Biogenic Silver-Copper-Aluminum Nanoparticles: A Green Nanotechnology Approach for Sustainable Food Security

T. P Ugosor[1*], M.M Toryem[1], I.J Tyoga[2]
[1*]Department of Chemistry, College of Education, Katsina-Ala, Benue State, Nigeria.
[2]Department of Biology, College of Education, Katsina-Ala, Benue State, Nigeria.
[2]Department of Chemistry, College of Education, Katsina-Ala, Benue State, Nigeria.
M.M Toryem ORCID ID: https ://orcid.org/0009-0008-6314-1448
I.J Tyoga ORCID ID: https://orcid.org/0009-0008-4276-0004
Corresponding author: paulugosor@gmail.com, 08032980396
T.P Ugoosor ORCID ID: https://orcid.org/0009-0001-1815-5057.
DOI: 10.56201/ijccp.vol.11.no6.2025.pg9.19

Citation: T. P Ugosor, T.M Toryem, I.J Tyoga (2025). Biogenic Silver-Copper-Aluminum Nanoparticles: A Green Nanotechnology Approach for Sustainable Food Security.

Abstract
Sustainable food security faces converging pressures from climate change, pests and pathogens, postharvest losses, and supply-chain disruptions. Again, due to the erroneous use of antimicrobial agents in the management of food pathogens, many pathogens have become resistant and as such, there has been a surge in multi-drug-resistant strains of food pathogens. Green (biogenic) nanotechnology, where metal or metal-oxide nanoparticles (NPs) are produced using plant, microbial or agro-waste extracts, has been proven to have enhanced antimicrobial activity against food pathogens. This paper examines biogenic synthesis of Silver-Copper-Aluminium trimetallic nanoparticles involving aqueous leaves extract of Azadirachta Indica Juss (Neem) at room temperature and characterization using UV-Visible, FTIR, XRD, and SEM-EDX spectrophotomerty. In vitro anti-microbial studies of the nanoparticles was performed on selected food pathogens. UV-Vis spectroscopy of the Ag-Cu-Al NPs revealed a characteristics peak maximum at 405 nm. XRD analysis showed the crystalline nature of the NPs of 36.10 nm average crystallite size. SEM and EDX analysis showed surface features and material composition of the NPs. FTIR analysis revealed functional groups in the extract as responsible for the reduction, coating, and stabilization of NPs. Data obtained from the inhibition zone (mm) was investigated using Version 20 statistical package for social sciences, SPSS. The data was evaluated using ANOVA with LSD post hoc test and recorded as Mean ± SD at $p < 0.05$. The Ag-Cu-Al NPs demonstrated antimicrobial action in a dose-response relationship against five pathogenic fungi: Aspergillus niger, Fusarium oxysporum, Aspergillus flavus, Rhizopus stolenifera, and Botryodioplodia theobromae as well as three bacteria: Klebsiella oxytoca, Pseudomonas aeruginosa, and Serratia marcenscens. The NPs compared positively with standard antifungal (Fluconazole) and antibacterial (Ciprofloxacin) agents used as positive controls for fungi and bacteria, respectively. The NPs could be used as a viable tool for combating multi-drug resistant food pathogens for sustainable food security.

Key Words: *Ag-Cu-Al-Nanoparticles; Azadirachta Indica Juss (Neem); Biogenic; food pathogens,); In vitro antimicrobial activity.*

1.0 Introduction

Ending hunger and improving diet quality requires interventions that reduce food losses, control contamination, and safeguard the supply chain. A United Nation's (UN) report on state of food Security and Nutrition in the world revealed that around 733 million people faced hunger in 2023 [1], underscoring the need for technologies that are both effective and sustainable for ensuring sustainable food security.

In order to address the challenges faced by hunger and food insecurity, there has been a rapid advance in nanotechnology. Multimetallic NPs have gained increasing attention amidst the different types of nanoparticles because when compared to monometallic or bimetallic NPs, multimetallic nanoparticles show more catalytic activity, enhanced antibacterial effect, different shapes, highly selectivity, detection, sensitivity, stability, and chemical changes [2, 3]. These encouraging characteristics are a result of the synergetic effects of these materials. Synergism in these multimetallic NPs have shown a significant increase in remarkable properties like, photo-induced catalysis, antimicrobial activity, corrosion inhibition potential and other medicinal properties [4, 5].

Biogenic synthesis of NPs harnesses phytochemicals, enzymes, or microbial metabolites as reductants, coating and stabilizing agents to functional nanomaterials under mild conditions, aligning with circular-bioeconomy principles (e.g., using agro-waste extracts). Recent reviews and demonstrations show biogenic silver nanoparticle (Ag NPs) synthesized with plant or microbial extracts yield nanoscale, high-surface-area materials with strong broad-spectrum antioxidant/antimicrobial action: reactive oxygen species (ROS) generation, membrane disruption, ion release, and excellent plasmonics [6, 7]. Copper/copper oxide nanoparticles (Cu/CuO NPs) are effective, low-cost antimicrobials (nanofungicides/bacterials) with distinct modes of action (Cu^{+}/Cu^{2+} release, Fenton-like ROS, protein/DNA binding). They are widely studied for antifungal and antibacterial packaging, crop-pathogen controls, and as electrochemical/Surface-Enhanced Raman Spectroscopy (SERS) labels in rapid food sensing platforms for the detection of Escherichia coli, Salmonella, antibiotic residues and pesticides [8]. Metallic aluninium nanoparticles (Al NPs) are common in foods, aluminium oxide (Al_2O_3) and Anodic Aluninium Oxide (AAO) are valued as chemically robust, degradation-resistant supports and nanoporous scaffolds [5]. Ag-Cu bimetallic NPs imbedded in Polylactic Acid (PLA) packaging films improved antimicrobial performance and extended chicken meat shelf-life versus controls, illustrating synergy and reduced dosage [3].

Various approaches have been reported for the production of trimetallic NPs but, the green synthesis or biological synthesis route has proven to be most affordable and environmentally friendly [4]. Plant parts such as roots, leaves, flowers, seeds, and stems, and barks can be used. Plant extracts serve as reductants, encapsulating, and stabilizing agents due to the presence of biomolecules such as alkaloids, terpenoids, tannins, flavonoids, saponins, steroids, enzymes, polyphenols and antioxidants [9].

Azadirachta indica Juss referred to as "nature's drug store," is of the mahogany family, Meliaceae [10]. The plant is among the local medicinal plants in south Asia and is also widely distributed in Nigeria. Each part of the plant has some medicinal properties. Extracts of bark, stems, seeds, flowers, leaves and roots of the Neem have strong physiological activities against insect pests, with very low toxic effects to mammals and the environment [11, 12]. Neem contains active biomolecules in almost every part (roots, seeds, leaves, branches, bark, trunk, and flowers) with diverse medicinal properties [11]. Phytochemical analysis of neem leaves revealed the presence of triterpenoids, saponins, tannins, flavonoids, phenols, alkaloids, proteins, glycosides, carbohydrates and alkaloids [12]. These phytochemicals generally help the plant to resist fungi, bacterial and viral infections, and also protect it against feeding by insects and other animals [10]. *Azadirachta indica* has been utilized for a long time as local medicine for household and treatment against different human

ailments in ancient times.
Combining Ag-Cu-Al with *Azadirachta Indica* Juss (Neem) at nonoscale can broaden antimicrobial spectra and exploit complementary release kinetics: stabilize nanostructures and reduce uncontrolled migration, enable integrated sensing (SERS hot-spot on AAO) within packaging, and enhance mechanical/barrier properties of bio-based films. By slowing microbial growth and oxidation, these materials can reduce postharvest losses, an essential lever of food security, while enabling smart packaging that can both protect and monitor food quality.
This research focuses on green preparation of Ag-Cu-Al NPs using hot aqueous leaves extract of *Azadirachta Indica* Juss (Neem) and *In vitro* antimicrobial studies against some selected food pathogens.

2.0 Materials and Methods

2.1 Materials and chemicals

Materials used: *Azadirachta Indica* Juss (Neem) leaves, collected within the campus of Fr. Moses Orshio Adasu University, Makurdi in May, 2025 and authenticated by a plant taxonomist. The pathogens used comprised of five fungi: *Aspergillus flavus, Aspergillus niger, Botryodioplodia theobromae, Fusarium oxysporum* and *Rhizopuss tolenifera* and three bacteria: *Klebsiella oxytoca, Pseudomonas aeruginosa Serratia marcescens* which were sourced from the Laboratory, Department of Biological Sciences, Rev. Fr. Moses Orshio Adasu University, Makurdi, Benue State.

The chemicals used: Analytical grade copper (II) chloride ($CuCl_2$), silver nitrate ($AgNO_3$), aluminium oxide (Al_2O_3). All chemicals and regents were sourced from BDH Chemicals, England, M&B laboratory, England, through Emole (NIG) LTD, Makurdi, Benue State. All chemicals utilized in this study were analytical grade and were used directly without additional purification. Solutions were freshly prepared with double distilled water and stored in dark conditions to minimize photochemical interference. The glass ware employed in the experiment were sterilized using a 10 % sodium hypochlorite solution, thoroughly rinsed in double distilled water and air-dried before use. Throughout the entire experimental process, aseptic techniques were strictly observed.

2.2 Methods

2.2.1 Drying and pulverization

Fresh neem leaves were carefully washed with distilled water to remove impurities and then air-dried in a shade at room temperature for two weeks to prevent chemical degradation. After complete drying, the leaves were ground into a fine powder using a clean wooden mortar and pestle. The resulting powder was stored in an air-tight plastic container for later use.

2.2.2 Plant extraction procedure

The plant material was extracted by adding 100 g of the fine powder to 500 mL of distilled water, heated at 60 °C for 30 minutes, allowing it to cool in a desiccator, and then filtering it. The extract was kept at 4 °C in the refrigerator for further analysis**.**

2.3.3 Culture media preparation

The Clinical and Laboratory Standards Institute performance standards (CSLI) for antimicrobial inhibition discs tests method, [13] was used without modifications.

2.3 Qualitative phytochemical Screening

Preliminary qualitative phytochemicals analysis was carried out using standard analytical procedures as described by [11] without any modification.

2.4 Synthesis of Ag-Cu-Al Trimetallic NPs

Trimetallic NPs was prepared by reducing the precursor salts of silver, copper and aluminum simultaneously using extract of *Azadirachta indica* Juss (Neem). Then, 30 mL of the freshly prepared leaves extract was added drop-by-drop to 150 mL of 0.1 M $AgNO_3$-$CuCl_2$-Al_2O_3 solutions (50 mL of each salt solution), stirring constantly using a magnetic stirrer. A colour change from grey to olive green indicated the formation of the trimetallic NPs. Centrifugation of the NPs at 1200 rpm for 5 minutes was performed, washed with distilled water to obtain pure particles, and then stored in a desiccator for further evaluation.

2.5 Ag-Cu-Al Trimetallic NPs Characterization

The NPs formation was investigated using UV-Vis absorption spectrum, Uv-3600 Plus, Shimadzu, Japan in the range of 200-800 nm; XRD-6000, Shimadzu, Japan, monochromatic Cu Ka radiation (1.5419 Å) at 40 kV and 30 mA at 2θ (10-70^o), speed of 4^o per minutes; Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX: JEOL-JSM5800, Japan), and Perkin Elmer FTIR Spectrophotometer-100, KBr pellet method, range of 500 - 4000 cm^{-1}).

2.6 Antimicrobial Inhibition Test

Method of [14] was used without modifications. The biosynthesized NPs was evaluated for antimicrobial action against five pathogenic fungi: *Rhizopus stolenifera, Aspergillus niger, Botryodioplodia theobromae, Aspergillus flavus* , and *Fusarium oxysporum,* and three bacteria: *Klebsiella oxytoca, Pseudomonas aeruginosa* and *Serratia marcenscens.*
Inhibition zone (mm) was recorded with a transparent plastic ruler after the incubation period. The zone of inhibition was calculated as follows and expressed in percentage:

$$\% \text{ Zone of Inhibition} = \frac{\text{Average diametre of pathogen colony}}{\text{Average diametre of pathogen in control}} \text{ x } 100\ \%.$$

The zone of inhibition was rated on the scale described by [14] as follows:
100 % inhibition (highly effective); 50 – 99 % inhibition (effective); 20 – 49 % inhibition (moderately effective); 0 – 19 % inhibition (slightly effective) and ≤ 0 % inhibition (not effective.

2.7 Statistical Analysis

The data obtained from the inhibition zone (mm) was evaluated using ANOVA with LSD post hoc test at $p < 0.05$. Results were recorded as Mean ± SD.

3. 0 Results and Discussion

3.1 Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical analysis of the extract of *Azdirachta indica* is shown in Table 1. The result revealed the presence of alkaloids, tannins, flavonoids, saponins, polyphenols, glycosides, resins, and steroids.

Table 1: Phytochemical screening result

Secondary Metabolite	Result
Alkaloids	+
Saponins	+
Flavonoids	+
Polyphenols	+
Tannins	+
Steroids	+
Resins	+
Glycosoides	+
Coumarins	-

Key: + = Presence of phytochemical; - = Absence of phytochemical.

3.2 Characterization of NPs

3.2.1 UV-Visible Spectroscopic Analysis

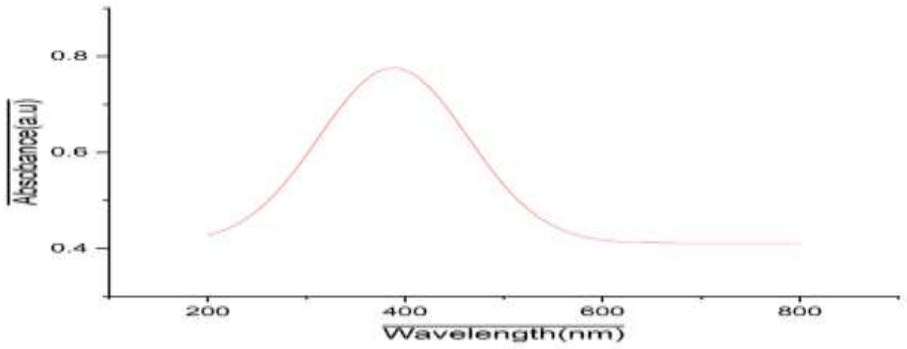

Figure 1: UV-vis spectrum of Ag-Cu-Al trimetallic nanoparticle

UV-vis spectrum of the NPs is shown in (Fig. 1). UV-visible absorption spectra of Ag-Cu-Al trimetallic nanoparticle shows absorbance at 400-407 nm and peak maxima of 401 nm, which is due to the presence of Ag-Cu-Al NPs formation. The symmetrical structure suggests that the nanoparticles are not uniformly distributed or homogenous. The irregularity of the nanoparticles is responsible for the broad absorption peak. Size, shape and encapsulating agent of nanoparticles influence the location of the SPR absorption band as shown the spectrum.

3.2.2 XRD Analysis

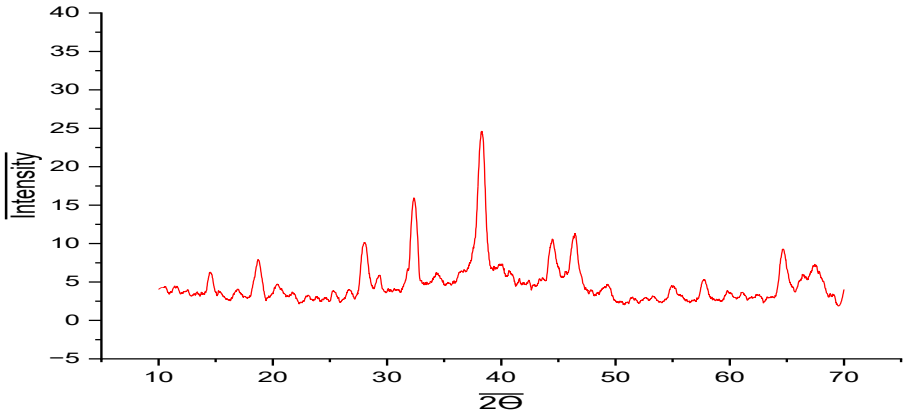

Figure 2: XRD pattern of the NPs

Figure 2 represents the XRD pattern of the NPs. Average crystallite size of the nanoparticles

was estimated utilizing the Debye-Scherrer equation:

$D = \frac{K\lambda}{\beta\cos\theta}$; where, D = crystallite size; k = constant/shape factor (0.94), ƛ = wavelength of the X ray (1.5418 Å), β = is the width at half maxima, θ = Bragg's angle.

XRD pattern shows that the Ag-Cu-Al NPs are crystalline in nature with sharp peaks. Diffraction peaks are shown at 38.48°, 44.68°, 47.13° and 65.24° which corresponds with the (111), (200) and (220) when compared with the standard powder and diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS). Face-centered cubic structures of silver, confirmed the presence of silver oxide in the NPs [14, 15] . Copper oxide peaks are shown at 28.86° and 33.27°, aluminum oxide at 57.76° and 58.61° because of the oxidation of copper and aluminum respectively [16]. The average crystallite size of the NPs was estimated to be 36.10 nm.

3.2.3 SEM-EDX Analysis

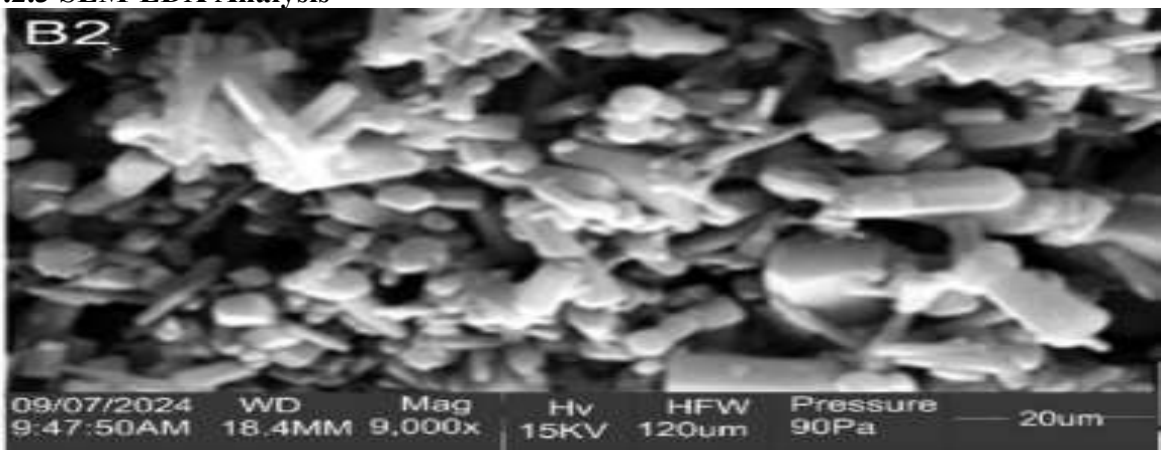

Fig. 3: SEM images of Ag-Cu-Al NPs

SEM image of the ZnO is presented in Figure 3. SEM analysis revealed an irregular crystalline structure characteristics of metallic nanocomposites formed as a result of the strong interparticle contact imposed by the high surface energy [17]. The images also showed the intricacy of the crystallite size of the densely packed cylindrical nature and rough surface texture, which might be attributed to biomolecules from the leaf extract that are bound to the Ag-Cu-Al NPs. This result is consistent with the XRD analysis..

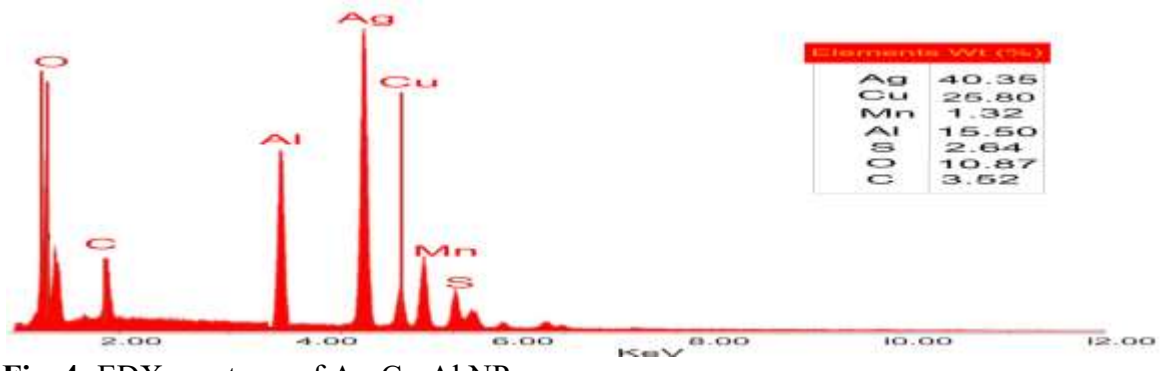

Fig. 4: EDX spectrum of Ag-Cu-Al NPs

EDX spectrum showed distinctive peaks and high elemental composition of Ag, Cu and Al, confirming the purity of the NPs [17].

3.2.4 FTIR Analysis

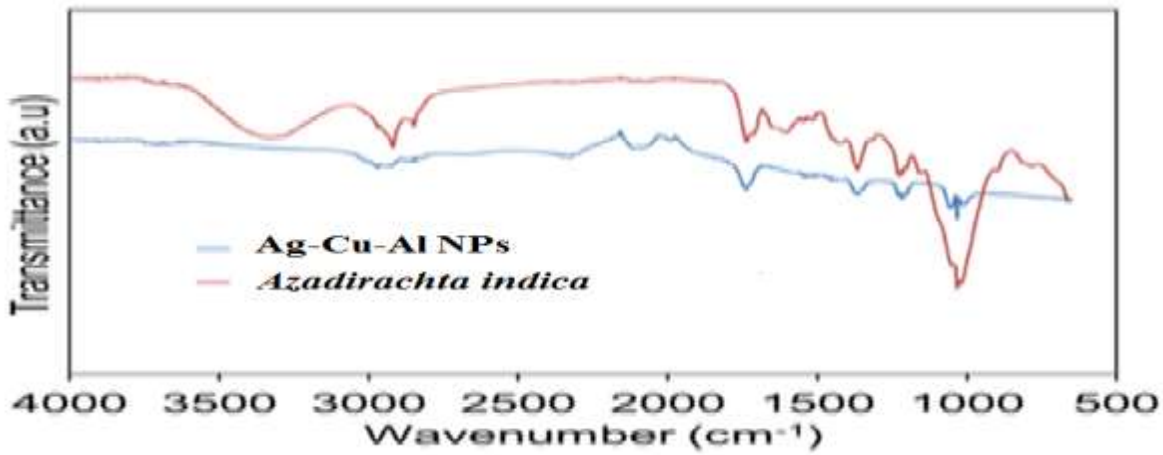

Fig. 5: FTIR spectra of Ag-Cu-Al NPs and *Azdirachta indica (juss)* leaves extract.

Fig. 5 shows FTIR spectra of the Ag-Cu-Al nanoparticles and *Azdirachta indica* (juss) leaves extract respectively. The peak at 3318 cm^{-1} is unique of stretching vibration of hydroxyl group (-OH) of polyphonols. C-H medium stretching for alkane is responsible for the bonds found 2938 cm^{-1}. Peak 2081 cm^{-1} can be assigned to C=C stretch of alkenes. 1736 cm^{-1} IR band is associated with C=O stretching of esters. 1367 cm^{-1} is strong band for N=O stretching for nitro compounds. While 1217 cm^{-1} and 1033 cm^{-1} are attributed for C-O stretch for esters and C-C bending stretch of alkanes respectively.
Leaves extract of *Azdirachta indica* (juss) is known to have phytochemicals such as alkaloids, tannins, steroids, saponins and flavonoids capable of reducing metal ions of silver, copper and Aluminium as well as serving as capping and stabilizing agents for nanoparticles [18].

3.3 Antimicrobial Inhibition Test Result

Table 2: Average inhibition zone (mm) of biosynthesized Ag-CU-Al NPs

	Ag-CU-Al NPs Concentration (mg/mL)				
	100	**75**	**50**	**25**	**Control**
Fungi					
A. niger	$6.91^{c} \pm 0.08$	$6.83^{c} \pm 0.42$	$5.83^{b} \pm 0.42$	$3.67^{a} \pm 0.52$	$8.16^{d} \pm 0.41$
A. flavus	$7.98^{c} \pm 0.04$	$7.50^{c} \pm 0.54$	$6.33^{b} \pm 0.51$	$5.20^{a} \pm 0.20$	$9.67^{d} \pm 0.52$
B. theobromae	$8.16^{c} \pm 0.42$	$7.50^{b} \pm 0.54$	$7.00^{b} \pm 0.63$	$6.67^{a} \pm 0.32$	$11.00^{d} \pm 0.89$
R. stolenifera	$13.67^{d} \pm 0.52$	$8.16^{c} \pm 0.98$	$7.50^{b} \pm 0.55$	$5.80^{a} \pm 0.41$	$18.00^{e} \pm 0.63$
F. oxysporum	$25.50^{d} \pm 0.55$	$20.33^{c} \pm 0.52$	$17.33^{b} \pm 0.52$	$12.33^{a} \pm 0.52$	$29.67^{e} \pm 0.52$
Bacteria					
K. oxytoca	$26.00^{d} \pm 0.89$	$20.33^{c} \pm 0.52$	$18.33^{b} \pm 0.52$	$15.67^{a} \pm 0.52$	$34.83^{e} \pm 0.75$
S. marcescens	$34.67^{d} \pm 0.82$	$26.00^{c} \pm 0.89$	$22.33^{b} \pm 0.52$	$18.33^{a} \pm 0.42$	$40.33^{e} \pm 0.52$
P. aeruginosa	$35.16^{d} \pm 0.42$	$30.83^{c} \pm 0.98$	$25.33^{b} \pm 0.52$	$18.33^{a} \pm 0.52$	$37.67^{e} \pm 0.52$

N= 5, values expressed as Mean ± SD. Values in the same row with different alphabetical letters (superscript) have significant correlation at $p < 0.05$.

Figure 7: Inhibition test plates

Table 3: Zone of inhibition (%) of the Ag-Cu-Al NPs

Concentration (mg/mL)	100	75	50	25
Fungi				
Aspergillus niger	84.68	83.70	71.45	44.98
Aspergillus flavus	82.52	77.56	65.46	53.77
Botryodioplodia theobromae	74.18	68.18	63.64	60.64
Rhizopus stolenifera	75.94	45.33	41.67	32.22
Fusarium oxysporum	85.95	68.52	58.41	41.56
Bacteria				
Klebsiella oxytoca	75.94	45.33	41.67	32.22
Serratia marcescens	85.95	68.52	58.41	41.56
Pseudomonas aeruginosa	74.65	58.37	52.63	44.99

Key:
a = 100 % inhibition (highly effective)
b = 50 – 99 % inhibition (effective)
c = 20 – 49 % inhibition (moderately effective)
d = 0 -19 % inhibition (mildly effective)
e = ≤ 0 % inhibition (not effective) [14].

3.4 Antimicrobial Study of the Ag-Cu-Al Nanoparticles

Table 2 shows the average zone of inhibition (mm) while Table 3 shows the zone of inhibition (%) of NPs versus the pathogens. In general, the results revealed that inhibitory effects of the NPs increased with increasing concentration ($p < 0.05$). The result showed average zones of inhibition (mm) that are significant at $p < 0.05$. Generally, the differences in inhibition between concentrations are statistically significant with bacteria more susceptible to the NPs than fungi. The biosynthesized NPs correlates positively with standard commercial antifungal (Fluconazole) and antibacterial (Ciprofloxacin) used as positive control agents.

4.0 Conclusion

Ag-Cu-Al nanoparticles was effectively prepared through a sustainable approach using *A. indica* leaves extract. *In vivo* antimicrobial analysis of the particles against the microorganisms exhibited dose-dependent antimicrobial activity with the highest activity (85.95 %) at 100 mg/mL. The Ag-Cu-Al NPs could be potentially used to control postharvest food losses for sustainable food security and management of multi-drug resistant variants of microorganisms.

ACKNOWLEDGEMENT

The authors highly acknowledge the staff of the Departments Biology, and Biological Sciences, College of Education, Katsina-Ala and Rev. Fr. Moses Orshio Adasu University, Makurdi respectively for the facilities provided and the assistance for the successful conduct of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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