

# **BIOSYNTHESIS OF SILVER-COPPER-ALUMINUM TRIMETALLIC NANOPARTICLES USING AQUEOUS LEAVES EXTRACT OF *Hierochloe odorata* AND EVALUATION OF EVALUATING ITS ANTIMICROBIAL ACTIVITIES**

## **ABSTRACT**

Due to the erroneous use of antibiotics, many pathogens have become resistant to them and as such, there has been a surge in multi-drug-resistant bacteria. This in turn has posed a great threat to living things and global economic development. Although various methods are presently being used to mitigate these effects however, most of these methods have some disadvantages such as high cost, low efficiency and risk of reintroducing more pollutants to the environment. Literature studies have revealed that nanoparticles, due to their large surface to volume ratio and excellent catalytic and antimicrobial properties, have been applied as antimicrobial agents. Furthermore, the synergistic effect of trimetallic nanoparticles has also increased its efficacy as an antimicrobial agent. In this study, silver-copper-aluminum nanoparticles (Ag-Cu-Al NPs) was successfully biosynthesized using aqueous leaves extract of *Hierochloe odorata* at room temperature. The biosynthesized Ag-Cu-Al NPs was first confirmed by the observation of a distinct colour change from grey to olive green after upon addition of the aqueous leaves extract to 0.1 M silver nitrate, copper chloride and aluminum oxide salts solution. The Ag-Cu-Al NPs was examined using UV-vis spectroscopy of the Ag-Cu-Al NPs revealed a characteristics peak maximum at 405 nm. FTIR analysis revealed the possible biomolecules present in the leaves extract responsible for bio-reduction, capping and stabilization of Ag-Cu-Al NPs the nanoparticles (NPs). XRD analysis revealed the crystalline nature and particle size of the Ag-Cu-Al NPs. SEM-EDS analysis revealed the surface morphology and elemental composition of the NPs. Furthermore, antimicrobial activity of Ag-Cu-Al NPs were also investigated which revealed outstanding compared favourably with standard antimicrobial agent (state the name of the antimicrobial agent here ) used as positive control antimicrobial activity against *Staphylococcus aureus*, *Salmonela sp.* *Aspergillus niger* and *Aspergillus flavus*. The biosynthesized NPs has potential to manage multi-drug-resistant microorganisms.

**KEYWORDS:** Ag-Cu-Al Nanoparticles, Trimetallic, Biosynthesis, *Hierochloe odorata*, Antimicrobial.

## **1. Introduction**

Globally, one of the leading causes of serious health challenges is foodborne disease caused by infectious agents. At the present time, antibiotics antimicrobial agents are the only source of treatment against disease causing microorganisms such as bacteria and fungi. However, due to the erroneous use of antibiotics antimicrobial agents, many pathogens have become resistant to them. The regularity of infectious disorders caused by bacteria resistant to many drugs has increased, especially methicillin-resistant *Staphylococcus aureus* and *Salmonella sp.*, penicillin-resistant *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* resistant to fluoroquinolones.

In contrast to the past, extensive research is being conducted in the field of nanotechnology because of its vast applications in both the industrial and health sectors. The use of nanotechnology in a variety of fields, including chemistry, biology, and engineering, has

brought about advanced, effective, and unique products with improved features, especially those that find biomedical applications [1–3]. The burgeoning field of nanoparticles is considered to be one of the ground-breaking approaches to addressing significant issues worldwide. Nanoparticles (NPs) offer high catalytic activity as well as a high surface-to-mass ratio [4]. Nanoparticles are adequate, cost effective, and environmentally friendly solutions to current processing materials [5]. These synthesized nanomaterials have distinguishable proficiencies that are useful for addressing gaps in sectors such as health, energy production, drug delivery systems, and wastewater treatment [6–8].

In order to address [the challenges of multi-drug-resistance by microorganisms](#), [these issues](#), the use of alternative ~~antibacterial~~ antimicrobial agents such as metallic nanoparticles have been of great focus. Metallic nanoparticles have powerful, targeted, and prolonged ~~antibacterial~~ antimicrobial interactions with [bacteria microorganisms](#) and biofilms at [lower tiny](#) dosage because of their smaller dimensions and high surface area-to-volume ratios. Multiple studies have revealed that trimetallic nanoparticles exhibit excellent antimicrobial activity compared to that of bimetallic and monometallic nanoparticles. In one such study, Au/Pt/Ag was synthesized via green synthesis method, by using leaf extract of *Lamii albi flos*. The synthesized Au/Pt/Ag nanoparticles demonstrated remarkable antimicrobial properties against harmful strains of bacteria such as *Enterococcus faecalis* and *Enterococcus faecium*, both planktonic as well as sessile [18].

There are various types of nanoparticles depending on their morphology and other traits. Metallic nanoparticles, for example gold and silver nanoparticles, have been used in numerous applications [such](#) as biosensors, efficient drug delivery systems, active food packaging [materials](#), and other medicinal uses [9]. Metallic nanoparticles have received increasing attention among the numerous types of nanoparticles due to the usefulness of their biological features, nontoxic nature, and their distinctive properties [10-11]. Due to their unique optical, electrical, and magnetic properties, metal nanoparticles have been investigated for a variety of applications [12]. Compared to monometallic nanoparticles, multimetallic nanoparticles, composed of various metals, provide a unified system that can display unique features. Furthermore, when compared to monometallic or bimetallic nanoparticles, trimetallic nanoparticles exhibit more catalytic activity, more antibacterial effect, diversified shapes, highly selective detection and sensitivity, high level of stability, and chemical transformation [13]. These promising features are a result of the combined effects of the three metals that make up trimetallic nanoparticles. Synergism in these trimetallic nanoparticles have been shown to significantly increase remarkable properties like, photocatalytic activity, antimicrobial activity, corrosion inhibition potential and other therapeutic activities [14-15].

Various methods have been reported for the synthesis of trimetallic nanoparticles but, the green synthesis or biological synthesis route has proven to be most affordable and environmentally friendly. Different plants, microorganisms, and biodegradable wastes can be used as a source to synthesize these nanoparticles via this route. Plant components, including leaves, seeds, roots, and flowers, can be employed. Plant extracts are considered to be reducing, capping, and stabilizing agents due to the presence of bioactive compounds such as flavonoids, terpenoids, enzymes, alkaloids, and antioxidants [16].

One of such plants that can be used in the synthesis of trimetallic nanoparticles is *Hierochloe odorata*. *Hierochloe odorata* or *Anthoxanthum nitens* (commonly known as sweet grass, Manna grass or Vanilla grass) is an aromatic herb native to Northern Eurasia and North

America. It is used as a smudge, in herbal medicine and in the production of distilled beverages. It owes its distinctive sweet scent to the presence of coumarin and possess high antioxidant properties. The name *Hierochloe odorata* is from the Greek and Latin. *Hierochloe* means "holy grass" and *odorata* means "fragrant". Some North American indigenous people burn Sweet Grass in ceremonies to attract good spirits. [17].

This study proposes Ag-Cu-Al trimetallic NPs, synthesized via green methods with aqueous leaves extracts of *Hierochloe odorata*, characterization of the NPs by UV-Vis spectroscopy, FTIR, XRD and SEM-EDS analysis, and its application as an alternative antimicrobial agent against some of these multi-drug resistant bacteria and fungi. The aqueous leaves extract of *Hierochloe odorata* would serve as capping and stabilizing agent due to the wide range of biomolecules present in the extract. Unlike existing antimicrobial agents, Ag-Cu-Al NPs combine silver's noble barrier, copper's catalytic reactivity, and aluminum's Al<sub>2</sub>O<sub>3</sub> passivation layer. Its antimicrobial effect can be attributed to the combined synergistic effects of the trimetallic nanoparticles (leading to the release of reactive oxygen species, ROS) and other activities towards the microbes, including their contact with cell membranes, resulting in structural changes, in permeability, and disruption, and damage to microbial DNA and RNA, production of free radicals, as well as inactivation of essential enzymes by reaction between thiol groups and metal ions released by the nanoparticles, causing cell paralysis and eventually, death and damage to microbial DNA as well as RNA damage [18].

The aqueous leaves extract of *Hierochloe odorata* used as capping and stabilizing agent was effective (due to the wide range of biomolecules present in the extract) in minimizing the average particle size and reduced the tendency of agglomeration of the biosynthesized Ag-Cu-Al nanoparticles. The biosynthesized trimetallic nanoparticles were characterized using UV-Vis spectroscopy, FTIR, XRD and SEM-EDS analysis. The biosynthesized trimetallic nanoparticles were further verified for their antimicrobial ability on some fungi and bacterial strains.

## 2. Materials and Methods

### 2.1. Materials and Chemicals

The materials used includes were *Hierochloe odorata* leaves, were collected and identified from the premises of Federal College of Forestry Jos, Plateau State and identified by a plant taxonomist. The bacterial and fungal strains of *Staphylococcus aureus*, *Salmonella sp*, *Aspergillus niger* and *Aspergillus flavus* were obtained from maintained at the laboratory of the department of Science Laboratory Technology of the University of Jos, Jos. All the chemicals and reagents were used as received without further purification

The chemicals used were analytical grade copper (II) chloride (CuCl<sub>2</sub>), silver nitrate (AgNO<sub>3</sub>), aluminum oxide (Al<sub>2</sub>O<sub>3</sub>), were obtained from Thermo Fisher Scientific Pittsburgh, PA, United States of America,. The bacterial and fungal strains of *Staphylococcus aureus*, *Salmonella sp*, *Aspergillus niger* and *Aspergillus flavus* were maintained at the laboratory of the department of Science Laboratory Technology of the University of Jos, Jos. All the chemicals and reagents were used as received without further purification

### 2.2. Methods

### **2.2.1 Preparation of Aqueous leaves extract of *Hierochloe odorata***

Exactly 10 grams of fresh uninfected leaves of *Hierochloe odorata* was weighed and then washed several times with running tap water and rinsed with deionized water for removal of dust particles. Afterwards, the leaves were chopped into smaller pieces. The chopped and weighed material was then transferred into a 500 mL beaker containing 100 mL of deionized water and then heat on a hotplate at 30 °C. The mixture was brought to stand for 15 minutes and allowed to cool after which it was filtered using Whatman filter paper No. 1. The filtrate was then kept for onward use.

### **2.2.2 Preparation of 0.1 M AgNO<sub>3</sub> and CuCl<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> Salt Solutions**

Exactly 1.70, 1.34 and 1.02 grams of silver nitrate, copper (II) chloride and aluminum oxide salts respectively were weighed and transferred into beakers containing 100 mL of deionized water. The mixtures were stirred to ensure that the salt dissolves properly after which the salt solutions were mixed together to obtain a homogenous mixture. The prepared salt solutions was then stored for onward use

### **2.2.3 Synthesis of Ag-Cu-Al Trimetallic Nanoparticles**

The trimetallic nanoparticles was synthesized by reducing the precursor salts of silver, copper and aluminum concurrently using aqueous leaves extract of *Hierochloe odorata*. 30 mL of the freshly prepared leaf extract was added drop wise to 150 mL of 0.1 M AgNO<sub>3</sub>-CuCl<sub>2</sub>-Al<sub>2</sub>O<sub>3</sub> solution (50 mL of each salt solution) with constant stirring on a magnetic stirrer. On addition of the extract and after a period of time, a colour change was observed from grey to olive green, indicating the formation of the trimetallic nanoparticles. The trimetallic nanoparticles formed was then centrifuged at 3000 rpm for 15 minutes, washed with distilled water, allowed to dry at room temperature and kept for further analysis.

### **2.2.4 Characterization**

The biosynthesized trimetallic nanoparticles was subjected to various characterization techniques to determine the size, morphology and composition of the biosynthesized nanoparticles. UV-Visible measurements of the nanoparticles was recorded using the T70 PG Instruments' UV-Spectrophotometer at different wavelength and absorption and. FTIR (Cary 630 Agilent Technologies) was used to identify the possible biomolecules present in the plant extract responsible for capping and stabilization of the nanoparticles. SEM-EDS analysis was carried out using Quanta 250 FEG instrument to investigate the surface morphology and elemental composition of the nanoparticles. The crystalline structure and particle size of the biosynthesized nanoparticles was analyzed using X-ray diffraction (XRD Empyrean Malvern Panalytical diffractometer). XRD spectra were obtained in the 2θ angle range of 20°–100°.

### **2.2.5. Applications**

#### ***Antimicrobial studies***

Antimicrobial susceptibility test of the biosynthesized Ag-Cu-Al trimetallic nanoparticles was carried out in the microbiology laboratory of the department of Microbiology, University of Jos Plateau state. The agar diffusion test (well diffusion method) was adopted [19]. The analysis was carried out against two strains each of bacteria and fungi; *Staphylococcus aureus*, *Salmonela sp* and *Aspergillus niger* and *Aspergillus flavus* respectively.

***(i) Preparation of Inoculum for Bacteria susceptibility Test***

The inoculum was prepared by emulsifying bacteria colonies from nutrient agar medium. Using a sterile wire loop, the cells were inoculated in 10 mL ml of sterile nutrient broth. The broth culture of the test bacteria isolates was then incubated at 37°C for 18 hours. After incubation the broth cultures were standardized using 0.5 MacFarland standard ( $1.5 \times 10^8$  cfu/mL) as described by Clinical and Laboratory Standard Institute, CLSI (2009). This was done by comparing the turbidity of the bacterial inoculum with the prepared 0.5 MacFarland standard reagent, so as to obtain equal turbid mixture.

***(ii) Antibacterial Activity Test of Nanoparticles***

A sterilize molten Muller Hinton Agar (HiMedia) was prepared according to manufacturers' instruction and dispensed into sterile Petri plates previously adjusted at 45 °C in a water bath. The agar plates were then left to solidify under Bunsen burner flame for 60 min. Subsequently, a sterile cotton swab was dipped into 18 hours old bacterial suspensions adjusted to turbidity of 0.5 McFarland Standard. The swab was then used to inoculate the solidified agar by evenly streaking cotton swab over the medium. After 30 min, equidistant wells were cut in the medium using a 6mm diameter flame sterilized cork borer and labeled. A 1ml of the test nanoparticles of the different concentrations (400, 200, 100 and 50 mg/mL) and controls were then dispensed into the wells and labeled. The plates were incubated at 37 °C for 24 h and diameters of growth inhibition zones around the wells formed were measured in millimeters. Antibiotic gentamicin ( $10 \mu\text{g mL}^{-1}$ ) was used as positive and 5% DMSO as negative control agents.

***(iii) Determination of Minimum inhibitory concentrations (MIC)***

The minimum inhibitory concentration (MIC) of the trimetallic nanoparticles was determined by broth dilution method according to the method adopted by [20]. Sample concentration was prepared from the stock solutions by two-fold dilutions in sterile nutrient broth. Seven dilutions of the samples ranging from 6.25, 12.5, 25, 50, 100, 200 and 400 mg mL<sup>-1</sup> were tested. The inoculums of test strains prepared from fresh overnight cultures were adjusted to 0.5 McFarland standards, which equals to  $1.5 \times 10^8$  CFU mL<sup>-1</sup> for bacteria. The bacterial suspension of 1 ml was added to each of the tubes and incubated at 37 °C for 24 h. The control tubes contained only the bacteria inoculum and sterile distilled water used with no test nanoparticles. After incubation, the visual turbidity was observed and recorded. The lowest concentration without turbidity observed was measured as a MIC of the extracts.

***(iv) Preparation of Inoculum for fungi susceptibility Test***

The inoculum was prepared by obtaining using the already obtained pure and fresh fungal cultures. Using a sterile inoculating needle, the fungal cells were inoculated via point inoculation on a sterile solidified agar plate. The culture of the test fungal isolates was then incubated at 27 °C for 3 – 5 days.

### (v) Antifungal Activity Test of Nanoparticles

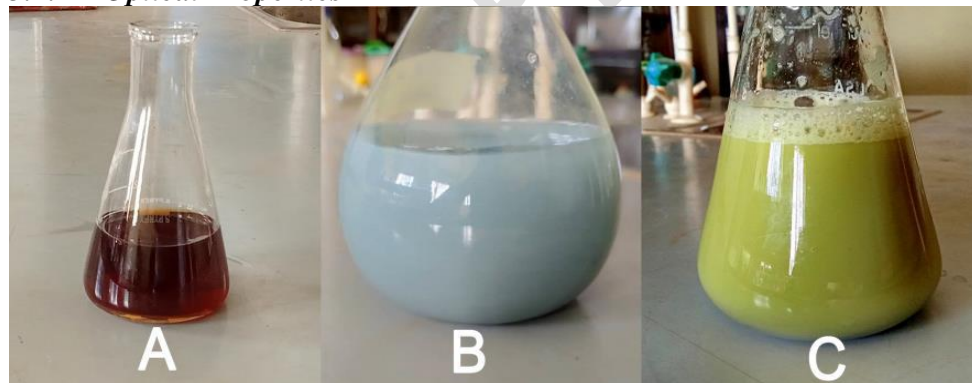
Antifungal activity of the trimetallic nanoparticles was evaluated using agar well diffusion assay. A sterilized molten Potato dextrose agar (PDA) was prepared according to manufacturers' instruction and dispensed into sterile Petri plates previously adjusted at 45 °C in a water bath. The agar plates were then left to solidify under Bunsen burner flame for 60 min. Subsequently, a sterile cotton swab was dipped into fungal culture. The swab was then used to inoculate the solidified agar by evenly streaking cotton swab over the medium. After 30 min, equidistant wells were cut in the medium using a 6mm diameter flame sterilized cork borer and labeled. A 1 mL of the dissolved nano particles of different concentrations (400, 200, 100 and 50 mg/mL) and controls (Fluconazole antibiotic) were then dispensed into the wells and labeled. The plates were incubated at 25 °C for 3 – 5 days and diameters of growth inhibition zones around the wells formed were measured. Antibiotic Fluconazole (10 µg mL<sup>-1</sup>) was used as positive and 5% Dimethyl Sulfoxide (DMSO) as negative control agents.

### (vi) Determination of Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration (MIC) of dissolved nanoparticles were determined by broth dilution method as adopted by [20]. Sample concentration was prepared from the stock solutions by two-fold dilutions in sterile PDA. The fungal suspension of 1 mL was added to each of the tubes and incubated at 25 °C for 3 – 5 days. The control tubes contained only the fungal inoculum and sterile distilled water used with no test nanoparticles. After incubation, the visual turbidity was observed and recorded. The lowest concentration without turbidity observed was measured as a MIC of the nanoparticles.

## 3. Results and discussion

### 3.1. Optical Properties



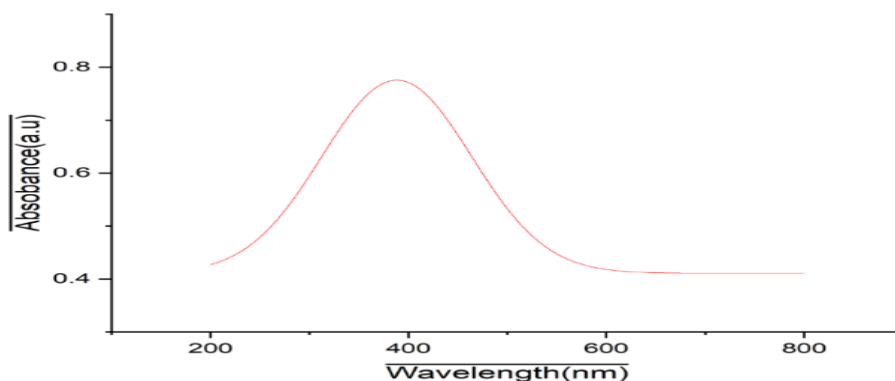
**Plate 1:** Hierochloe odorata leaves extract (A), Ag-Cu-Al trimetallic salt solution (B), Ag-Cu-Al nanoparticle solution (C)

One of the distinct characteristic properties of nanoparticles is their opto-electronic properties. The optical property of the trimetallic salt solution of Ag-Cu-Al, Hierochloe odorata, leaf extracts and the trimetallic nanoparticles of Ag-Cu-Al are shown on plate 1. After addition of 30 mL of aqueous leaf extract of Hierochloe odorata the colour of the 150 mL of the 0.1 M trimetallic salt solution of Ag-Cu-Al changed from grey to olive green indicating the

formation ~~creation~~ of Ag-Cu-Al trimetallic nanoparticles. The excitation of the surface plasmon resonance action in the nanoparticles is responsible for the colour variations. The size and shape of metal surface plasmon oscillation, as well as their optical properties are well understood.

### 3.2. UV-Visible Spectroscopy Analysis

More information about Ag-Cu-Al trimetallic nanoparticles was obtained through the use of UV-visible spectroscopy, which was used to observe the reduction of aqueous metal ions after treatment with *Hierochloe odorata* leaves extract (Figure 1). In the UV-visible absorption spectra of Ag-Cu-Al trimetallic nanoparticle, the typical surface plasmon resonance (SPR) is observed with an absorbance of approximately 400-407 nm with peak maxima of 405 nm, which can be attributed to the presence of Ag-Cu-Al trimetallic nanoparticles. The nearly symmetrical structure of the plasmon band suggest that the nanoparticles are not uniformly distributed and homogenous. This non-uniformity of the nanoparticles is responsible for the slightly broad absorption peak. The size, shape and capping agent of nanoparticles influence the position of the SPR band

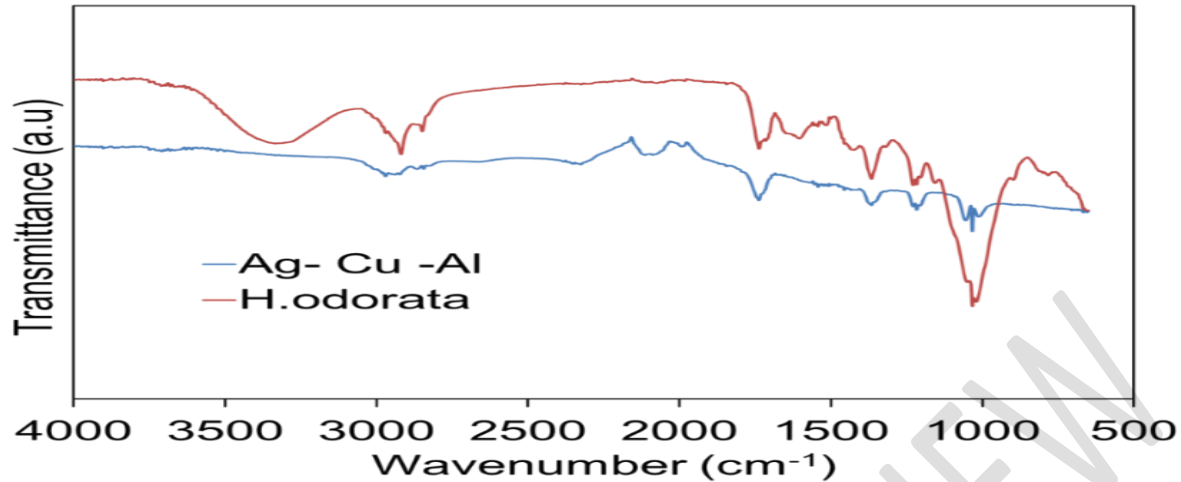


**Figure 1:** UV-visible spectrum of Ag-Cu-A trimetallic nanoparticle

### 3.3. Fourier Transform Infra-Red Spectroscopy Analysis

Figure 2 shows the FTIR spectra of *Hierochloe odorata* aqueous leaf extract mediated synthesis of Ag-Cu-Al trimetallic nanoparticles. It shows the distinct absorption bands for the functional groups involved in the reduction of the trimetallic ions. Absorbance peaks were recorded at 3317, 2938, 2080, 1735, 1367, 1217 and 1033  $\text{cm}^{-1}$ . The band at 3317  $\text{cm}^{-1}$  corresponds to secondary amine (N-H), C-H medium stretching for alkane is responsible for the bonds found 2938  $\text{cm}^{-1}$ . The 2080  $\text{cm}^{-1}$  can be attributed to C=C stretch for alkenes, 1735  $\text{cm}^{-1}$ . IR band is associated with C=O stretching for esters. 1367  $\text{cm}^{-1}$  is strong band for N=O stretching for nitro compounds. While 1217  $\text{cm}^{-1}$  and 1033  $\text{cm}^{-1}$  are attributed for C-O stretch for esters and C-C bending stretch for alkanes respectively.

Aqueous leaves extract of *Hierochloe odorata* is known to contain phytochemicals such as tannins, alkaloids and carbohydrates, saponins and flavonoids which are rich in antioxidant properties capable of donating atoms of hydrogen and protein moieties that can bind with metallic ions and capable of reducing metal ions of silver, copper and Aluminium and also serve as stabilizing agents to the synthesized nanoparticles [21].



**Figure 2:** Comparative FTIR spectra of Ag-Cu-Al nanoparticles and *Hierochloe odorata* leaves extract.

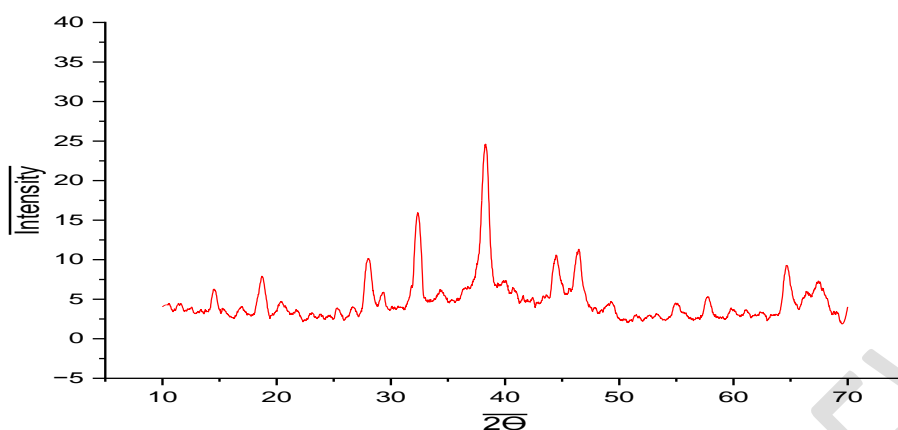
### 3.4. X-ray Diffraction (XRD) Analysis

The X-ray diffraction pattern is used in the determination of the size, shape, fraction analysis and parameter determination of the sample. The position of the peaks obtained from diffraction pattern also gives information about the translational size and shape of the crystal. The average size of the silver nanoparticles was calculated using the Debye-Scherrer equation;

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where, D, is the size thickness of the crystal, k = constant/shape factor (0.94),  $\lambda$  = wavelength of the X ray's incident on the crystal (1.5418 Å),  $\beta$ = is the width at half maxima at (111) reflection at Bragg's angle  $2\theta$ , while  $\theta$  is the Bragg angle. and the constant (k) has a value of 0.94 and the wavelength ( $\lambda$ ) of 1.5418

From the XRD patterns of the biosynthesized Ag-Cu-Al nanoparticles as shown in Figure 3, it is clear that the diffraction pattern of the trimetallic nanoparticles were essentially crystalline as shown with the sharp peaks [22]. Estimated diffraction peaks observed at 38.47°, 44.67°, 47.12° and 65.23° corresponds to the (111), (200) and (220) standard face centered cubic structures of silver which confirms the presence of silver oxide in the sample [22, 23]. The XRD pattern also shows peaks of copper oxide at 28.85° and 33.26°, aluminum oxide at 57.75° and 58.60° brought about by the oxidation of copper and aluminum particles respectively [24]. The average size of the biosynthesized nanoparticles was determined to be 35.92 nm, which was calculated from (Table 1). The values of the crystals planes of the biosynthesized Ag-Cu-Al nanoparticle observed were compared with the standard powder and diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS) which showed that the biosynthesized Ag-Cu-AL nanoparticles exhibit a Face center cubic crystals. The other peaks shown in the diffraction pattern might be as a result of some bioorganic compounds or proteins present in the plant extract [25].



**Figure 3: XRD spectrum of Ag-Cu-Al nanoparticles**

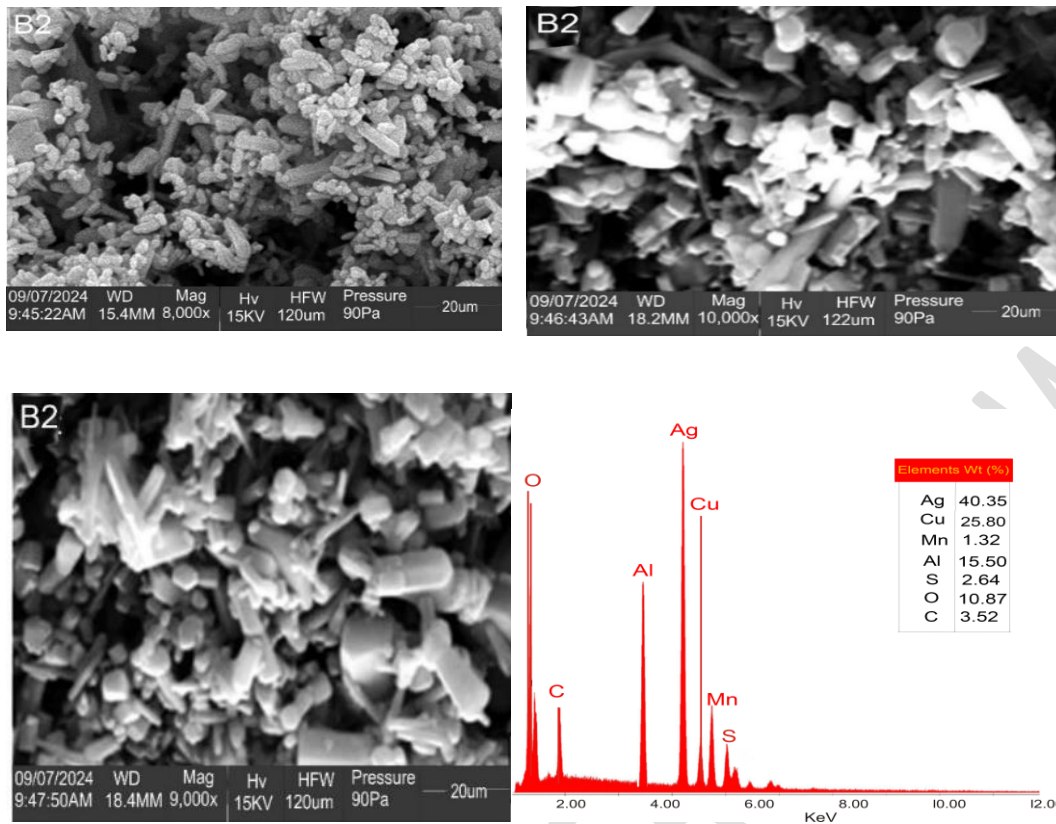
**Table 1: Average size analysis of Ag-Cu-Al nanoparticles from XRD analysis**

Position (nm)	Height (Abs)	FWHM (radians)	d-Spacing (Å)	Particle size (nm)
15.195	6.103	0.725	0.173	9.275
19.157	8.234	0.636	0.137	21.977
29.781	9.890	0.720	0.317	32.955
33.292	15.106	0.210	0.278	39.677
38.789	23.207	0.265	0.387	45.012
45.016	11.026	0.425	0.167	51.072
47.129	11.221	0.328	0.241	55.596
57.321	4.476	0.352	0.195	63.047
65.230	9.652	0.346	0.268	67.786

### **3.5. Scanning Electron Microscope - Energy-Dispersive X-ray Spectroscopy Analysis**

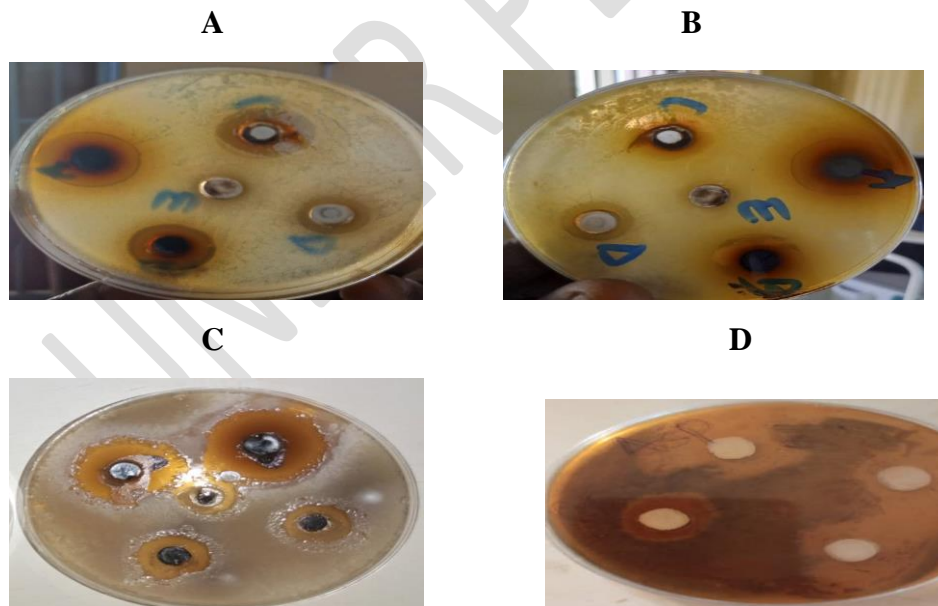
The SEM-EDS analysis was utilized to examine the surface roughness and morphology of the Ag-Cu-Al nanoparticles as shown in plate 2. The morphology shows an irregular crystalline structure which is typical for metallic nanocomposites due to the strong interparticle contact imposed by the high surface energy. It also reveals the intricacy of the particle size to be densely packed and also the cylindrical nature of the nanoparticles. The image also shows a surface roughness which might be attributed to biomolecules from the leaf extract that are bound to the Ag-Cu-Al nanoparticles.

The EDS also revealed the elemental composition of the nanoparticles with silver which is easily displaced and hence more active and having the highest composition thereby forming the outer shell while aluminium forms the inner core shell.



**Plate 2: SEM-EDS images of Ag-Cu-Al nanoparticles**

### 3.6. Antimicrobial Activities



**Plate 3: Antimicrobial activity of Ag-Cu-Al NPs against (a) *Salmonella sp.* (b) *Staphylococcus aureus* (c) *Aspergillus niger* (d) *Aspergillus flavus***

Antimicrobial activities of the biosynthesized Ag-Cu-Al NPs were evaluated by using standard assay. The nanoparticles showed very good inhibition zone against the two strains of bacteria and fungi (*Salmonella sp.* and *Staphylococcus aureus*), (*Aspergillus niger* and *Aspergillus flavus*) when compared with the standard antimicrobial agent (state the name of the antimicrobial agent here ) used as positive control under study as presented in plates 3a and 3b. The zone of inhibition of Ag-Cu-Al NPs obtained against *Salmonella sp.* and *S. aureus* are 23 mm and 18 mm, respectively while 11 mm and 18 mm were obtained for *Aspergillus niger* and *Aspergillus flavus* respectively. It was observed that the trimetallic nanoparticles showed dose-dependent inhibition against the two fungal and two bacterial pathogens as shown in tables 2 and 4. This result is comparable to that obtained by Kannaiyan *et al.*, [26].

MIC test was obtained at 100 mg/mL for both *Salmonella sp.* and *S. aureus*, while the MIC test was obtained at 200 mg/mL and 400 mg/mL for *Aspergillus niger* and *Aspergillus flavus* respectively. From the above results, biosynthesized Ag-Cu-Al trimetallic nanoparticles using aqueous leaves extract of *Hierochloe odorata* have demonstrated very effective antibacterial ability against two bacterial species, *Salmonella sp.* and *Staphylococcus aureus*, and two fungal species, *Aspergillus niger* and *Aspergillus flavus*. All four pathogen species showed dose-dependent inhibition.

**Table 2: Antibacterial Sensitivity Assay**

Organism	400 mg/mL	200 mg/ mL	100 mg/ mL	50 mg/ mL	Control (? mg/mL)
<i>Salmonella sp.</i>	23 mm	16 mm	11 mm	4 mm	25 mm
<i>S. aureus</i>	18 mm	13 mm	7 mm	-	25 mm

**Table 3: Minimum Inhibitory Concentration (MIC) test**

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	Control (? mg/mL)
<i>Salmonella sp.</i>	+	+	+	-	100 mg/mL
<i>S. aureus</i>	+	+	+	-	100 mg/mL

**Table 4: Antifungal Sensitivity Assay**

Organism	400 mg/ mL	200 mg/ mL	100 mg/ml	50 mg/ mL	Control (? mg/mL)
<i>Aspergillus niger</i>	11 mm	7 mm	-	-	17 mm
<i>Aspergillus flavus</i>	18 mm	12 mm	7 mm	-	19 mm

**Table 5: Minimum Inhibitory Concentration (MIC) Test**

Organism	400 mg/ mL	200 mg/ mL	100 mg/ml	50 mg/ mL	Control (? mg/mL)
<i>Aspergillus niger</i>	+	+	-	-	200 mg/

					mL
<i>Aspergillus flavus</i>	+	-	-	-	400 mg/ mL

#### 4. Conclusion

The use of plant extracts in the synthesis of nanoparticles as a green approach has gained widespread attention due to its affordability, facile nature and being environmental friendly. These plants extract contains bioactive components such as flavonoids, alkaloids, terpenoids, antioxidants and enzymes that acts as reducing, capping and stabilizing agents in nanoparticles synthesis [27]. In this study, aqueous leaves extract of *Hierochloe odorata* was used to successfully biosynthesize Ag-Cu-Al trimetallic nanoparticles at room temperature. The biosynthesized Ag-Cu-Al NPs when compared with standard antimicrobial agent used as positive control showed effective antimicrobial activity against *Staphylococcus aureus*, *Salmonella sp.* *Aspergillus niger* and *Aspergillus flavus*. The NPs have outstanding potential antibacterial activity against *Staphylococcus aureus*, *Salmonella sp.* *Aspergillus niger* and *Aspergillus flavus*. This result confirms that aqueous leaves extract of *Hierochloe odorata* can be used in the biosynthesis of Ag-Cu-Al trimetallic nanoparticles and applied which can be used as a viable tool in the treatment of some multi-drug resistant strains of microbial pathogens. as shown in this study.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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