

Nickel nanoparticles have attracted a lot of attention because of their unique magnetic, chemical, and physical properties, as well as their potential applications in a variety of technological fields, such as catalysis (Bibi *et al*., 2017), battery manufacture (Cheng *et al*., 2020), novel ink for nanotube printing (Abdel Fattah *et al*., 2016), incorporation in textiles (Jiao *et al*., 2019), enhanced pseudo-capacitance (Kiran *et al*., 2020).

Nickel nanoparticles, compared to other magnetic nanoparticles, have a lot of promise as catalysts, propellants, and sintering ingredients in coatings, polymers, and fibers (Hill *et al*., 2019). Because of its relative abundance in the earths crust (Bian *et al*., 2017), Ni is more cost-effective as a catalyst than most other metals. Nickel's electrical conductivity allows it to be used in a variety of applications (Sagasti *et al*., 2019). For instance, nickel nanoparticles are available in high purity, ultra-high purity, passivity, coated, and dispersed forms for usage as nanofluids (Wang *et al*., 2019). Nickel nanoparticles are used in lithium ion batteries, electrochromic test devices, super-capacitors, smart windows, photocatalysis for water remediation, electrochemical sensors, and chemical process catalysis (Sone *et al*., 2016; Khalil *et al*., 2018; Mayedwa *et al*., 2018).
Recent increases in microbe resistance to a variety of antibiotics, as well as rising health-care costs, have prompted the development of more cost-effective novel ways of producing nanoparticles with specific physical, chemical, and resistance qualities (Pankhurst *et al*., 2009). Nanoparticles' antibacterial properties have been linked to their small size and high surface-area-to-volume ratio, which allow them to interact closely with the membranes of viruses, fungi, and bacteria (Morones *et al*., 2007). Metal nanoparticles such as Ag, Cu, Ni, and Co (and their oxides) have previously been shown to have antimicrobial properties (Ravikumar *et al*., 2012). In the chemical manufacture of nanoparticles, stabilizing and protecting substances interact chemically with the surface of nickel nanoparticles, changing their shape, electrical, and magnetic properties (Liu *et al*., 2008).

In recent years, there has been a growing emphasis on green synthesis of metal nanoparticles due to its applicability in the use of nontoxic renewable chemicals and the reduction of created waste (Pandian *et al*., 2016). Polymeric nanoparticles, such as chitin nanoparticles, have also been discovered to be low-cost biodegradable materials that can be used to safeguard the environment (Dhananasekaran *et al*., 2016). However, the single-step production of plant-mediated nanoparticles for human medicinal applications is quick, inexpensive, environmentally friendly, and safe (Huang *et al*., 2007). As a result, in this study, sunflower leaf extracts were employed to produce nickel nanoparticles, and their antibacterial efficacy was tested against clinical bacterial isolates.

2. METHODOLOGY

2.1Plant collection

The plants (sunflower leaves) were obtained in Akoda, Ede South Local Government, Osun State, Nigeria, from open spaces. 7.42' 59.2" N and 4.27' 9.99" E are the latitude and longitude of the location, respectively.

2.2 Preparation of Leaf Extract

Sunflower leaves were carefully cleaned with distilled water before being used in the nanoparticle synthesis. Plant parts (leaves) were finely chopped and homogenized with a mortar and pestle for each plant. Extraction was done at room temperature with distilled-deionized water at a ratio of 1:5 (w/v). After that, the mixture was filtered through Whatman No. 1 filter paper. According to Ahmad and Sharma (2012), the filtrate was collected and stored at 4°C for nanonickel and hybrid syntheses. The technique was followed for each and every plant that was used.

2.3 Synthesis of Nickel Nanoparticles

At a ratio of 1: 10 (v/v), 10 mL of each plant extract was added to 100 mL of the various doses of aqueous nickel nitrate solution (0.5-2.0 mM). The resulting mixture was continually stirred and gradually heated until the color of the reaction solution changed. Using a UV-Vis spectrophotometer, samples were taken at 5, 10, 15, 20, 30, 45, and 60 minute intervals to monitor the bioreduction of Ni+ ions to Ni0 (double beam thermo scientific GENESYS 10S model). The absorbance peak was determined by placing the sample in a quartz cuvette with a resolution of 1 nm. Plant extracts were used as reducing, capping, and stabilizing agents in the experiment (Ahmad & Sharma, 2012).

2.4 Isolation of the Biosynthesized Nickelnanoparticles

Centrifugation and multiple washes with distilled deionized water were used to remove nanoparticles from the reaction mixture. The biosynthetic nanoparticles were gathered using a centrifuge type 0508-1 that was run at 5000 rpm for 30 minutes. To purify the nanoparticle suspension, it was redispersed in distilled deionized water to eliminate excess organics, then centrifuged for 10 minutes at 5,000 rpm. The suspension was dried in the oven and preserved for subsequent analysis. In addition, the supernatant was stored at 4°C for toxicity testing and nanoparticle recovery. This method was utilized to isolate nickel and nickel hybrid nanoparticles from plant extracts (Shankar *et al*., 2004).

2.5 Characterization of Nanoparticles

The sizes, shapes, and optical measurements were determined using a twin beam thermo scientific Genesys 10S UV-Vis spectrophotometer with wavelengths ranging from 200 to 800 nm (Wiley, McLellan, Siekkinen & Xia, 2006; Swarnalathan, Christina & Payas, 2012). Each aliquot sample taken at time intervals was placed in a quartz cuvette with a resolution of 1 nm, with distilled-deionized water as the reference solvent. A Perkin-Elmer 55 spectrophotometer was used to measure the photoluminescence of the biosynthesized nanoparticles. In a 1 1 cm quartz cell, the samples were inserted.

2.5.1 Sample Preparation for UV-Vis Spectroscopy

To make the black colloid, a freshly created solution of l-cysteine capped nickel nanoparticles was microwaved for 60 seconds (the color of the solution changed from clear/transparent to brown, then dark black). A set of tongs would be used to delicately remove the hot black colloidal dispersion from the microwave oven. The sample would then be swiftly chilled in an ice water bath, and UV-Vis spectra would be taken as soon as it reached room temperature. Using 1 cm quartz cuvets and a Perkin-Elmer Lambda 2 UV-Vis spectrometer, optical spectrum characterization of these colloids was carried out.

2.6 Antimicrobial Activity

2.6.1 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, 1998). The optical equivalent of BaSO4 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 H_2SO_4 with constant stirring. A pg instrument UV-Vis spectrophotometer model T90+ with a 1 cm light path and matched cuvette was used to determine the optical density of the turbidity standard at a wavelength of 625 nm. For the 0.5 McFarland standards, which is equivalent to 1.5 x 108 bacterial cells per mL, the permissible range is 0.08–0.13. The standard was divided into screw cap tubes of the same size and volume, much like the bacterial inocula were grown or diluted. To avoid evaporation, the tubes were firmly sealed. After that, they were kept at room temperature in the dark. Before usage, a vortex mixer was used to vigorously agitate the turbidity standard. The standard has a six-month potency; the emergence of big particles in the standard indicates that it is about to expire (BSAC, 1998).

2.6.2 Preparation of Inocula

The tested organisms (*E. coli* and *Proteus sp*.) came from a clinical source. 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 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 used. The turbidity of the McFarland 0.5 standard was comparable to that of a bacterial culture containing 1.5×10^8 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). To avoid population growth, the suspension was used within 5 minutes.

2.6.3 Sensitivity of Test Organisms

The antibacterial properties of the biosynthesized nanoparticles were studied using a modified version of Aida's approach (Aida, Rosa, Blamea, Thomas & Salvador, 2001). The test organisms were collected on sterile agar slants 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 recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).

2.6.4 Agar Well Diffusion Method

The antibacterial activity of produced nanoparticles was assessed 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 injected onto a 9-cm Mueller hinton agar plate. Each of the standardized test organisms (1 mL) was flooded into the plate and stirred. The inoculum that was left over was properly decanted. On the agar plates, a sterile cork borer was used to produce 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 previously been infected with the test organisms (*E. coli* and *Proteus sp*). However, each extract was tested in duplicate with 0.1 mL of 5 g/mL ciprofloxacin as a bacterium positive control; these were then placed on the bench for 1 hour to allow appropriate nanoparticle diffusion (NCCLS, 1993). The plates were then cultured for bacteria at 37°C for 24 hours. The antimicrobial activity of each nanoparticle obtained from the plant extract was assessed by measuring the zone of inhibition surrounding each well (excluding the well width). For each organism, double experiments were carried out.

3. RESULTS AND DISCUSSION

3.1 UV-Visible absorption of Nickel Nanoparticle

A method called UV-Visible absorption was used to investigate the optical characteristics of nickel nanoparticles (Figure 1). The sample was dispersed in distilled water for 10 minutes at a vortex. These spectra can be seen in two distinct peaks. Figure 2 shows the first peak, which corresponds to the creation of nickel nanoparticles (Manisha and Neetu, 2016), and the second peak, which is assigned to 675 nm (Figure 2). It could be the surfactant.

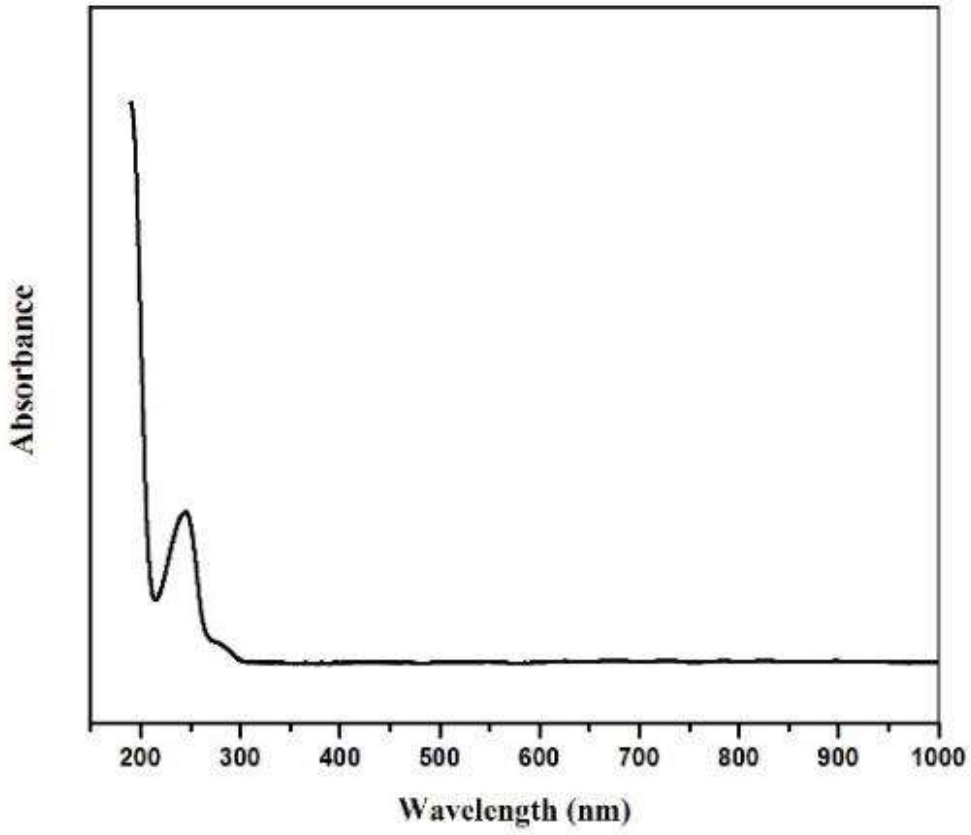

Figure 1: UV-Visible absorption profile of Nickel nanoparticles synthesized using sunflower leaves

3.2 Antibacterial Studies

The antibacterial activity of nanoparticles is typically thought to be proportional to the surface area in contact with microbes. At the surface of a chemical or substance, reactions take place. As a result of the smaller size and higher surface-to-volume ratio, or increased surface area, there is more interaction with the bacteria. Against *E. coli*, leaf extract (sunflower) and manufactured nickel nanoparticles produced inhibitory zones of 5 mm and 2 mm, respectively. This suggests that nanoparticles are more effective at inhibiting *E. coli* than sunflower leaf extract (Plate 1 and Table 1). Leaf extract (sunflower) and manufactured nickel nanoparticles had no zone of inhibition against *Proteus sp.* According to the findings (Figure 1), both manufactured nickel nanoparticles and sunflower leaf extract are ineffective in inhibiting *Proteus sp*., according to the findings.

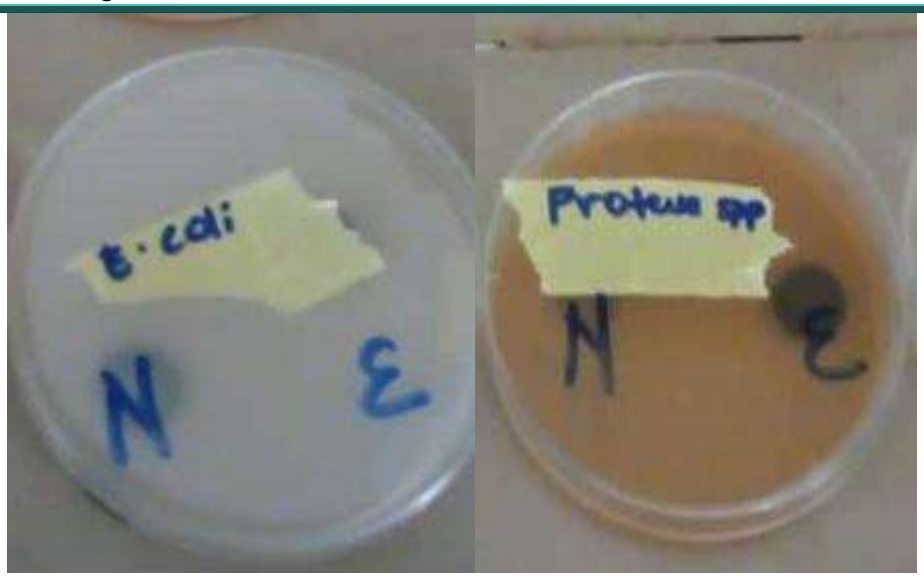

Plate 1: Zone of inhibition of leaves extract of sunflower and synthesized Nickel nanoparticle against *E.coli* and *Proteus s.*

Table 3: Antibacterial activity of sunflower leaves extract and Nickel nanoparticle against *E.coli* and *Proteus species*

ORGANISMS	Nanoparticle	Sunflower Extract
E.coli	**5mm**	**2mm**
Proteus species	**Nil**	**Nil**

4. CONCLUSION

The nature of the plant, such as its phytochemical composition, particular adaptability, and medicinal relevance, influences nanoparticle synthesis. In this study, we used sunflower leaf to evaluate an environmentally friendly and cost-effective green production of nickel nanoparticles. By converting nickel ions to nanosized nickel particles, water-soluble organic chemicals found in leaf and callus extracts were primarily responsible for the creation of nickel nanoparticles. The antibacterial activity of the nickel nanoparticle was then tested against E. coli and Proteus species. In comparison to gram-negative bacteria, Proteus species displayed resistance to sunflower leaf extract and nickel nanoparticles (*E. coli*). According to UV-visible spectroscopic investigations, the nickel nanoparticles generated were found to be crystalline in nature, spherical in shape, with sizes ranging from 42 to 50 nm, and stable. The antibacterial efficacy of the produced nickel nanoparticles and sunflower leaf extract against E. coli was shown, but not against Proteus species. Chemical, physical, and microbial-mediated methods for producing nickel nanoparticles can all be replaced with this green, economical, and simple method.

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