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BIOGENIC SYNTHESIS OF NICKEL IRON OXIDE NANOPARTICLES AND ITS ANTI-MICROBIAL STUDIES USING NEEM *(Azadirachta indica)* LEAF EXTRACT

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ABSTRACT

The biosynthesis of nickel ferrite ($NiFe_2O_4$) nanoparticles is investigated in this work using an aqueous extract of neem (*Azadirachta indica)* leaves as a natural capping agent. The antibacterial properties of the nanoparticles are assessed in vitro against S*almonella typhi, Pseudomonas aeruginosa, Escherichia coli*, and *Staphylococcus epidermidis*. Active substances including flavonoids, alkaloids, terpenoids, saponins, steroids, and tannins were confirmed by phytochemical screening to be present in the neem extract, indicating their possible function in the creation and stability of nanoparticles. Using a green process that involved calcination and metal salt reduction, $NiFe_2O_4$ nanoparticles were created, and their structural characteristics were described using SEM, FTIR, and UV-Vis studies. FTIR verified the presence of organic surface functional groups and Ni–Fe–O bonding, while UV-Vis spectroscopy demonstrated distinctive d-d transitions. SEM pictures showed micro-sized crystalline morphology. Using disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) experiments, antibacterial activity was evaluated. Broad-spectrum antibacterial activity was demonstrated by both neem extract and $NiFe_2O_4$ nanoparticles, with the nanoparticles showing larger inhibition zones. When compared to neem extract, $NiFe_2O_4$ showed better potency, with MIC and MBC against *S. aureus* and *P. aeruginosa* as low as 25 mg/mL. These results imply that $NiFe_2O_4$ nanoparticles mediated by Neem may be excellent options for antibacterial uses, especially against species of bacteria that are resistant. We make use of neem leaves extract as capping agent to control the synthesis of the $NiFe_2O_4$ nanoparticle and explore significant in vitro potential application against *S.epidermidis, P.aeruginosa, E. coli, and salmonella typhi*.

Keywords: Nanoparticles, Nickel Iron Oxide, *Azadirachta indica*, Green synthesis, Antibacterial activity, Phytochemical analysis

INTRODUCTION

Nanotechnology has garnered significant interest over time. The study and the development of atoms, molecules, or macromolecules at nanoscale is known as nanotechnology (Biswas & Yu Wu, 2012). The fundamental unit of nanotechnology is nanoparticles. Nanoparticles are organic or inorganic, metal, carbon, or metal oxide particles that are between one and one hundred nanometers in size. Hasan S. (2015).The nanoparticles exhibit unique physical, chemical, and biological properties at the nanoscale that set them apart from their counterparts at greater dimensions. The origins of these phenomena include increased mechanical strength, more stability or reactivity in a chemical reaction, a relatively larger surface area to volume, etc. Nanoparticles are employed in a wide range of applications due to these properties. The nanoparticles differ in size, shape, and dimensions in addition to their composition (Cho *et al.*, 2013). There are four different kinds of nanoparticles: one-dimensional (like graphene), two-dimensional (like carbon nanotubes), three-dimensional (like gold nanoparticles), and zero-dimensional (like nano dots), which have length, width, and height fixed at one point. Different nanoparticles have different sizes, shapes, and structures. They can be irregular or spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc., and range in size from 1 to 100 nm. The surface may be uniform or uneven, depending on surface differences. One or more crystal solids may be free or clumped together in crystalline or amorphous nanoparticles. Nanotechnology is the key to a clean and sustainable future (Ealia, & Saravanakumar, 2017)

Generally speaking, nanoparticles fall into three classes: the organic (such as ferritin, liposomes, dendrimers, and micelles) (Tiwari *et al.,* 2008). Inorganic nanoparticles that are typically classified as metal and metal oxide-based nanoparticles, Carbon-based particles, which are made completely of carbon (Bhaviripud *et al.*, 2007). They are classified as fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers, carbon black, and even activated carbon at the nanoscale (Ealia, & Saravanakumar, 2017).

Potential Applications

Strong antibacterial activity is demonstrated by the green-synthesised $NiFe_2O_4$ nanoparticles made with Neem (*Azadirachta indica)* extract, suggesting that they have potential for a number of practical uses. These consist of:
Cosmetics and sunscreens; active ingredient delivery (Hodoroaba *et al.*, 2014), electronics; used in transistor (Teng *et al.*, 2008), catalysis; used in fuel cells (Crooks *et al.*, 2001), medicine; used in drug delivery (Ganesh & Archana, 2013), food; used in food packaging (Laad & Jatti, 2016), renewable energy; increasing solar cells efficiency (Syukri & Singh, 2024) etc
Neem *(Azadirachta indica)* is a plant that belongs to the Indian subcontinent's Meliaceae (Mahogany) family (Chopra *et al.*, 1952). Later, it was brought to numerous tropical nations in Africa and America, where it now stands in populations of 18 to 30 million trees (Mathieu *et al.*, 2007). Neem is a small to medium-sized, evergreen tree that grows quickly (5 to 20 m high). It usually loses most of its leaves during the dry season before blooming in full foliage. A broad variety of geographic and climatic circumstances can be

tolerated by the tree. It grows well in shallow, dry, stony soils, as well as in hard clay or calcareous soils. According to (Keithure *et al.*, 2015) and (Ogbuewu *et al.*, 2011), neem trees need little water and lots of sunlight.

Neem trees have been shown to grow affixed to the roots of native plants in Katsina State, where they compete with them for water and nutrients (Bello *et al.*, 2010, 2019). This stresses the native trees and causes them to die sooner. Neem has been shown to be allelopathic in other places (Bello *et al.*, 2010) Judd. (2004), harmful to a wide variety of plant species, and a fierce rival. According to Rice. (1984), allelopathy is the detrimental effect of one plant or microbe on another. Thus, the purpose of this study was to look into how the Neem tree affected the local species in Katsina State's savanna area.

Figure 1: Neem *(Azadirachta indica)* leaves

In this research we make use of neem leaves extract as capping agent to control the synthesis of the $NiFe_2O_4$ nanoparticle and test it's in vitro potential application against *S.epidermidis, P.aeruginosa, E. coli, and salmonella typhi.*

Background of the Study

The development of bacterial strains resistant to antibiotics has prompted a pressing quest for substitute antimicrobial medicines. A promising path is provided by nanotechnology, especially the creation of metal oxide nanoparticles, because of the special physicochemical characteristics of materials at the nano scale. For their antibacterial, catalytic, and magnetic properties, nickel ferrite ($NiFe_2O_4$) nanoparticles have garnered interest. However, traditional synthesis techniques frequently use a lot of energy and hazardous substances. A sustainable method for getting over these restrictions is green synthesis, which uses plant-based extracts. An eco-friendly alternative for the manufacture of nanoparticles is (*Azadirachta indica)*, also known as Neem, a medicinal plant that is abundant in bioactive chemicals. This study aims to contribute to the creation of safe and effective nano materials for biomedical applications by examining the biosynthesis of $NiFe_2O_4$ nanoparticles using Neem leaf extract and assessing their antibacterial activity.

Research Gap

Few research have examined the combined usage of $NiFe_2O_4$ nanoparticles and Neem (*Azadirachta indica)* using green synthesis techniques, despite the latter's promising potential and *Azadirachta indica's* well-known antibacterial qualities. The structural properties and antibacterial activity of Neem-mediated $NiFe_2O_4$ nanoparticles, especially against clinically significant pathogens, have not been thoroughly studied.

MATERIALS AND METHODS

Every chemical utilized was of analytical quality and were used withoutany additional purification.

Method

The methodologies adopted in this study include extraction, phytochemical screening, nanoparticles synthesis and antimicrobial analysis:

Collection of Neem leaves sample

The Neem leaves, were collected from Kofar Kwaya behind Sir, Usman Nagogo College of Arabic and Islamic Studies, (SUNCAIS) Katsina State Nigeria, and their identity were confirmed by botanist, in the Department of Biology Faculty of Natural and Applied Science, Umaru Musa Yar'adua University Katsina.

Extraction method

The obtained leaf samples were put into a mortar and processed into a powder. Then1040 milliliters of boiling distilled water were mixed with 40 grams of the leaf powder. For three days at room temperature, the mixture was put into a 500 ml conical flask that was covered with aluminum foil to prevent evaporation and light exposure. For twenty-four hours, the Ericsmeyer flask was set up on a platform shaker at 70 rpm. A filter was used to filter the mixture, and a magnetic stirrer was used to stir it (Balamurugan *et al.*, 2019).

Phytochemical Analysis of Neem (*Azadirachta indica)* leaves extract

Chemical test for the identification of bioactive chemical constituents in the neem leaves extract was carried out as shown in the following procedure below:

Test for Phenols and Tannins
When 2 milliliters of the extract were combined with 3 milliliters of a 2% $FeCl_3$ solution in a test tube, the blue hue would be visible. Sivanandham, (2015).

Test for Terpenoids (Salkowski's Test)
The 3 milliliters of chloroform were combined with 2 milliliters of the extract. After that, 3 milliliters of sulfuric acid concentrate was added with caution and shook gently. A reddish-brown tint that developed during the interphase indicated that terpenoid was present (Balamurugan *et al.,* 2019).

Test for Glycosides
The Three milliliters of glacial acetic acid containing three drops of 2% $FeCl_3$ were combined with two milliliters of the extract. The mixture was transferred to another test tube that included three milliliters of sulfuric acid concentrate. Glycosides were identified by a brown ring at the interphase (Balamurugan *et al.,* 2019).

Test for Flavonoids (Shinoda Test)
The Five magnesium ribbon pieces were combined with two milliliters of the extract, and a drop of strong hydrochloric acid was added while the color change was being observed. Flavonoids were recognized by pink or orange coloring (Balamurugan *et al.*, 2019).

Test for Saponins
After placing 4 milliliters of the solvent extract in a test tube and giving it a good shake, the observations were noted. The presence of saponins was suggested by the production of a stable form (Balamurugan *et al.,* 2019).

Biogenic synthesis of $NiFe_2O_4$ Nanoparticles Using *Azadirachta indica* (Neem leaves) Extract
In a 250 mL flask, the biosynthesis of $NiFe_2O_4$ NPs using an aqueous extract of Neem (*Azadirachta indica*) was started using 4.0 mM of $NiCl_2$ and $FeCl_3$ each. 150 mL of Neem (*Azadirachta indica*) extract solution was first heated to 95°C. Next, $NiCl_2$ solution was added gradually while being vigorously stirred, and the reaction was allowed to proceed in the dark for two hours. The reaction solution's brownish hue gave way to a blackish suspension. After that, 100 mL of Neem (*Azadirachta indica*) extract was added to the reaction suspension for two hours while the $FeCl_3$ solution was gradually added while retaining the previous reaction temperature. As a result, the reaction suspension was cooled, and 1.0M NaOH solution was continuously added to the acidic reaction suspension until the pH reached about 10. After obtaining the precipitant solution, it was centrifuged at 4,000 rpm. The precipitate was rinsed three times with methanol and several times with double-distilled water. The resulting precipitate was cleaned and then dried in an oven. A mortar and pestle were used to crush the dried goods into a fine powder. For two hours, the powder was calcined at 700°C. Several methods were used to acquire and characterize the calcined powder of $NiFe_2O_4$ NPs (Loo *et al.,* 2018).

Anti-bacterial activity
Preparation of Inoculates
In order to create final inoculums, the bacterial strains of S. epidermidis and P. aeruginosa from the culture plates were standardized by comparing the turbidity of the culture to 0.5 McFarland standards. These were then diluted in fresh broth (peptone water) and incubated at 37°C for 24 hours (Ezouberi *et al.,* 2005).

Serial Dilution
To achieve a concentration of 100 mg/ml, one (0.5g) of the extract and synthesized nanoparticles were weighed using a balance and then poured into a sterile, clean test tube with five milliliters of distilled water. It was then transferred to another test tube with 2.5 milliliters to obtain 50, 25, and 12.5 mg/ml, and it was stored for additional analysis (Ezouberi *et al.,* 2005).

Discs preparation
After using a puncher to create filter paper with a diameter of about 6 mm, it was autoclaved for 15 minutes at 1210 °C, poured into each concentration, and left to absorb for an hour (Bauer *et al.,* 1966).

Sensitivity test using disc diffusion method
The solidified Muller Hinton agar was inoculated with 0.2 cc of each of the 39 grams of (MHA) that were manufactured. The dish remained on the bench. Ciprofloxacin was used as a control, and discs with varying concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml were seeded. The diameter zone of inhibition was then measured after the plate was incubated for 24 hours at 37 °C (Ezouberi *et al.,* 2005).

Determination of minimum inhibitory and minimum bactericidal concentration
The standardized bacterial inoculum was added to test tubes containing 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml of the liquid medium and extract using dilution tube procedures. After that, the tubes were incubated aerobically for 24 hours at 37°C with an equal setup for the positive control. MIC was found in the tubes that grew the least. The lowest dose of the chemotherapeutic drug that prevented the subculture from growing was recorded when the MIC were subcultured into nutritional agar plates devoid of antibiotics. Thins stands for the chemotherapeutic agents' MBC (Ezouberi *et al.,* 2005).

RESULTS AND DISCUSSION
Results
Table 1: Result for Qualitative Analysis of Leaves of *Azadirachta indica* (Neem)

Phytochemical Test	Leaves
Alkaloid	Positive (+)
Flavonoids	Positive (+)
Saponins	Positive (+)
Terpenoids	Positive (+)
Steroids	Positive (+)
Tannins	Positive (+)

From table 1, the leaves extract shows the presence of alkaloid, terpenoids, flavonoids, saponins, tannins, steroids denoted by the symbol '+'. The secondary metabolites present will be the natural capping agents that will be supplied by the Neem leave extract to influence the formation of the Nickel Iron Oxide nanoparticles as reported by (Raj & Lawerence, 2018).

UV Analysis Result

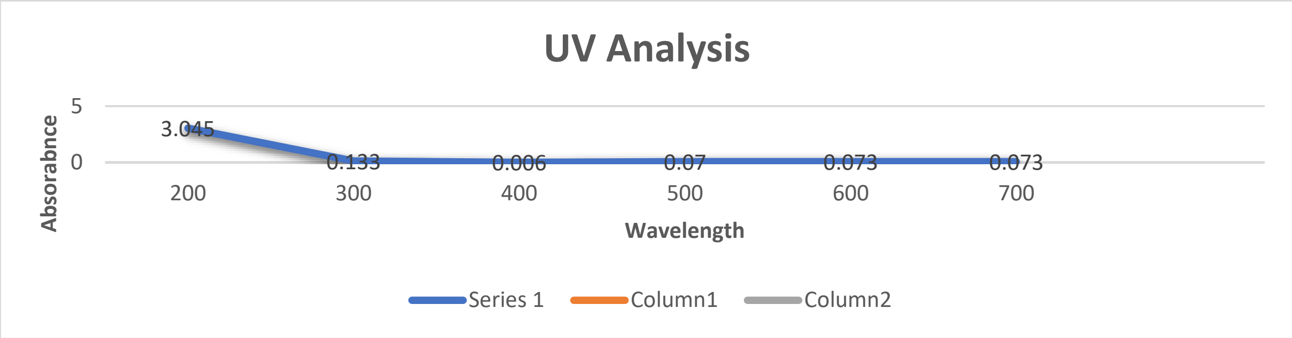

Figure 2. UV-Analysis Result

According to the literature reported by (Raj & Lawerence, 2018), the wavelength range covers from 200nm to 800nm both UV and visible region.
At 200nm, the peak typically indicates transition from bonding pi to ant-bonding pi orbital, with the absorbance of 3.045 commonly associated with aromatic rings, C=C bonds. At 300nm, the peak often corresponds to the transitions from non-boding electron to anti-bonding pi orbital, with the absorbance of 0.133 likely from C=O. At 400nm – 500nm, the peak indicates the d-d transition in synthesized nanoparticles. At 500nm – 700nm, No significant broad peak revealed.

FTIR of the Synthesized $NiFe_2O_4$
Table 2: Wavenumber and intensity

Peak Number	Wavenumber (cm^{-1})	Intensity
1	1028.74524	84.79077
2	1416.38838	98.91546

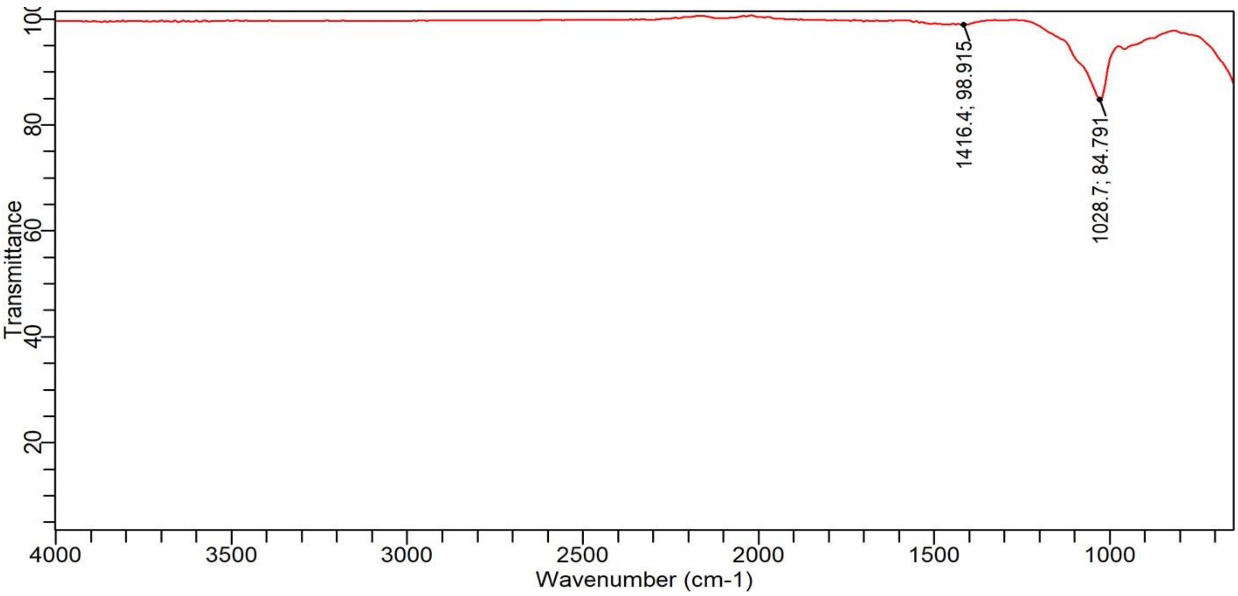

Figure 3: FTIR of the Synthesized $NiFe_2O_4$

It was approximately reported by (Raj & Lawerence, 2018) that the, absorption peak at 1028.74524 cm^{-1} confirms the presence of Ni-Fe-O bond. The higher wave number range was due to the presence of surface impurities in the sample. The FTIR analysis also suggests that the Nickel Iron Oxide nanoparticles may have a surface modification or capping with organic compounds from the Neem leaves extract. This surface modification may influence the optical, electrical, and catalytic properties of the synthesized nanoparticles.

SEM of Synthesized $NiFe_2O_4$

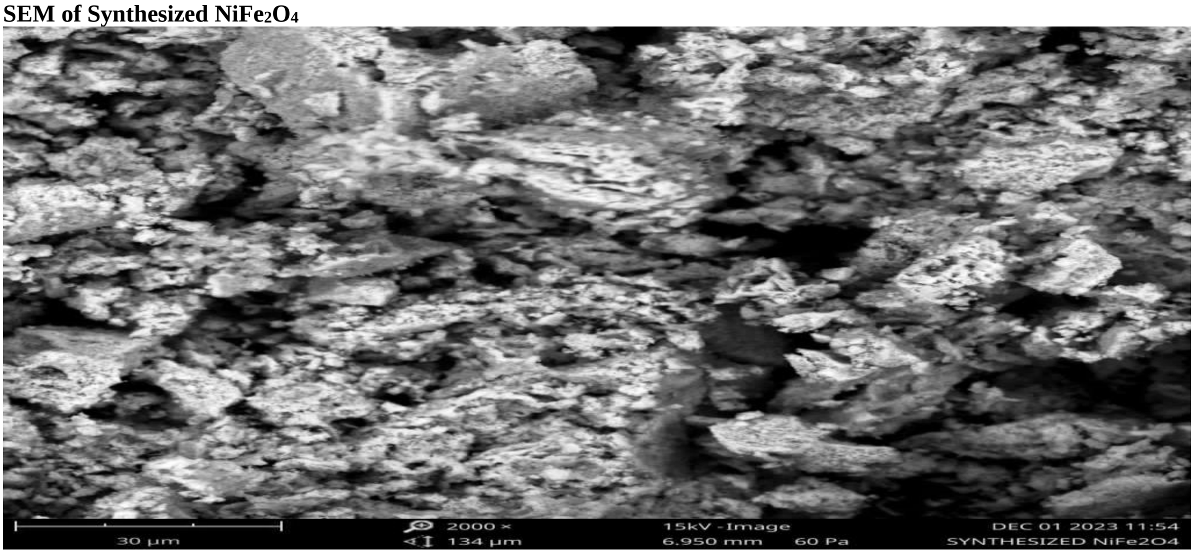

Figure 4: Photomicrograph of SEM analysis of synthesized $NiFe_2O_4$ nanoparticles at 200,00x magnification scale in the size range of 134 µm

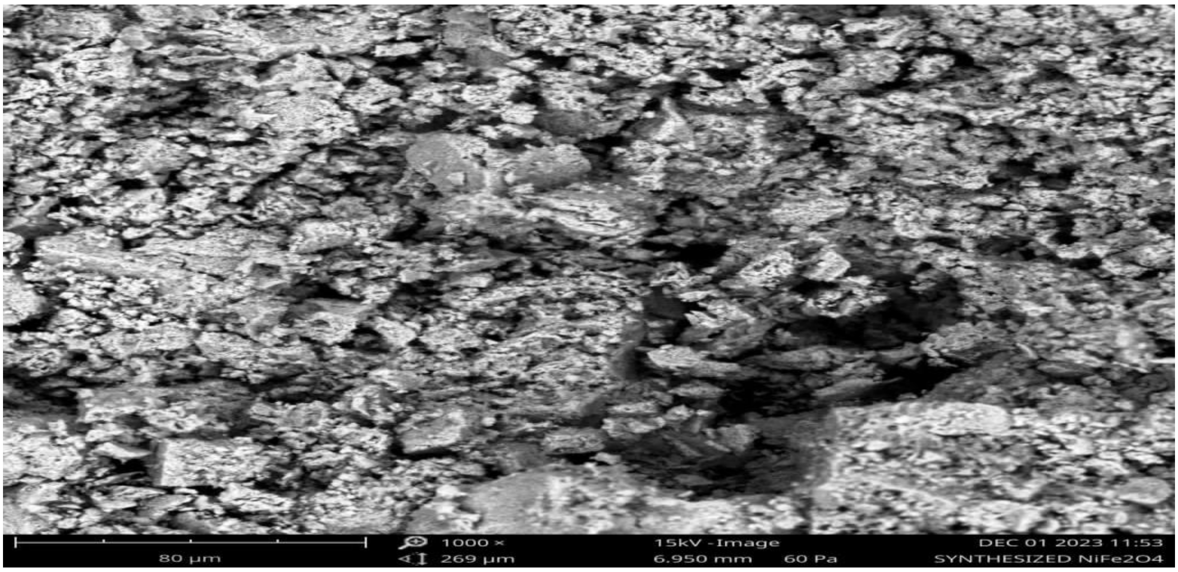

Figure 5: Photomicrograph of SEM analysis of synthesized $NiFe_2O_4$ nanoparticles at 1000x magnification scale in the size range of 269 µm

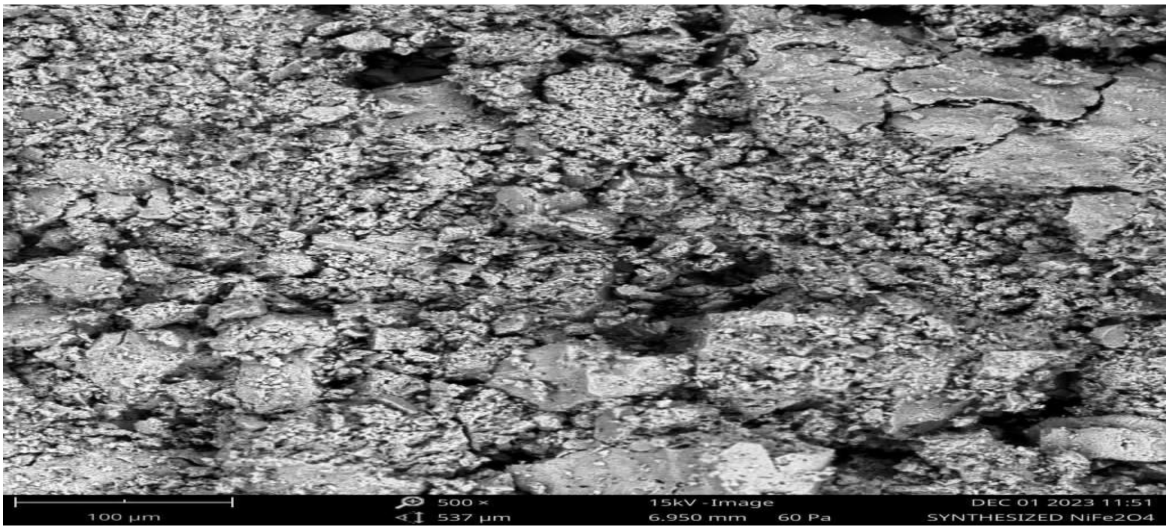

Figure 6: Photomicrograph of SEM analysis of synthesized $NiFe_2O_4$ nanoparticles at 500x magnification scale in the size range of 537 µm

The scanning electron microscope revealed the morphological characteristics of the synthesized Nickel Iron Oxide nanoparticles, displaying the presence of spherical crystals of synthesized Nickel Iron Oxide nanoparticles crystals according to the literature reported by (Raj & Lawerence, 2018). This suggested the how particle size analysis predicts the actual size of the synthesized Nickel Iron Oxide nanoparticles crystals.

Antibacterial Activity

Antibacterial Activity of Neem leaves Extract

Table 3: Zone of inhibition

Organisms	100 mg/ml	50 mg/l	25 mg/ml	12.5 mg/ml	Controls
S.epidermidis	11	9	8	ND	25
P.aeruginosa	13	10	9	7	29
S.aureus	13	10	8	7	24
Salmonella typhi	11	8	6	7	26
E.coli	11	10	8	7	26

Key: ND =Not detected

Table 4: Antibacterial activity of Neem Leaf Extract (Zone of Inhibition in mm, Mean ± SD)

Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
S. epidermidis	11.3 ± 0.4	9.0 ± 0.5	8.0 ± 0.2	ND
P. aeruginosa	13.1 ± 0.3	10.1 ± 0.4	9.1 ± 0.3	8.0 ± 0.2
S. aureus	11.4 ± 0.5	10.2 ± 0.3	8.1 ± 0.2	7.1 ± 0.3
Salmonella typhi	11.4 ± 0.4	10.0 ± 0.2	6.1 ± 0.3	7.2 ± 0.4
E. coli	11.2 ± 0.4	10.1 ± 0.3	8.0 ± 0.3	7.0 ± 0.2

Key: ND =Not detected

Table 5: Minimum inhibitory Concentrations

Organisms	12.5 mg/ml	25 mg/l	50 mg/ml	100 mg/ml	Controls
S.epidermis	-	-	+	+	+
P.aeruginosa	+	+	+	+	+
S.aureus	-	+	+	+	+
Salmonella typhi	-	+	+	+	+
E.coli	-	-	+	+	+

Key: + = Absence of growth

- = Presence of growth.

According to the literature reported by (Bindhu & Umadevi, 2014), the minimum inhibitory concentration (MIC) of a Neem leaf extract reveals the following;

The *S.epidermis* grows at 12.5 and 25mg/ml but the growth inhibited by Neem leaves extracat at 50 and 100mg/ml. Therefore, the minimum inhibitory concentration (MIC) of *S.epidermis* against Neem leaves Extract is = 50mg/ml.

The *P.aeruginosa* does no grow at all concentration, including the lowest concentration.

The *S.aureus* grows at 12.5mg/ml but the growth inhibited by Neem leaves extracat at 25mg/ml, 50gm/ml and 100gm/ml. Therefore, the minimum inhibitory concentration (MIC) of *S.aureus* against Neem leaves extract = 12.5mg/ml.

The *Salmonella typhi* grows at 12.5gm/ml but the growth inhibited by Neem leaves extracat at 25mg/ml, 50gm/ml and 100gm/ml. Therefore, the minimum inhibitory concentration (MIC) of *Salmonella typhi against* Neem leaves extract = 12.5mg/ml.

The *E.coli* grows at 12.5mg/ml and 25mg/ml but the growth inhibited by Neem leaves extracat at 50gm/ml and 100gm/ml. Therefore, the minimum inhibitory concentration (MIC) of *E.coli against* Neem leaves extract = 50gm/ml.

Table 6: Minimum Bactericidal concentrations (MBC)

Organisms	12.5 mg/ml	25 mg/l	50 mg/ml	100 mg/ml	Controls
S.epidermidis	-	-	-	+	+
P.aeruginosa	-	-	+	+	+
S.aureus	-	+	-	+	+
Salmonella typhi	-	-	-	+	+
E.coli	-	-	+	+	+

Key: + = completely killed

- = presence of growth

Based on the literature reported by (Bindhu & Umadevi, 2014), the minimum bactericidal concentration (MBC) of a Neem leaf extract reveals the following;

The *S.epidermis* was completely killed by Neem leaves Extract at 100mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *S.epidermis* against Neem leaves Extract = 100mg/ml.

The *P.aeruginosa* was completely killed by Neem leaves Extract at 50mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *P.aeruginosa* against Neem leaves extract = 50mg/ml.

The *S.aureus* was completely killed by Neem leaves Extract at 100mg.ml. Therefore, the minimum bactericidal concentration (MBC) of *S.aureus* against Neem leaves extract = 100mg/ml.

The *Salmonella typhi* was completely killed by Neem leaves Extract at 100mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *Salmonella typhi against* Neem leaves extract = 100mg/ml.

The *E.coli* was completely killed by Neem leaves Extract at 50mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *E.coli against* Neem leaves extract = 50gm/ml.

Antibacterial Activity of a Synthesized Nickel Iron Oxide Nanoparticles, $NiFe_2O_4$

Table 7: Zone of inhibition

Organisms	100 mg/ml	50 mg/l	25 mg/ml	12.5 mg/ml	Controls
S.epidermidis	16	11	8	8	25
P.aeruginosa	15	10	10	7	29
S.aureus	18	14	10	8	31
Salmonella typhi	15	10	10	ND	26

Key: ND =Not detected

Table 8: Antibacterial activity of $NiFe_2O_4$ Nanoparticles (Zone of Inhibition in mm, Mean $\pm$ SD)

Organisms	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
S. epidermidis	16.1 $\pm$ 0.6	11.2 $\pm$ 0.5	8.3 $\pm$ 0.3	8.1 $\pm$ 0.4
P. aeruginosa	15.4 $\pm$ 0.4	10.1 $\pm$ 0.3	10.0 $\pm$ 0.2	10.0 $\pm$ 0.3
S. aureus	18.3 $\pm$ 0.5	14.6 $\pm$ 0.4	10.3 $\pm$ 0.4	10.0 $\pm$ 0.5
Salmonella typhi	15.0 $\pm$ 0.3	10.2 $\pm$ 0.3	8.1 $\pm$ 0.2	ND

Key: ND =Not detected

Table 9: Minimum inhibitory Concentrations

Organisms	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	Controls
S.epidermidis	+	+	+	+	+
P.aeruginosa	-	+	+	+	+
S.aureus	-	+	+	+	+
Salmonella typhi	-	+	+	+	+

Key: + = Absence of growth
= Presence of growth.

Similarly, the report by (Bindhu & Umadevi, 2014) corresponded to the minimum inhibitory concentration (MIC) of the synthesized Nickel Iron Oxide Nanoparticles and displays the following;
The *S.epidermis* does no grow at all concentration, including the lowest concentration.
The *P.aeruginosa* grows at 12.5mg/ml but growth inhibited by Nickel Iron Oxide Nanoparticles at 25mg/ml, 50gm/ml and 100gm/ml. Therefore, the minimum inhibitory concentration (MIC) of *P.aeruginosa* against Nickel Iron Oxide Nanoparticles = 25mg/ml.
The *S.aureus* grows at 12.5mg/ml but growth inhibited by Nickel Iron Oxide Nanoparticles at 25mg/ml, 50gm/ml and 100gm/ml. Therefore, the minimum inhibitory concentration (MIC) of *S.aureus* against Nickel Iron Oxide Nanoparticles = 25mg/ml.
The *Salmonella typhi* grows at 12.5gm/ml but the growth inhibited by Nickel Iron Oxide Nanoparticles at 25mg/ml, 50gm/ml and 100gm/ml. Therefore, the minimum inhibitory concentration (MIC) of *Salmonella typhi* Nickel Iron Oxide Nanoparticles = 25mg/ml.

Table 10: Minimum Bactericidal concentrations (MBC)

Organisms	12.5 mg/ml	25 mgm/l	50 mg/ml	100 mg/ml	Controls
S.epidermidis	-	-	+	+	+
P.aeruginosa	-	+	+	+	+
S.aureus	-	+	+	+	+
Salmonella typhi	-	+	+	+	+

Key: + = completely killed
- = presence of growth

Approximately, the literature reported by (Bindhu & Umadevi, 2014) shows that, the minimum bactericidal concentration (MBC) of the synthesized Nickel Iron Oxide Nanoparticles displays the following;
The *S.epidermis* was completely killed by Nickel Iron Oxide Nanoparticles at 50mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *S.epidermis* against Nickel Iron Oxide Nanoparticles = 50mg/ml.
The *P.aeruginosa* was completely killed by Nickel Iron Oxide Nanoparticles at 25mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *P.aeruginosa* against Nickel Iron Oxide Nanoparticles = 25mg/ml.
The *S.aureus* was completely killed by Nickel Iron Oxide Nanoparticles at 25mg.ml. Therefore, the minimum bactericidal concentration (MBC) of *S.aureus* against Nickel Iron Oxide Nanoparticles = 25mg/ml.
The *Salmonella typhi* was completely killed by Neem leaves Extract at 25mg/ml. Therefore, the minimum bactericidal concentration (MBC) of *Salmonella typhi against* = 25mg/ml.

CONCLUSION

The eco-friendly synthesis of $NiFe_2O_4$ nanoparticles utilizing Neem *(Azadirachta indica)* leaf extract was successfully demonstrated in this study, underscoring the function of phytochemicals as stabilizing and reducing agents. UV-Vis, FTIR, and SEM investigations were used to evaluate the produced nanoparticles, verifying their organic surface modifications and structural integrity. According to antibacterial tests, the produced nanoparticles and the neem extract both have strong antibacterial properties. Nevertheless, $NiFe_2O_4$ nanoparticles shown increased effectiveness, especially against Salmonella typhi, S. aureus, and P. aeruginosa, with minimum bactericidal doses as low as 25 mg/mL.

RECOMMENDATIONS

From this research the following recommendations could be made:

Based on these results, it is recommended to further investigate the potential therapeutic applications of neem leaf extracts against S. epidermidis and P. aeruginosa infections.Future studies could focus on identifying the active compounds responsible for the antibacterial activity and evaluating the safety and efficacy of neem leaf extracts as a potential alternative treatment option.

Similar research should be undertaken thereby furthering the characterization technique in order to ascertain full efficacy of the Nickel ferrite nanoparticles using *Azadirachta indica* leaves extract. i.e XRD analysis and XRF analysis

The synthesized $NiFe_2O_4$ NPs should also be used in the fields of magnetic data storage, biosensor, catalyst and nanotechnology

It is recommended that further studies should be carried out on the Phytochemicals profiling of the *Azadirachta indica* leaves to probe the detailed antibacterial activity of the constituent molecules using modern chromatography techniques such as Gas chromatography and High-performance liquid chromatography (HPLC).

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