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Green Synthesis of Ag-Fe Bimetalic Nanoparticle from Mahogany Leaf (*Khaya Senegalensis*) Extract and its Antimicrobial Applications

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Abstract
Researchers from all over the world have become increasingly concerned about bimetallic nanoparticles in recent years. In particular, these hybrid species are quite attractive for some technological purposes, particularly in the study of antibiotics. This study used a green method to create Ag–Fe bimetallic nanoparticles from khaya seneglences leaf extract, which were then characterized using variety of methods, such as UV, FT-IR, SEM, magnetic susceptibility and EDX. The UV results of Ag–Fe bimetallic nanoparticles displayed a distinctive UV–Vis spectrum, with the wavelength of 400 nm corresponding to the maximum absorbance peak at 0.375. The FT-IR bands that were detected were 872.19705 cm−1, 1021.29057 cm−1, 1408.93370 cm−1, 1565.48189 cm−1, 2952.0516 cm−1, and 3369.51340 cm−1. The EDX analysis affirms that Ag and Fe nanoparticles were formed because their respective peaks show up at 68.24 concentration (Ag) and 12.39 concentration, respectively. Effective antibacterial activity against pathogenic bacteria was shown by the antimicrobial research of Ag-Fe Bimetallic Nanoparticles made from khaya seneglences extract. Pseudomonas aeroginosa showed a larger zone of inhibition for every concentration examined than other pathogens. On the basis of complex, F.W (g/mol), Phase State, Shape, and Color, a few physical characteristics of the particle were also established. The complex, which is paramagnetic by nature, was also subjected to a magnetic suspectibility test. The solubility test was tested using four different solvents, DMSO, water, peptone, and ethanol, with DMSO being shown to be the most appropriate solvent.

Keywords: Antimicrobial, Nanoparticles, *Khaya senelensis,* Synthesis, Materials, *Susceptibility*

Introduction
The plant Mahogany, *K. Senegalensis,* a Savannah tree with various regional names (Madachi, Oganwa, Ona), is easily recognizable due to its rounded evergreen canopy, shiny pinnate leaves, and distinctive round seed pods. This majestic tree stand 30m tall with a 3m girth featuring a majestic crown, rests upon a sturdy trunk, its dark, scaly bark a testament to resilience. The tree's striking bark reveals dark pink slashes, while its compound leaves comprise 5 pairs of elliptical leaflets, measuring 5-10 cm in length and 2.5cm in width (Irvine, 1961; Keay *et al.*, 1989).

However, Nanotechnology as the name implies deals with atom or matter at the nano scale level. Nanoparticles are the main building blocks of nanotechnology which involve the study and application of materials at the atomic and molecular level to create innovative product and solution. Core components of nanotechnology, nanoparticles showcase distinct attributes

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arising from their compact size, augmented surface area, and quantum effects, culminating in unpredictable behavior (Rotello, 2004). Nanoparticles vary in types, and they can be classified according to their architectural structure, dimension or origin (Mazhar *et al.,* 2017). Noble metal-based nanoparticles stand out for their superior properties, including stability, biocompatibility, and large scale production potential, making them ideal for biomedical and environmental uses (Klebowski *et al.,* 2018).

In addition, the translation of metal-based nanoparticles into practical application is impeded by limitation stemming from toxicity, size, cellular uptake, and chemical instability, underscoring the need for target optimization. Combining the limitations of single metal nanoparticles offer a promising solution, leveraging the synergetic relationship between two metals to unlock new properties and expand their potential uses (Mazhar *et al.,* 2017). This method has demonstrated an improved effectiveness and possible ways to limit the monometallic nanoparticles.

Also, the unique combination of physical, chemical, thermal, mechanical, optical, catalytic and magnetic properties has made them increasingly attractive in recent years (Behers *et al.,* 2020). By combining two metals, bimetallic nanoparticles achieve unprecedented performance, surpassing the limitations of individual metal nanoparticles. In this research, we focus on the synthesis and antimicrobial activity of Ag-Fe bimetallic nanoparticles, utilizing an eco-friendly and sustainable approach by employing the leaf extract. This method not only help to reduce the environmental footprint associated with nanoparticle synthesis but also harnesses the inherent reducing and stabilizing capabilities of phytochemicals present in the mahogany leaf extract. (Arora *et al.,* 2020). However, researchers have conduct research on the antimicrobial potential of bimetallic nanoparticles (Ayagun *et al.,* 2022).

The antimicrobial properties of Ag-Fe nanoparticles have made them suitable for coatings on surface in healthcare settings and food packaging materials to enhance the development of bacteria and fungi. Scientific data reported that traditional medicine utilizes the leaves and stem of *khaya seneglensis* to alleviate diarrhea symptoms Olayinka *et al.*, 1992). Antimicrobial testing reveal that the extract was effective against bacterial which include: *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp*., *Salmonella spp.* and *Bacillus subtilis*. Abdullahi *et al*., 2016) reported a Minimal Inhibitory Concentration (MIC) of 50 mg/mL for *K. senegalensis* extract on *E. coli* suggest its promising therapeutic potentials in treating diarrhea-causing bacterial infections.

Materials and Methods

Weighing balance, magnetic stirrer, centrifugation machine, Drying Oven, Thermometer, incubator, Refrigerator, motor and pestle, FTIR, Uv, SEM (model Phenom ProX Phenom World Q150R), EDX (model PRO:X:800-07334 Phenom World and serial number MVE01570775) $AgNO_3$, $FeCl_3.6H_2O$, DMSO, distilled water were used, All the reagents used are of analytical grade.

Sample Collection

Khaya seneglencies fresh leaves were collected at Federal University Dutse, Jigawa State, and was identified by Dr. Mohammed Isa Auyo a taxonomist from the Department of Biology at Federal University Dutse with the code (FUDHAN: 004/43/1). The leaves were washed and rinsed with distilled water to get rid of unwanted dust particles and then it was shade dried at room temperature, the leaves were cut and grounded into small sizes using mortar and pestle.

Extraction and Preparation of Leaves extract

About 200 mL of hot distilled water was used to dissolve 5g of the ground leaves. The mixture was then magnetically stirred at 500°C for 60 minutes. After cooling, it was filtered through Whatman No. 1 filter paper and kept in a refrigerator for 24 hours at a temperature of about 4°C. Little changes were made to this process from Padilla-Cruz1 et al. (2022).

Synthesis of Ag-Fe Bimetallic nanoparticles from leaf extract

After mixing 100 ml of *Khaya seneglensis* extract with 200 mL $AgNO_3$ and 0.02M $FeCl_3.6H2O$ under magnetic stirring for 30 minutes at 50 °C, the liquid changed to a uniform brown color that ranged from light to deep, confirming the presence of nanoparticles. After cooling, the mixture was centrifuged for ten minutes at 4000 rpm, and the supernatant was disposed of. A 24-hour drying period was given to the product at room temperature (Sivamaruthi et al., 2019; Kamli et al., 2021; Yoro et al., 2022).

Characterization of Ag-Fe Bimetallic nanoparticles and khaya Seneglencies leaves extract.
UV-visible spectrophotometry

Various dilutions were prepared using a solution containing 1 mg/mL of Ag-Fe Bimetallic nanoparticles; the second dilution was prepared by measuring 1 mL of the stock solution into 2 mL of distilled water, and each subsequent dilution was prepared successively using the immediate solution. These solutions were analyzed using a UV-visible spectrophotometer, and absorbance at different wavelengths (200–800 nm) were recorded.

The FT-IR spectroscopic analysis

The FTIR analysis was performed on the Ag-Fe Bimetallic nanoparticles and the Khaya Seneglencies leaves extract at the Department of Biochemistry Laboratory at Umaru Musa Yar'adua University Katsina State, Nigeria.

Scanning electron microscopy

The scanning electron microscopy was done at Umaru Musa Yar’adua University, Katsina State, Nigeria, in the Central Research Laboratory with a scanning electron microscope model PROX: 800-07334 Phenom World and serial number MVE01570775.

The SEM machine was allowed to boot for 23 hours before it was used for analysis while the settings were done in the computer system. It consists of three basic elements: an X-ray tube, a sample holder, and an EDX-ray detector. X-rays were generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. The sample was placed on a sample holder and inserted into the machine. An image of a typical compound microscope was displayed on the monitor, which was then converted into a scanning electron microscope after the brightness and contrast were adjusted to produce a highly clear image. Lastly, the image was captured at magnifications of 50 and 100 micrometres.

Antimicrobial Assay

Antimicrobial discs were used to prepare different concentrations of the nanoparticles leaf extract (10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, and 30 mg/mL). Equal volume (1mL) of DMSO was dispersed in each test tube labeled and arrange as (10 mg/mL, 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL). To each of the test tubes the weighed Ag-Fe bimetallic nanoparticles extract was added, it was then mixed vigorously and left at room temperature for 3-6 hours for complete dissolution of the particles. A 6 mm in diameter of filter paper was prepared as a disc. Aseptically, to each of the disc a 25 mL of the solution of nanoparticles at different concentration were added. The discs were dried in hot air oven at 37°C for 30 minute and at 2-20°C under refrigeration. Bacterial pure culture colonies of *Staphylococcus aureus*, *Streptococcus pneumonae*, *Pseudomonas aerognosa, Escherichia coli* were inoculated onto different Muller Hinton agar using streaking method and incubated at 37°C for 24 hours. The plates were read using a ruler in millimeter and the zone of inhibition were measured and recorded. This is in line with EUCAST guide line as adapted and modified (Abbasi *et al.*, 2019).

Results and Discussion

The results of physical properties of the synthesized complex is presented in Table 1 and 2 respectively.

*Table **1: Physical Properties of the Complexes***

Complex	F.W (g/mol)	Phase State	Shape	Color
Ag-Fe	187.76	Crystaline	Irregular	Brown

*Table **.2: Solubility tests of the Complexes***

Solvent	solubility	observation
DMSO	+++	Completely soluble
water	-	Insoluble
peptone	++	Partially Soluble
Ethanol	++	Partially Soluble

Table 3: Magnetic susceptibility test for Ag-Fe nanoparticles

Sample ID	M(mg)	L(cm)	R	R_O	W_1	W_2
Ag-Fe nanoparticle	233	2.00	463	-025	610	843

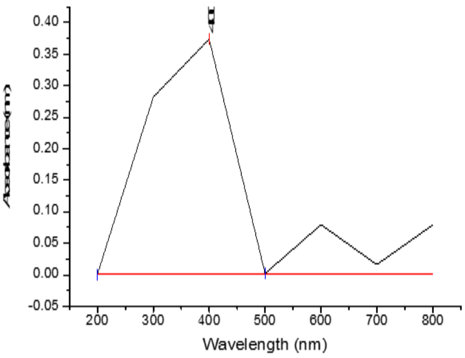

Figure 1: The Uv-vis of Ag-Fe Bimetallic Nanoparticles

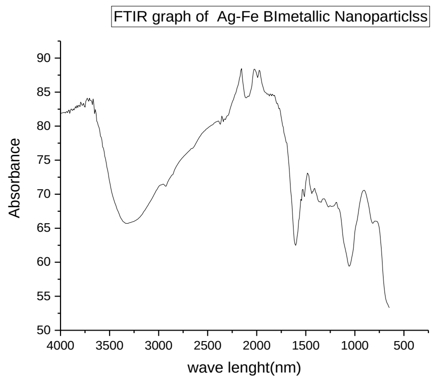

Figure 2: The FTIR of Ag-Fe Bimetallic Nanoparticles

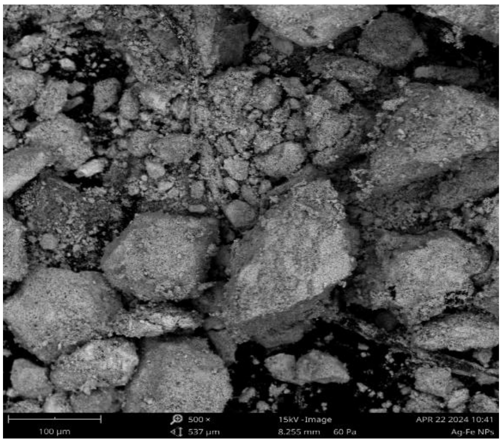

Figure 3: SEM of Ag-Fe bimetallic nanoparticles

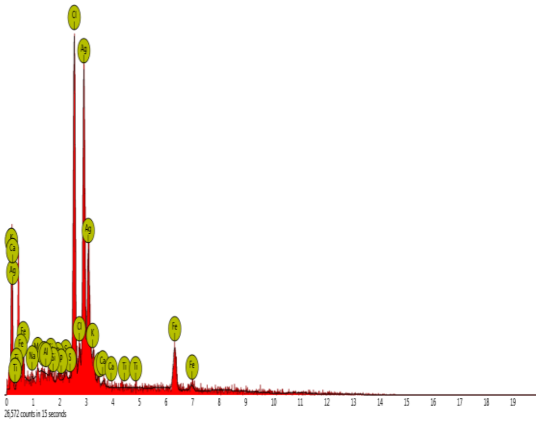

Figure 4: The EDX Ag-Fe Bimetallic Nanoparticles

Table 3: The EDX of Ag-Fe Nanoparticles

Element Number	Element Symbol	Element Name	Atomic Conc.		Weight Conc.
47	Ag	Silver	44.81		68.24
17	Cl	Chlorine	34.82		17.43
26	Fe	Iron	15.72		12.39
12	Mg	Magnesium	1.02		0.35
15	P	Phosphorus	0.75		0.33
16	S	Sulfur	0.71		0.32
14	Si	Silicon	0.77		0.31
19	K	Potassium	0.41		0.23
20	Ca	Calcium	0.37		0.21
11	Na	Sodium	0.60		0.20

Table **4:** ***The Antimicrobial Activity of Ag-Fe bimetallic Nanoparticles***

Ag-Fe BNP Test For Organism	concentration (Mg/mL)					control	Ceftriazon
	10 mg/ mL	15 mg/ mL	20 mg/ mL	25 mg/ mL	30mg/ mL		25 mg/mL
E.Coli	12mm	13mm	14mm	15mm	16mm	CEF	15mm
P. Aeroginosa	22mm	23mm	24mm	25mm	26mm	CEF	23mm
S. pneumoniae	7mm	8mm	9mm	11mm	12mm	ERY	8mm
Staphy Aureus	7mm	8mm	9mm	10mm	12mm	ERY	12mm
Kandida Albica	10mm	11mm	12mm	15mm	28mm	FLU	18mm

The complex consists of silver and iron, which are both transition metals. The combination of these two elements lead to interesting magnetic and electronic properties. The Ag-Fe complex is likely to exhibit a synergistic effect, where the presence of one element enhances the properties of the other.

The Ag-Fe complex is relatively high, indicating that the complex has a significant molecular weight which could be due to the presence of silver and iron atoms in the complex, or the incorporation of other elements or ligands.

The irregular shape of the Ag-Fe complex suggests that it may have a non-uniform morphology which could be due to the synthesis method, where the complex is formed through a process that does not produce uniform particles. The irregular shape could also be influenced by the presence of defects or impurities in the complex.

The brown color of the Ag-Fe complex is likely due to the presence of iron, which is known to impart a brown or reddish-brown color to materials. The color could also be influenced by the oxidation state of the iron, with higher oxidation states leading to a more intense brown color. Some physical properties of Ag-Fe nanoparticle synthesized from *khaya seneglensis* were identified which involve Complex, F.W (g/mol), phase state, shape, and color. The properties are in line with report provided by Lue. 2007.

The Solubility tests of the Ag-Fe nanoparticles complexes of different solvents were carried out to identify the solubility of Ag-Fe nanoparticles synthesized from mahogany leaves extract. The solvents used include: DMSO, water, peptone, ethanol. The complex dissolved completely in DMSO, insoluble in water and peptone but partially soluble in ethanol. The DMSO is chosen due to its suitability to dissolve Ag-Fe nanoparticle complex for antimicrobial activity. This is in line with Li and Lenhart (2012) report.

The Magnetic Susceptibility for Ag-Fe nanoparticle is found to be approximately 3.98×10^{-9} which is a good attribute to the complex.

The UV result of Ag–Fe bimetallic nanoparticles showed the characteristic UV–Vis spectrum, with highest peak at 0.375 for the absorbance at 400 nm as the wavelength which is in line with the report according to Anuradha *et al.*, 2020). An absorption spectrum resembling that of iron nanoparticles was observed, this suggests the formation of silver-iron core–shell bimetallic nanoparticles. Similar findings have been reported in the synthesis of diverse bimetallic nanoparticles (Padilla-Cruz1 *et al.*, 2021; Garcia *et al.*, 2014).

The nano size of the synthesized Ag-Fe BMNPs in Fig 1 was confirmed by the peak or band at around 400 nm as seen in UV spectrum and the result is in concordance with that of other researchers (Padilla-Cruz1 *et al.*, 2021). The average nano size of the synthesized Ag-Fe BMNPs was 18 nm which was confirmed by the peak at wavelength of 400 nm as seen in UV spectrum and this result is in concordance with that of other researchers (Padilla-Cruz1 *et, al.*, 2021).

Nanoparticles in Fig 2 shows the spectra of the generated Ag-Fe nanoparticles at 872 cm^{-1}, 1021 cm^{-1}, 1408. Cm;$^{-1}$, 1565 cm,$^{-1}$, 2952 cm^{-1}, and 3369 cm^{-1}.The hydroxyl group stretching is represented by the band at the 3369–3000 cm^{-1} area (OH). The aliphatic hydrocarbon (C–H) stretching may be the cause of the band at 295 cm^{-1}. The C=C and C=O stretching can be attributed to the bands at the 1408 and 1565 cm^{-1} regions, respectively. The stretching of C-O may be responsible for the band at 1021 cm^{-1}, and C-H bending vibration may be responsible for the band at 872 cm^{-1}. Other FT-IR results by researches are consistent with this study (Kotval *et al.,* 2018; Padilla Cruz1 et al., 2021; Yoro *et al.,* 2022).

The SEM of Ag-Fe bimetallic nanoparticles in respect of the size, shape, and morphologies of the formed Ag-Fe bimetallic nanoparticles were characterized. According to the SEM images of the Ag-Fe particles, these nanoparticles had a heterogeneous surface morphology. Furthermore, the Ag-Fe nanoparticles was credible uneven or irregular in sharp filled with cavities, the Ag-Fe nanoparticles look like grabbles both in size and shape. This indicate the surface morphology of the Ag-Fe bimetallic nanoparticles synthesized by green method from the extract of Mahogany leaves observed with the aid of scanning electron microscope and it reveals that the Mahogany leaves have a great potency to synthesize Ag-Fe bimetallic nanoparticles which are irregular in shape as compared with the surface morphology of the scanning electron microscope for Mahogany leaves (*Khaya Seneglencies)*. The result of Fig 3 agrees with some researchers' report in the earlier literatures (Kotval *et al.*, 2018; Ruíz-Baltazar *et al.*, 2014; Maghsoudy *et al.*, 2019).

The EDX spectroscopy was used to investigate the presence of elemental composition in the synthesized Ag-Fe nanoparticles as shown in Fig 3.

The composition and presence of elements in the biosynthesized Ag-Fe nanoparticles, energy Dispersive X-ray (EDX) spectral analysis was carried out to determine the elemental composition of the as-synthesized Ag-Fe bimetallic nanoparticles as shown in Table 3. The EDX analysis confirms the formation of Ag and Fe nanoparticles as their corresponding peaks at 64.24 concentration (Ag) and 12.39 concentration (Fe), endorsing the development of Ag-Fe bimetallic Nanoparticles. The Ag_{core}-Fe_{shell} bimetallic nanoparticle formation was initially suggested by Brightness contrast between the nanoparticles' core and shell regions (Table 3). The Prepared core-shell Ag-Fe bimetallic nanoparticles show that Ag signals in the EDX spectrum dominated the Fe signals, resulting in the enhancement of the Ag peaks, resulting in the high density of Ag in the shell region. According to the analysis of EDX spectrometers, the presence of the elemental Ag and Fe signals, corresponding to the Ag -Fe bimetallic nanoparticles, was confirmed as reported by (Maghsoudy *et al.*, 2019; Shailesh *et al.,* 2018).
The Antimicrobial Activity of Ag-Fe nanoparticles by the traditional medications available in pharmaceutical industries play imperative part to treat different microbial ailments, but are associated to problems, i.e., resistance against antibiotics. Hence, researchers are exploring the alternative ways to design alternate materials in order to combat against antibiotic resistance as well as to minimize the risks of spread of diseases.

The result presented in Table 4 is the antibacterial investigation of Ag-Fe bimetallic nanoparticles against *Staphylococcus aureus*, *Streptococcus Aeroginosa*, pseudomonas *and Escherichia coli, candida Albicans.* Throughout the studies, Erythromycin, Ceftriaxone and fluconazole were used as control at concentration of 23 mg/mL, 30 mg/mL and 25mg/mL respectively for the gram-positive isolate, gram negative isolate and fungal isolate. Different concentrations of 10 ml/mL, 15mg/mL, 20 mg/mL, 250 mg/mL and 30 mg/mL of Ag-Fe Bimetallic Nanoparticles was tested against each pathogen. This report agrees with the one observed by other researchers (Padilla *et al.,* 2021; Ruíz-Baltazar *et al.,* 2014). For each concentration investigated, *Pseudomonas arigenoso*, demonstrated higher zone of inhibition at 30mlg/mL as compared to other pathogens. The results of this research therefore indicated that Ag-Fe Bimetallic Nanoparticles synthesized from mahogany leaf extract demonstrated effective anti-microbial activity on different isolates. (Patil Shriniwas, 2017; Abbasi *et al.,* 2019; Yoro *et al.,* 2022).

Conclusion

The synergistic bactericidal and fungicidal effects against both Gram-positive and Gram-negative bacteria were demonstrated by the assessment of bimetallic nanoparticles' antimicrobial activity. The findings show that magnetic bimetallic nanoparticles made of metals that enhance their antibacterial properties may be designed and produced using green synthesis techniques. Additionally, this work provides information on how to treat drug-resistant illnesses and other biological uses by creating new and more potent antimicrobial compounds that may have bactericidal and fungicidal properties. When applied to pathogenic bacteria and fungi, the green produced Ag-Fe Bimetallic Nanoparticles from Mahogany leaf extract showed strong antibacterial action, *with Pseudomonas Aeuroginosa* and *candida albicans* exhibiting a larger zone of inhibition than other pathogens.

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