

Green Synthesis of Silver Nanoparticle Using *Okoubaka Aubrevillei* for Antimicrobial Treatment

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Abstract

This study prepared silver nanoparticles via the green approach using novel seed pulps of *Okoubaka aubrevillei*, a medicinal herb. The synthesized silver nanoparticles (AgNPs) were characterized by SEM-EDX, TEM, FTIR, UV-VIS and antimicrobial properties. The surface morphology showed an amorphous and polydispersed spherical shape in a random arrangement in the range of 10 – 50 nm. The nanoparticle shapes observed by TEM were spherical in shape with narrow particle size distribution. The FTIR showed that the extract contains carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups which aid in the reduction of Ag⁺ to Ag⁰ nanoparticles. The extracts contained a carbonyl group of amino acids which acts as a stabilizing agent to prevent agglomeration. UV-visible spectra of the AgNPs synthesized by methanolic extract indicated that AgNPs surface Plasmon band occurred in the range of 360 – 380 nm in an aqueous medium and the strongest absorbance bands at 370 nm. The antimicrobial study showed that the fungi; *C. albicans* was more susceptible than the bacteria; *E. coli*, *S. aerus*, and *Ps. aeruginosa*, while *Ps. aeruginosa* is a less susceptible microbe. The synthesized AgNPs were shown to be very effective against *C. albicans*, and recommended for further application.

Keywords: Extraction, Synthesis, medicinal, plant extracts and, UV-absorption.

1. INTRODUCTION

Silver nanoparticles (AgNPs) have become the subject of intense investigation in the scientific community due to their vast applications in fields such as catalysis, antimicrobials, optics, and biomaterial creation. [1]. Depending on their size, shape, and dispersion, AgNPs display new or better characteristics. Many ways based on plant extracts have been employed to create metal nanoparticles. These methods offer several benefits over chemical, physical, and microbial synthesis [2]. Many research papers have reported the synthesis of AgNPs using plant extracts such as *Helianthus annuus* [3], *Basella alba* [4], *Oryza sativa* [5], *Saccharum officinarum* [6], *Morinda lucida* [7], *Jatropha curcas* seed [8] and *Zea mays* [9] due to the absence of elaborated processes of culturing and maintaining the cell, high energy requirements, wasteful purifications, or

hazardous chemicals. Interestingly, research studies are yet to investigate the extraction, characterization and synthesis of *Okoubaka aubrevillei* having been reported to have medicinal properties such as anti-poison and indigestion and diarrhoea.

Globally and especially within the study area, there is virtually no available literature on the studies of *Okoubaka aubrevillei*. Additionally, the plant is quite rare and there is a paucity of data on its application. However, lots of work has been done on the synthesis, extraction and characterization of other plants which can elucidate the data of *Okoubaka* and antimicrobial activity. Therefore, the work will convey its global importance (knowledge gap), and scientific knowledge within the study region and provide potential grounds for further research in the emerging field of silver nanoparticle synthesis. Hence, there is a

need for an elaborate study of *Okoubaka aubrevillei* and its antimicrobial potential. The plant extract *Okoubaka aubrevillei* known as Anunuebe demonstrates distinct characteristics which differentiate it from traditional plant extracts used for nanoparticle synthesis. The phytochemical profile of *Okoubaka aubrevillei* produces antimicrobial and antioxidant effects along with bioactive properties that distinguish it from typical plant extracts. The synthesis process benefits from secondary metabolites such as alkaloids, saponins, tannins, flavonoids, terpenoids, and cardiac glycosides that reduce metal ions and maintain nanoparticle stability [10-11]

Okoubaka aubrevillei stands out because it contains unusually high levels of saponins and tannins. The plant source contains elevated levels of saponins at 33.80% and tannins at 1741.82 mg/100 g which surpass the values found in traditional plant materials. The compounds work to boost reduction potential while providing superior nanoparticle capping and stabilization which results in uniform sizes and prevents aggregation. The antimicrobial properties of *Okoubaka aubrevillei* extracts show effective action against Gram-positive and Gram-negative bacteria and fungi which enhances the potential of synthesized nanoparticles for antimicrobial applications [12].

The plant's dual parasitic behaviour and chemical defence mechanisms through root haustoria create an ecological perspective that is uncommon in nanoparticle synthesis research. The plant's complex environmental interaction produces distinctive bioactive compounds which improve nanoparticle functionalization capabilities. Among plants utilized for nanoparticle synthesis including neem and green tea, the *Okoubaka aubrevillei* plant offers superior bioactive compound diversity and stronger antimicrobial properties. The plant shows potential as an excellent green synthesis method for nanoparticles that could lead to improved biomedical and environmental applications. The investigation of

phytochemical synergies in this plant holds the potential to create revolutionary advancements in sustainable nanotechnology [11-13]

The antibacterial effect of Ag has been documented since ancient times, and Ag is currently used in a variety of applications such as catheters, dentures, and wound healing formulations, and the antibacterial and bacterial-killing properties of Ag-based formulations [14–16]. Plant-based AgNP production outperforms traditional physical and chemical methods. Plants have capping and reducing agents such as terpenes, polysaccharides, vitamins, polyphenols, and more, and when included with AgNPs, they have boosted antibacterial activity [17] Green production of nanoparticles utilising plants and microbes is physiologically safe, ecologically beneficial, and cost-effective. Plants and microbes have developed the ability to consume and collect inorganic metal ions from their surrounding habitats [18]. Biological entities may synthesize nanoparticles both intracellularly and extracellularly. The potential of a biological organism to employ its inherent organic chemistry processes to modify inorganic metal ions into nanoparticles has revealed a hitherto unknown field of research [19]. AgNPs are effective because of their antibacterial activity against bacteria, viruses, and other eukaryotic microorganisms. AgNPs, in particular, are playing an important role in nanotechnology and materials research. Colloidal silver is of special interest in aqueous solutions because of its unique chemical stability, thermal stability, excellent conductivity, and catalytic and antibacterial activity [20]. The use of plant extract to synthesise AgNPs is a key aspect of green production of nanoparticles [21]. In this context, we utilized *O. aubrevillei* seed extracts for the synthesis of AgNPs for antimicrobial treatment; having been described as a new homoeopathic medicine [22], The study offered a simple, effective, approach for green synthesis of Ag metal nanoparticles to reduce toxicity and

increase the bio-formulation potential for treating antimicrobial-resistant organisms.

2. EXPERIMENTAL APPROACH

2.1. Chemicals and Reagents

Silver nitrate (AgNO₃; 99.9%), and methanol (99.9%) were purchased from Kenton Chemicals LTD, Owerri, Imo State, Nigeria, and used as purchased. The plant extract served as the reducing and capping agents. Deionized (DI) water of 18.2 M Ω resistivity at 25 °C was used for preparing the standard solutions.

2.2. Plant Materials

Okoubaka aubrevillei seeds were collected and identified by a botanist at the School of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Imo state, Nigeria. The young green Okoubaka aubrevillei seeds were taken from the parent plant and cleaned with tap water. The washed plant materials were air dried for 30 days duration to remove air pockets and moisture at laboratory conditions (29-32 oC and 55-60 % RH). The dried matter was ground to a powder with a manual grinder. About 200 g of the dried plant matter was introduced into a soxhlet device followed by 300 mL of methanol added as the solvent and operated continuously for 72 hr. The extracted material (extract) was filtered with Whatman filter paper No. 1 and then methanol was evaporated in the oven operated at 40 oC overnight. The drying temperature of 40 °C was optimized to preserve heat-sensitive bioactive compounds crucial for reducing and stabilizing silver ions. Higher temperatures degrade these compounds, while lower temperatures prolong drying and risk contamination to enhance nanoparticle yield, stability, and antimicrobial efficacy, aligning with green chemistry principles. The dried samples were sterilised with a UVC lamp (180 nm) with a protective shield at a distance of 0.05 m for 30 s. [17, 23].

2.3. Synthesis of Silver Nanoparticles (AgNPs)

The silver nanoparticles (AgNPs) of *O. aubrevillei* seeds were prepared with silver nitrate (AgNO₃) as a precursor. AgNO₃ of 1 mM concentration (90 mL) was prepared in deionized water and 10 mL of 10 % (w/v) methanol *O. aubrevillei* seeds extract was slowly added with continuous stirring. The 10% methanolic extract concentration was chosen to ensure optimal bioactive compound availability without causing nanoparticle aggregation, as higher concentrations risk instability while lower concentrations yield incomplete reduction. Additionally, methanol has high efficacy in extracting key phytochemicals essential for nanoparticle synthesis. As the process proceeded, the colour of the admixture slowly changed into golden yellow which revealed the appearance of AgNPs. The mixture was placed in the dark for 24 hr for the equilibration of AgNPOs. The separated mixture was decanted and the settled part was transferred into a magnetic stirrer at 10,000 for 30 min after which the clear supernatant was discarded and the settled matter was washed in deionized water for 10 min at 5000 rpm. The washing approach was repeated and then ethanol was used to wash the settled material at 5000 rpm for 10 mins. Afterwards, the filtrate was discarded and steeled layer was dried in the oven at 70 oC for 12 hr. [17, 23]

2.4. Characterization

The Fourier transform Infrared Spectroscopy (FTIR) (Shimadzu IR-Affinity-IS-spectrometer) was used to study the chemical and structure elucidation of the membrane. The samples were prepared as pellets of solids that were dispersed in KBR for data acquisition. The nano-morphology was examined by Shimadzu EDX-7000 Energy dispersive X-ray- fluorescence spectrometer. The specimens were mounted on a copper grid coated with carbon dispersed in methanol and ultrasonicated for 45 min before loading into the instrument. The morphology of the AgNPs

was studied using Shimadzu (scanning electron microscope-energy diffraction xray) 550-SEM-EDX superscan The EDX acquired the elemental composition using an Oxford micro-analyzer having an accelerating voltage of 30 kV and upto 3.5 μm resolution. To determine the time point of maximum production of silver nanoparticles, the absorption spectra of the samples were taken 300–540 nm using a UV–vis spectrophotometer (Shimadzu SPD-20 A prominence UV/VIS detector) and The de-ionized water was used as the blank [23, 24].

2.5. Antimicrobial Analysis

The antimicrobial activity was carried out using four different strains which include *C. albicans*, *E. coli*, *S. aureus*, and *Ps. aeruginosa* microorganisms. The antibacterial performance of synthesized AgNPs was determined using a disc diffusion method selected for this assay. Each microbe inoculum was prepared according to McFarland 0.5 standards and contained 108 CFU mL⁻¹. Each strain's lawn was created by distributing 100 μL of the relevant inoculum on Petri plates of Muller Hinton Agar medium. AgNPs disc, Ciprofloxacin 5 mcg/disc (positive control), and DMSO disc (negative control) were placed on each plate. All studies were performed in triplicates with standard deviation and the zone of inhibition was calculated in mL after 24 h of incubation at 37 °C [23, 25].

3. RESULTS AND DISCUSSION

3.1. AgNPs Characterization

The FT-IR spectrum obtained for the crude extract of *Okoubaka aubrevillei* K Schum is shown in Fig. 1. which displayed several absorption peaks, reflecting its complex nature. Strong absorption peaks at 3354.6 cm^{-1} resulted from stretching of the -NH band of amino groups or are indicative of bonded (-OH) hydroxyl group. The absorption peaks at about 2922.2 cm^{-1} are assigned to stretching vibrations of -CH₂ and CH₃ functional groups. The peaks at

1699.7 and 1515.3 cm^{-1} showed the fingerprint region of CO, C-O, and O-H groups. The intense band at 1021.3 cm^{-1} is allocated to the C-N stretching vibrations of aliphatic amines.

The spectrum bands at 1581 and 1446.2 cm^{-1} were identified as amide I and amide II which was due to carbonyl (C=O) and amine (-NH) stretching vibrations in the amide linkages of the proteins, respectively. The absorption band at 1446.2 cm^{-1} could be attributed to methylene scissoring vibrations from the proteins. Therefore, the chemical analysis indicated that the carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) groups in *Okoubaka aubrevillei* K Schum extract were mainly involved in the reduction of Ag⁺ ions to Ag⁰ nano-particles.

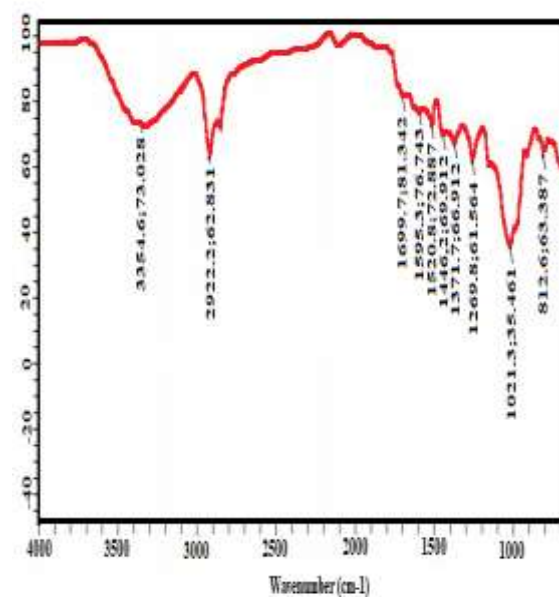


Figure 1. Fingerprint and functional groups of *Okoubaka aubrevillei*.

The FT-IR spectroscopic confirmed that the protein present in *O. aubrevillei* K Schum acted as a reducing agent and stabilizer for the silver nanoparticles and prevented agglomeration. The carbonyl group of amino acid residues has a strong binding ability with metal, suggesting the formation of a layer covering silver nanoparticles and acting as a stabilizing agent to prevent agglomeration in the aqueous medium [26].

SEM-EDX in Fig. 2. was carried out to determine the surface morphology of the AgNPs and the elemental composition of NPs. The surface morphology concerning resolution showed an amorphous and polydispersed spherical shape as a result of its random arrangement, with AgNPs in the range of 10-50 nm, having an average size of 20 nm. Furthermore, the antimicrobial properties will exert a greater effect on bacteria because of their particle sizes. Hence, the scan result strongly suggests the

presence of nanoparticles because the higher the magnification, the smaller the particle sizes. Moreover, the SEM-EDX confirmed the elemental composition of nanoparticles as silver. Additionally, the optical absorption band between the range of 4-6 Kev is typical for the absorption of metallic silver nano-crystallites. Similarly, the EDX confirmed the strong presence of silver and oxygen atoms needed for AgNP compositions as obtained similar study [27].

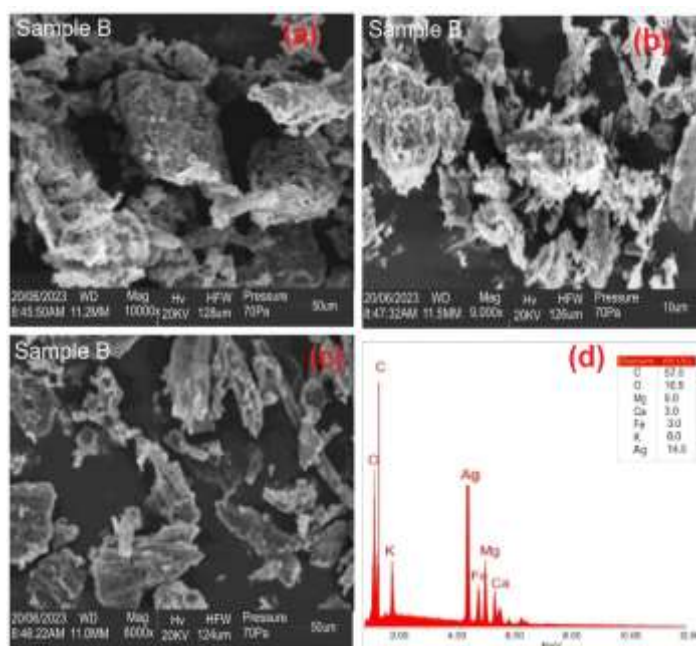


Figure 2. The SEM-EDX profiling of *O. aubrevillei* AgNPs.

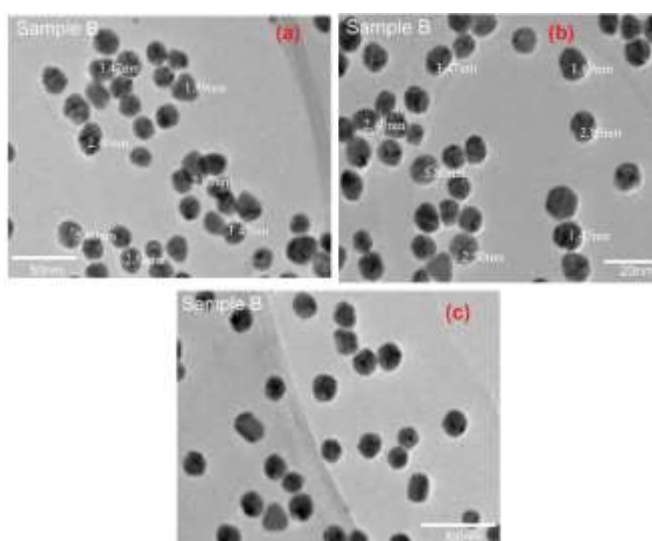


Figure 3. The TEM profiling of *O. aubrevillei* AgNPs.

The nano-size distribution of AgNPs in the aqueous solution was evaluated using (transmission electron microscope) TEM and shown in Fig 3. The size distributions of AgNPs were obtained by measuring nanoparticle diameter in the images using different magnifications that sowed nanodispersion and nano aggregations of respective particle sizes. The nanoparticle shapes observed by TEM were similar to spherical shapes with particle sizes ranging from 1.47 - 5.80 nm with a relatively narrow particle size distribution. Furthermore, AgNPs with an average size ≤ 10 nm showed a higher prospect for electronic effect that greatly increases their bactericidal activity. The dispersion offers phase stability which improves the properties of AgNPs significantly. Furthermore, the grey and dark sections in the black circle are mineral elements. The results obtained are in agreement with previous studies that reported spherical AgNPs synthesized extract and obtained 70 - 190 nm in size [27, 28]

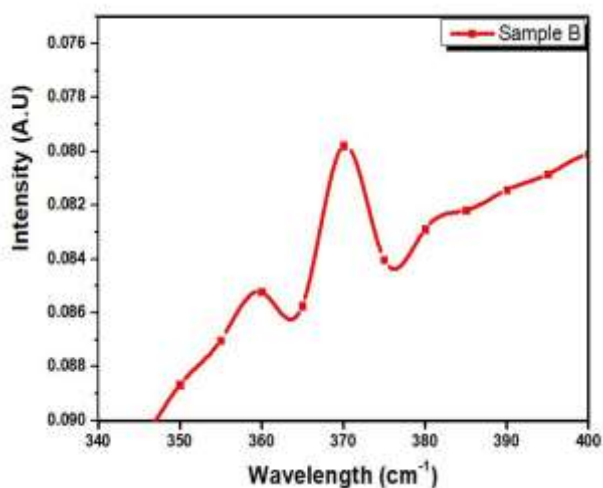


Figure 4. UV-Visible Synthesized AgNPs of *O. Aubrevillei*.

UV-visible spectroscopy analysis of synthesized AgNPs of methanolic extract of *Okoubaka aubrevillei* is shown in Fig 4. The UV-visible spectroscopic analysis provided a spectrum that justified the formation and stability of metal

nanoparticles in aqueous solution. UV-visible spectra of the AgNPs synthesized by methanolic extract of *O. umbrevillei* indicated that AgNPs surface Plasmon band occurred in the range of 360 nm and 380nm in an aqueous medium. Also, UV-visible absorption spectra indicated the strongest absorbance band at 370 nm for AgNPs formed by the synthesized samples. Furthermore, the UV-visible spectra (UV-vis) results obtained showed a blue shift or hypsochronic effect which can be attributed to shorter wavelength due to a reduction in particle size and higher surface Plasmon energy. Hence, the antimicrobial properties will be improved. However, from the results obtained, it bolstered cost-effectiveness because the absorbance is nearer to the visible light region. Interestingly; the formed NPs were highly stabilized with no complexity of the formed AgNPs. It is known that the samples contained some reducing agents which released into solutions and are responsible for the formation of AgNPs [29].

Comparing our study and previous findings, the synthesized silver nanoparticles (AgNPs) exhibited spherical shapes with sizes ranging from 10 to 50 nm, averaging around 20 nm. This morphology aligns with findings from other green synthesis studies. For instance, AgNPs synthesized using *Vitex agnus-castus L.* fruit extract were also spherical, with sizes between 30 and 60 nm [30]. Similarly, AgNPs produced with *Eugenia roxburghii DC.* leaf extract displayed spherical shapes, though specific size ranges [31]. The UV-visible spectroscopy analysis of the current study revealed a surface plasmon resonance (SPR) band at 370 nm, indicating the formation of AgNPs. This observation is consistent with previous reports where SPR bands appeared between 415 and 439 nm [32]. The slight blue shift in our study suggests smaller particle sizes, which can enhance antimicrobial properties. These comparisons underscore the effectiveness of *Okoubaka aubrevillei* extract in

producing stable, spherical AgNPs with potent antimicrobial activity [33].

3.2. Antimicrobial Evaluation of *O. Aubrevillei*.

The Effects of different concentrations of *O. aubrevillei* on each of the Test Organisms are shown in Fig 5.0. The evaluation showed that *O. aubrevillei* extract had different levels of activity against all the test organisms at concentrations of 200 mg/mL and 100 mg/mL. At concentrations of 50 mg/mL and 25 mg/mL, activity was observed against only 3 organisms; *S. aureus*, *Ps. aeruginosa*, and *C. albicans*, while at the lowest concentration of 12.5 mg/mL, only *S. aureus* and *C. albicans* were inhibited. The positive control had activity against *E. coli* and, *C. albicans*, but not *S. aureus* and *Ps. aeruginosa* [34].

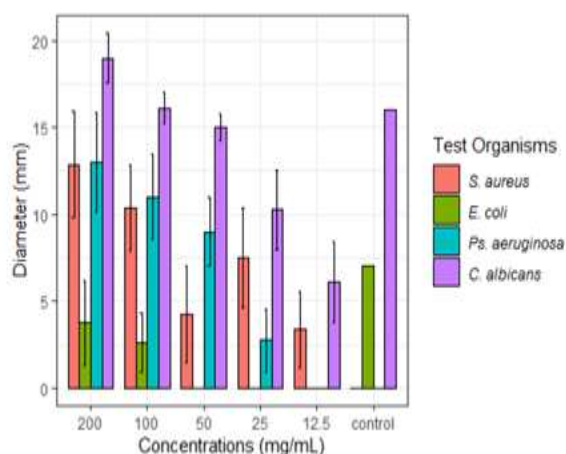


Figure 5. Effect of different concentrations of *O. aubrevillei* on the inhibition zone of organisms.

For all the different concentrations of *O. Aubrevillei* extract, the highest inhibition zone was against *C. albicans*, which compares well with the positive control where the highest inhibition zone was also against *C. albicans*. The observed difference in the inhibition effect of *O. aubrevillei* extract against each of the test Organisms was statistically significant ($P < 0.007$) at the different concentrations. At concentrations of 200 mg/mL and 100 mg/mL, the inhibition zone diameter of *O.*

aubrevillei extract was significantly ($P < 0.05$) higher than that of the control, which had no inhibition against *S. aureus*. The inhibition zone diameter of the control against *E. coli* was significantly ($P < 0.05$) higher than those of 50 mg/mL, 25 mg/mL and 12.5 mg/mL concentrations of *O. aubrevillei* extract, but not 200 mg/mL and 100 mg/mL. Results from *Ps. aeruginosa* at the inhibition zone diameter of 200 mg/mL of *O. aubrevillei* extract was significantly higher than that of 25 mg/mL ($P < 0.005$), and 12.5 mg/mL and the control ($P < 0.001$); both of which had no inhibition effect [35].

That of 100 mg/mL was significantly higher than that of 25 mg/mL ($P < 0.04$), as well as those of 12.5 mg/mL and the control ($P < 0.002$); both of which showed no inhibition. Also, at 50 mg/mL, it was higher than the 12.5 mg/mL zone and the control ($P < 0.02$); both of which had no inhibition effect. The observed difference in the inhibition effect of *O. aubrevillei* was statistically significant ($P < 0.001$) at the different concentrations. Against *C. Albicans*. The inhibition zone diameter of the plant extract at 200 mg/mL, 100 mg/mL, and 50 mg/mL were significantly higher ($P < 0.05$) than that of 12.5 mg/mL, which had no inhibition effect. Equally the inhibition zone diameter of the control was significantly higher ($P < 0.05$) than that of 12.5 mg/mL of the plant extract, but not those at 200 mg/mL, 100 mg/mL, and 50 mg/mL. The control had inhibition activity against only two of the test organisms; *E. coli* and *C. albicans* while the plant extract inhibited all the test organisms at concentrations of 200 mg/mL and 100 mg/mL. The screening of the antimicrobial activity of plant extracts is therefore a step towards determining the potential of the plants as sources of antimicrobial compounds that are effective against human pathogenic microorganisms [23].

Furthermore, the table below summarizes the inhibition zone diameters and their statistical significance for the different concentrations of *O.O. aubrevillei* extract,

compared to the control for each microorganism. The positive control was used to evaluate the antimicrobial activity against specific organisms (*E. coli* and *C. albicans*), while the negative control was used to assess the baseline (no antimicrobial activity). The value X indicates the observed inhibition zone diameters, which can be carefully deduced from Fig 5. The

study was performed with multiple replicates (n = 3) for each concentration and organism to ensure the reliability and consistency of the results. This was done to minimize experimental error and improve the robustness of the findings. Therefore the study outlines the statistical tests used to assess the significance of the observed differences in inhibition zones.

Table 1. Inhibition zones and statistical significance.

Concentration (mg/mL)	<i>S. aureus</i> (mm)	<i>Ps. aeruginosa</i> (mm)	<i>C. albicans</i> (mm)	<i>E. coli</i> (mm)	Statistical Significance (P-value)
200	x	x	x	18	P < 0.05 Compared to control
100	x	x	x	x	P < 0.05 Compared to control
50	x	x	x	x	P < 0.02 Compared to control
25	x	x	x	x	P < 0.05 Compared to control
12.5	x	x	x	x	No inhibition effect compared to control
control	No inhibition	No inhibition	x	x	P < 0.05 Compared to control

The present study therefore evaluated the antimicrobial properties of *O. aubrevillei* fruit extracts. The findings showed that the activity of the plant extracts is concentration-dependent as the inhibition zone diameter increases with increasing concentrations of the extract. This agrees with the other previous studies [36]. At the highest concentration of 200 mg/mL and 100 mg/mL, the plant extract had activity against all the test organisms. At these concentrations, the plant extract performed better than the control which had activity against only *E. coli* and *C. albicans*. Considering that at high concentrations, phenolic compounds denature the proteins present in microbial cells, it is, therefore, possible that the phenolic compounds present in the plant extract might have also

contributed to the strong antimicrobial activity of the plant extract at higher concentrations.

C. albicans was the most susceptible organism in the different concentrations of the plant extracts and the control, thus, *O. aubrevillei* fruit extract can be effectively used against fungal infections as similarly recorded by Buchheim-Schmidt et al., (2021) study that observed that the activities of organisms towards metabolites varies greatly depending on the extracting solvent. In addition, it further confirms that increased concentration enhanced antimicrobial activity observed by Singh et al., (2023) The present study also showed that the antimicrobial activity of the *O. aubrevillei* fruit extracts varied with the different extraction solvents [38-39]. The

strong antimicrobial activity was observed in the crude extract. The strong antimicrobial activity of the ethyl acetate solvent in the present study contradicts the findings of a previous study investigating the antimicrobial activity of different extracts of *Magnifera indica*, which observed the least antimicrobial activity in the ethyl acetate extracts [40].

4. CONCLUSION

The optimum synthesis of methanol was achieved as the methanolic extract was fabricated by silver nitrate (AgNO₃) as a precursor of which 90 mL of 0.05 mM AgNO₃ was prepared in deionized water and 10 mL of 10% (w/v) methanolic seed. The surface morphology concerning resolution showed an amorphous and polydispersed spherical shape while the TEM showed spherical shapes with particle sizes ranging from 1.47 - 5.80 nm. FTIR study indicates that the carboxyl (–C=O), hydroxyl (–OH), and amine (NH) groups in *O. aubrevillei* extracts are mainly involved in the reduction of Ag⁺ ions to Ag⁰ nanoparticles. The UV–visible spectra of the AgNPs indicated that the AgNPs surface Plasmon band occurred at the strongest absorbance band at 370 nm. The present study showed a strong activity of the aqueous extract on only *S. aureus*, but not *E. coli*. It also did not show any activity against *Ps. aeruginosa*. Except for the aqueous extract which had no activity

against *Ps. aeruginosa*, the solvent used showed antibacterial activity against both the Gram-positive and Gram-negative bacteria, an indication of the presence of broad-spectrum antibiotic compounds in the plant extracts. The study also showed that the fungi; *C. albicans* was more susceptible than the bacteria; *E. coli*, *S. aerus*, and *Ps. aeruginosa*. The findings of this study showed a cost-effective synthesis which indicated the presence of broad-spectrum antimicrobial compounds in the plant extract, in addition, Xray diffraction (XRD) and xray photoelectron spectroscopy XPS instrumentation can be applied to elucidate the mechanism and interaction of the reaction pathway with antimicrobials. A limitation of this study is the exclusive use of the disc diffusion method for antimicrobial analysis. Future research should include additional assays, such as biofilm synthesis assays, to provide a more comprehensive understanding of the antimicrobial mechanisms and efficacy of AgNPs synthesized from *Okoubaka aubrevillei*.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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