

Valorization of African Walnut (*Tetracarpidium Conophorum*) Husks for Green Synthesis of Silver Nanoparticles and Sustainable Control of Indoor Mycotoxigenic Fungi

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Research Article

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Abstract

Indoor mycotoxigenic fungi pose significant health and structural risks to building occupants, necessitating sustainable remediation strategies. This study investigated the green synthesis of silver nanoparticles (AgNPs) using African walnut (*Tetracarpidium conophorum*) husk extract and evaluated their antifungal efficacy against indoor toxigenic molds. The AgNPs were synthesized through photo-activation of walnut husk extract with silver nitrate solution and characterized using UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Energy Dispersive X-ray (EDX) analysis, and Transmission Electron Microscopy (TEM). UV-Vis spectroscopy confirmed nanoparticle formation with maximum absorbance at 430 nm, while FTIR analysis revealed the presence of functional groups including amines, amides, alkyl groups, and carbonyl compounds responsible for bio-reduction and stabilization. EDX analysis confirmed silver as the predominant element (75.20%), and TEM micrographs revealed predominantly spheroidal nanoparticles with well-defined edges. The biosynthesized AgNPs demonstrated exceptional antifungal activity against three mycotoxigenic *Aspergillus* species (*Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) isolated from poorly ventilated indoor environments, achieving inhibition rates exceeding 95% at 50 µg/mL and 98% at 100 µg/mL. In contrast, the precursor silver nitrate and plant extract alone showed minimal antifungal activity (below 50%). These findings demonstrate that African walnut husk-derived silver nanoparticles represent a promising, eco-friendly alternative to conventional chemical biocides for controlling indoor mycotoxigenic fungi, with potential applications in the formulation of sustainable antimicrobial building materials.

Introduction

Indoor air quality has emerged as a critical public health concern, with fungal contamination representing one of the most significant and pervasive threats to human health and building integrity worldwide [1, 2]. Toxigenic molds, particularly species belonging to the genera *Aspergillus*, *Penicillium*, and *Stachybotrys*, are ubiquitous in indoor environments where they proliferate under favorable moisture and temperature conditions, releasing substantial quantities of airborne spores that can reach concentrations exceeding 10^4 colony-forming units per cubic meter in severely contaminated buildings [3, 4]. These microscopic propagules, measuring typically 2–10 micrometers in diameter, possess remarkable resilience and dispersal capabilities, allowing them to penetrate deep into respiratory systems and establish persistent contamination cycles that are extraordinarily difficult to eliminate through conventional cleaning methods [1, 5]. The economic and health burden of mold-related building damage and health complications continues to escalate, with recent estimates suggesting that approximately 21% of asthma cases in the United States may be attributable to residential dampness and mold exposure, highlighting the urgent need for innovative and sustainable remediation strategies [2, 6, 7].

The health implications of chronic exposure to toxigenic mold spores in indoor environments are profound and multifaceted, encompassing both acute respiratory responses and long-term systemic

health effects that disproportionately affect vulnerable populations including children, elderly individuals, and immunocompromised patients [8, 5]. Exposure to damp and moldy environments may cause a variety of health effects including stuffy nose, sore throat, coughing or wheezing, burning eyes, or skin rash, with people who have asthma or are allergic to mold experiencing severe reactions [4]. Recent research has expanded understanding beyond traditional allergic responses to include nonallergic effects such as nervous-system effects, suppression of the immune response, hemorrhage in the mucous membranes of the intestinal and respiratory tracts, rheumatoid disease, and loss of appetite [3, 4]. Certain molds are toxigenic, meaning they can produce toxins (mycotoxins), with molds that produce mycotoxins like *Stachybotrys chartarum* requiring the same careful consideration as other molds that can grow in houses or workplaces [4, 9]. Furthermore, mold-related health effects are particularly concerning because as mold grows, spores, cells, fragments, and unstable organic compounds can enter the air, producing allergens, irritants, and mycotoxins that can be toxic, especially to individuals who have a sensitivity to them [1, 4, 5, 9].

Current approaches to indoor mold remediation rely heavily on chemical biocides, UV irradiation, and physical removal methods that often prove inadequate for achieving complete spore elimination while simultaneously raising concerns about environmental toxicity, development of resistant fungal strains, and potential harm to human health from residual chemical contaminants [10]. Conventional antifungal agents, including quaternary ammonium compounds, phenolic disinfectants, and synthetic fungicides, frequently demonstrate limited efficacy against spore-forming molds due to their robust protective cell walls and metabolic dormancy, necessitating repeated applications at high concentrations that can compromise indoor air quality and pose additional health risks to occupants [9, 11]. Moreover, the increasing prevalence of antifungal resistance among indoor mold populations has rendered many traditional remediation approaches progressively less effective, creating an urgent demand for novel antimicrobial strategies that can overcome these limitations. The environmental persistence and bioaccumulation potential of synthetic biocides also present significant ecological concerns, as these compounds can leach into soil and groundwater systems, contributing to broader environmental contamination and disruption of beneficial microbial communities [4, 10, 12].

In response to these challenges, green nanotechnology has emerged as a promising alternative approach, with green synthesis of silver nanoparticles (AgNPs) using biological resources representing the most facile, economical, rapid, and environmentally friendly method that mitigates the drawbacks of chemical and physical methods [6, 13, 14]. Recent advances in plant-mediated synthesis have demonstrated that green synthesis of silver nanoparticles using plant extracts has emerged as a sustainable and eco-friendly alternative to conventional physical and chemical methods, with walnut-derived materials showing particular promise. Studies have confirmed AgNP formation using walnut septum waste as an underutilized bioactive-rich by-product, with characteristic surface plasmon resonance bands confirming successful nanoparticle synthesis [15]. The antifungal potential of green-synthesized silver nanoparticles has been increasingly recognized, with recent research demonstrating that silver nanoparticles are gaining significant attention in agriculture due to their potent antifungal properties [11, 14, 16] and outstanding physicochemical properties and demonstrated biological and

antimicrobial efficacy [14]. This study aims to evaluate the antifungal efficacy of green-synthesized silver nanoparticles derived from walnut husk extract against three clinically important toxigenic molds commonly isolated from indoor environments, providing a sustainable and environmentally benign approach to addressing the growing problem of indoor fungal contamination and its associated health risks.

Materials and Methods

Methodology

Sample Collection and Preparation of Sample

African walnut (*Tetracarpidium conophorum*) samples were procured from New Wazo Market (8.1652⁰ N, 4.2568⁰ E) within Ogbomoso metropolis, Oyo State, Nigeria, and were conveyed to the laboratory in a sterile polythene bag. The samples were crushed to separate the husk from the edible part, after which the husks were dried at room temperature $\pm 28^{\circ}\text{C}$ for 48 hours, then pulverized aseptically into powdery form. One gram (1g) of the pulverized sample was weighed and suspended in 100 ml of distilled water and heated in water bath at 60 ⁰C for 1 hour. The extract was filtered using Whatman No. 1 filter paper and then centrifuged at 4000 rpm for 15 minutes to obtain clear supernatant of the extract while the pellet was discarded.

Preparation and Synthesis of Green Synthesized Nanoparticles

Preparation of Silver Nitrate (AgNO_3) Solution and Synthesis of Silver Nanoparticles

Exactly 1mM of silver nitrate salt was dissolved in 1000ml of distilled water and was stored in amber bottle and kept in a dark cupboard at ambient temperature ($\pm 28^{\circ}\text{C}$). Two milliliters of the sample supernatant was reacted with 20ml of silver nitrate, the solution was then photo-activated for 30 minutes by exposure to direct sunlight as it enhances the formation of the silver nanoparticles. As the experimental control, a solution of the sample supernatant alone was also maintained. The formation of silver nanoparticles (AgNps) was visually observed by checking the colour change [17].

Characterization of the Synthesized Silver Nanoparticle

UV-Vis spectrum analysis

Two milliliters of the synthesized silver nanoparticles were poured into quartz cuvette and readings were taken on a spectrophotometer using wavelengths between 180–500 nm and bandwidth of 2.0nm.

Fourier Transform Infra-red (FTIR) Spectroscopy

The silver nanoparticles were subjected to FTIR spectroscopy in order to discover the functional groups or biomolecules responsible for the bio-reduction and capping of the synthesized nanoparticles. The silver nanoparticles colloidal suspension was subjected to centrifugation at 7000 rpm for 10 min. The supernatant was discarded while the resulting pellet was washed in sterile distilled water twice and thereafter; freeze dried. The crystals were then mixed with KBr (Merck for spectroscopy) and then pulverized in an agate mortar to form a homogeneous powder from which, under a pressure of 7 tons, the appropriate pellet was prepared. Using the Perkin Elmer 3000 MX spectrometer, all spectra were recorded from 4000 to 400 cm^{-1} . There were 32 scans per spectrum with a resolution of 4 cm^{-1} and the spectra were analyzed using the spectroscopic software Win-IR Pro Version 3.0 with a peak sensitivity of 2 cm^{-1} .

Energy Dispersive X-ray (EDX) Analysis

The elemental composition of the nanoparticle was carried out using Espirit 1.8.5 software. This technique was used to determine the most prevalent element in the nanoparticle. A droplet of silver nanoparticles was placed onto a clean silicon wafer surface. The droplet was allowed to dry by evaporation at ambient temperature, and a thin carbon film was placed on the dried nanoparticle to prevent them from charging, thereafter the compositions of the elements present in the samples were determined.

Transmission Electron Microscopy (TEM)

The size and shape of the silver nanoparticles were evaluated. Using Leo 912 AB high resolution transmission electron microscope operating at an accelerating voltage of 120 kV, a sample of the synthesized silver nanoparticles solution was placed on the carbon-coated copper grid and dried prior to microscopy.

Test Organisms Collection

Three mycotoxigenic molds; *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) isolated from indoor (walls) environment with poor ventilation were collected from Nigerian Stored Products Research Institute (NISPR), Ilorin, Kwara State.

Antifungal activity of the silver nanoparticles

The antifungal potentials of the silver nanoparticles were determined employing the Mycelial Inhibition Method reported by Oribhabor and Uguoke [18], 8 mm plug of 48-h old pure cultures of *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) were inoculated on potato dextrose agar plates incorporated with 50 and 100 $\mu\text{g/ml}$ of the

synthesized silver nanoparticles and 100 µl/ml of the sample aqueous extract. For the negative control experiments, the fungal plugs of the test molds]were inoculated on PDA plates with 1mM AgNO₃ and PDA plates without the incorporation of the silver nanoparticles. The plates were incubated at 27 ± 2⁰ C for 72 hours. The mycelial diameters of test fungal growths were measured in all the plates and the percentage growth inhibitions was determined as follows:

$$Mycelial\ inhibition\ (\%) = \frac{D_{control} - D_{test}}{D_{control}} \times 100$$

Where D is the diameter of the fungal mycelial growth on the potato dextrose agar plates

Results and Discussion

Green synthesis and characterization of the silver nanoparticles

The green synthesis of the silver nanoparticles was bio-fabricated through the mediation of the bio-reducing molecules present in the reducing and capping agent, aqueous extract of African walnut (*Tetracarpidium conophorum*) husk. The bio-reduction and stabilization of the silver nanoparticles was evident in the colour change as illustrated in Fig. 1. The bio-reduction and capping of the silver nanoparticles occurred in the process of photo-activation. The reaction was initially colourless, then started turning into light orange which intensified to deep orange colour within 30 minutes, Lima *et al.* [19] also reported bio-fabrication of silver nanoparticles within 30 minutes at photoactivation. The retained colouration of the reaction lent credence to the stabilization of the silver nanoparticles. Sakaray *et al.* [20] reported a change in colouration from pale yellow colouration to dark brown at the stabilization of the silver nanoparticles synthesized from *Acalypha indica* plant extract. Asefian and Ghavam [21] also reported a change in colour from light yellow to brown at stabilization of the silver nanoparticles synthesized from *C. longa* *M. chamomilla* and *A. wilhelmsi* extracts. The progressive increase in the bio-fabrication of AgNPs, which is directly proportional to the reaction time indicates the reduction of silver (Ag⁺) to colloidal silver (Ag⁰), a surface plasmon resonance phenomena [21, 22, 23].

In order to further establish the bio-fabrication of the silver nanoparticles, the stabilized reaction was subjected to Uv-Vis spectroscopy which displayed maximum absorbance at the wavelength of 430.0 nm as shown in Fig. 2. Giri *et al.* [24] also reported the silver nanoparticles synthesized from *Eugenia roxburghii* with Uv-Vis spectrum wavelength of 417nm. Lima *et al.* [19] also reported the silver nanoparticles synthesized from different parts of *Paullinia cupana* with Uv-Vis spectrum wavelength of 450nm.

The FTIR spectrum of the silver nanoparticles showed prominent broad peaks at 3778.31, 3376.0, 2918.0, 2853.01, 2360.17, 1595.0, 1369.0, and 1032.0 cm⁻¹ (Fig. 3). The vibrational stretch around 3778.31, and 3376.0 cm⁻¹ is ascribe to N-H amines, amides and O-H group. The peak at 2918.0,

2853.01, and 2360.17 cm^{-1} is assigned to C-H stretch of the alkyl group, and $\text{C} \equiv \text{C}$ from alkyne and the peak at 1595.0, 1369.0, and 1032.0 cm^{-1} confirms the presence of conjugation effects of $\text{C} = \text{O}$ stretching, carbonyl groups or esters and N-H binding of protein. Sakaray *et al.* [20] also reported silver nanoparticles from *Acalypha indica* extract IR peaks at 3285.56 to 1637.84 cm^{-1} which indicated the presence of phenolic and carbonyl groups. The observed bands are lattice vibrational modes showing the adsorption of biomolecules functional groups on nanoparticles [20, 25].

The Energy Dispersive X-ray (EDX) result in Fig. 4 showed that silver is the most occurring metal observed in the nanoparticles sample through the strong peak (75.20%) observed at 3 KeV on the EDX spectrum, Hemlata *et al.* [26] also reported a strong absorption peak of silver at 3 keV, which was due to silver being the major constituent. The Transmission Electron Microscope has been employed in the shape and size analysis of the silver nanoparticles which is shown in Fig. 5, the TEM micrograph taken at 20 and 50 nm magnifications showed that the silver nanoparticles have a predominantly spheroidal morphology with well-defined edges, According to Mie's theory, which states that the presence of only a single maximum absorption band in UV-Vis spectrum of a metallic nanoparticles is considered characteristic of spherical nanostructures [19, 27].

Antifungal activity of the silver nanoparticles

The silver nanoparticles showed significant effect against the growth of three molds: *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) isolated from indoor (walls) environment with poor ventilation. The silver nanoparticles showed antifungal activity of 97.94, 95.05 and 98.01% at 50 $\mu\text{g/mL}$ against *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) respectively. At 100 $\mu\text{g/mL}$, the silver nanoparticles showed excellent antifungal properties of 98.38, 98.19 and 98.25% at 100 $\mu\text{g/mL}$ against *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) respectively. The precursor (1 mM silver nitrate) showed poor antifungal properties of 14.20, 9.50 and 48.30% against *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) and *Aspergillus salisburgensis*-IDM3 (PX430758) respectively, while the extract also showed poor activities of 7.50, 9.50 and 7.30% at 100 $\mu\text{g/mL}$ against *Aspergillus flavus*-IDMM1 (PX430759), *Aspergillus fumigatus*-IDM2 (PX430757) respectively (Table 1, Fig. 6). Ogar *et al.* [6] reported the antifungal properties of silver nanoparticles against *S. chartarum* and *C. globosum* isolated from the surface of gypsum drywall, and it was observed that the AgNPs at 30 and 200mg/L significantly inhibited the growth of the fungi.

Table 1
Antifungal activities of the bio-fabricated silver nanoparticles

Test Organisms	Silver Nitrate	African walnut (<i>Tetracarpidium conophorum</i>) husk aqueous extract	Bio-fabricated Silver nanoparticles	
	(1 mM)	($\mu\text{L/mL}$)	($\mu\text{g/mL}$)	
		100	50	100
	(%)			
<i>A. flavus</i>	14.20	7.50	97.94	98.38
<i>A. fumigatus</i>	9.50	9.50	95.05	98.19
<i>A. salisburgensis</i>	48.30	7.30	98.01	98.25

It has been reported by Campana *et al.* [9] that cementitious building materials, even paints, rich in inorganic and organic compounds, represent a good nutrient source or substrate for the viability of molds. Some conditions determine the growth of molds on building materials, especially cementitious ones, such as moisture, water availability, temperature, nutrient, and pH. molds attack these surfaces contributing to their erosion and deterioration, thereby reducing the life of the structure itself and negatively affecting the health of the inhabitant through inhalation, ingestion and dermal contact with the molds' spores [5, 12]. It is of great importance to use building materials that are capable of inhibiting the growth of mycotoxigenic molds [6, 7, 9]. These results showed that the bio-fabricated silver nanoparticles is a potent agent against some indoor myco-toxigenic fungi, and this could be exploited for the formulation of antifungal agents in building materials.

Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles using aqueous extract of African walnut (*Tetracarpidium conophorum*) husks as both reducing and capping agents. The bio-fabrication process was confirmed through visual color change from colorless to deep orange, with comprehensive characterization validating the formation of stable, spheroidal silver nanoparticles. The UV-Vis spectroscopy peak at 430 nm, FTIR functional group analysis revealing bioactive compounds, EDX confirmation of 75.20% silver content, and TEM visualization of well-defined spheroidal morphology collectively established successful nanoparticle synthesis. The biosynthesized silver nanoparticles exhibited remarkable antifungal efficacy against three clinically relevant mycotoxigenic *Aspergillus* species commonly found in indoor environments with poor ventilation. At relatively low concentrations of 50 and 100 $\mu\text{g/mL}$, the AgNPs achieved inhibition rates of 95–98%, significantly outperforming both the silver nitrate precursor and the plant extract alone. This superior performance underscores the synergistic effect of nanoparticle formation, wherein the enhanced surface area-to-volume ratio and unique physicochemical properties of AgNPs enable potent antimicrobial action against resistant fungal species. The utilization of African walnut husks, which are typically discarded as agricultural waste, for

nanoparticle synthesis represents a dual achievement in environmental sustainability: valorization of biomass waste and production of eco-friendly antifungal agents. This approach addresses the limitations of conventional chemical biocides, including environmental toxicity, health concerns, and the development of fungal resistance, while offering a sustainable solution to the growing problem of indoor mold contamination. The exceptional antifungal properties demonstrated by these green-synthesized nanoparticles, combined with their environmentally benign production method, position them as promising candidates for incorporation into building materials, paints, and coatings to prevent mycotoxigenic fungal colonization and improve indoor air quality. This research contributes to the expanding field of green nanotechnology and provides a practical pathway toward sustainable indoor environmental management.

Declarations

Ethics Declaration

Not Applicable

Consent to Publish Declaration

Not Applicable

Consent to Participate Declaration

Not Applicable

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Funding

No funding was received for this research work.

Data Availability

All data that support the findings of this study are available from any of the corresponding authors upon request.

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Figures

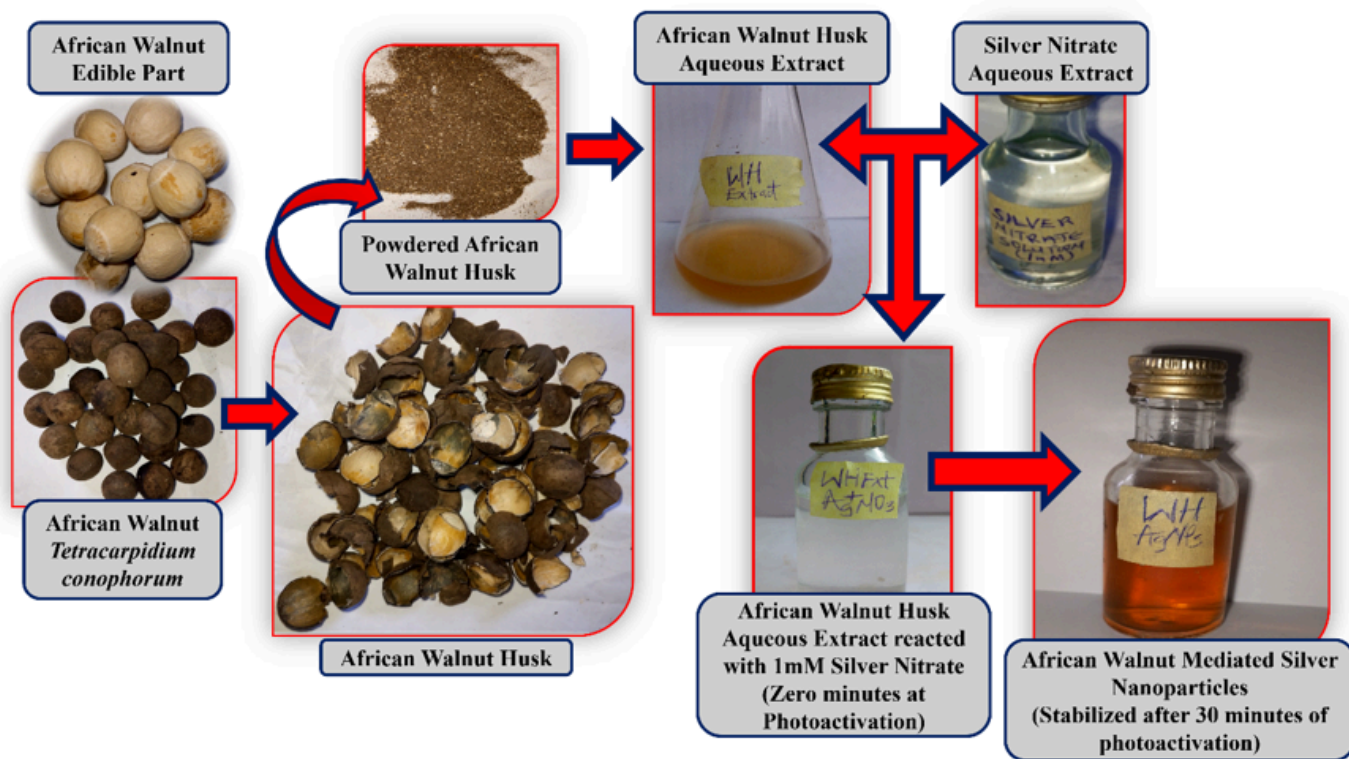


Figure 1

Sample preparation and green synthesis of silver nanoparticles from African walnut (*Tetracarpidium conophorum*) husk aqueous extract.

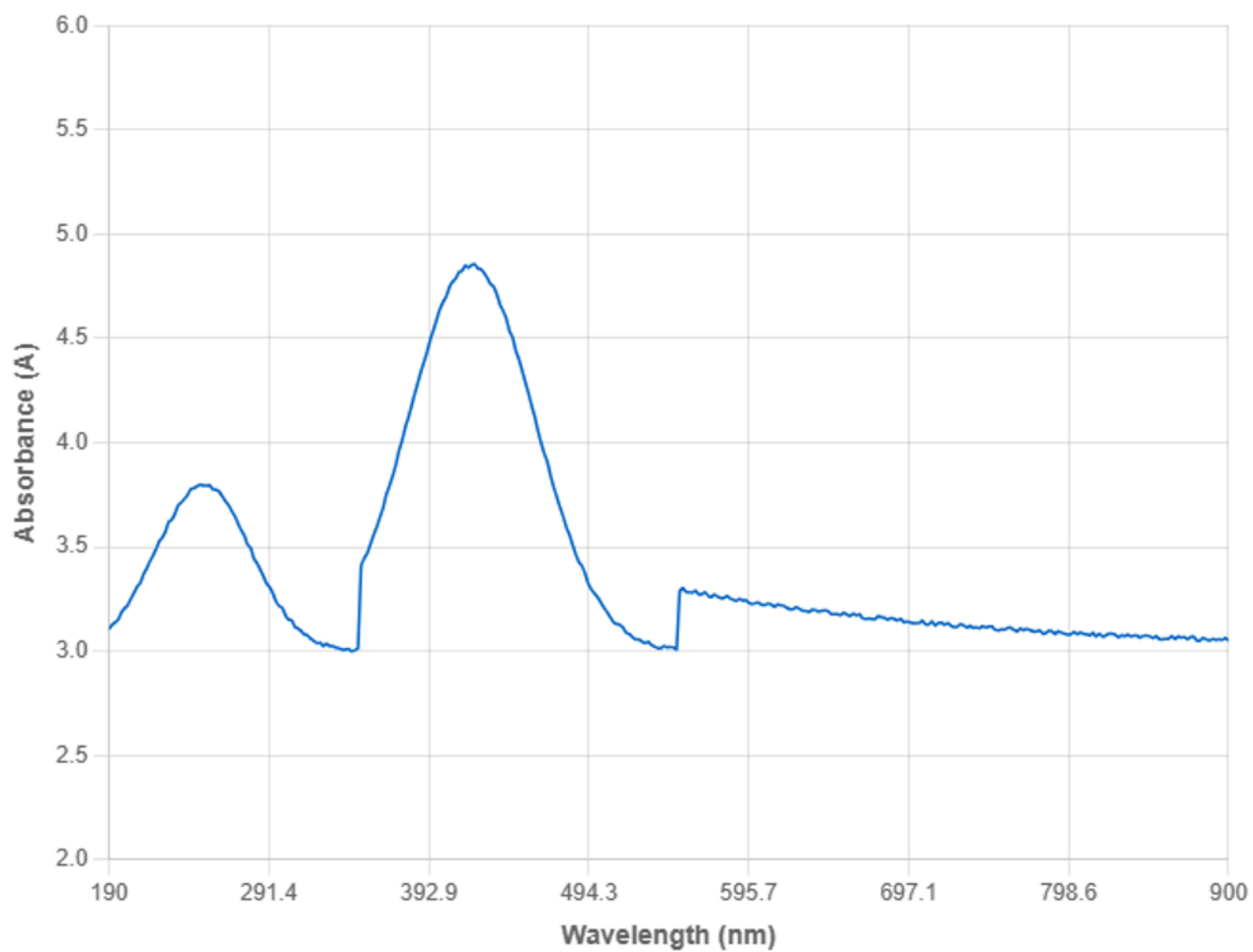


Figure 2

UV-visible spectrum of the biosynthesized silver nanoparticles.

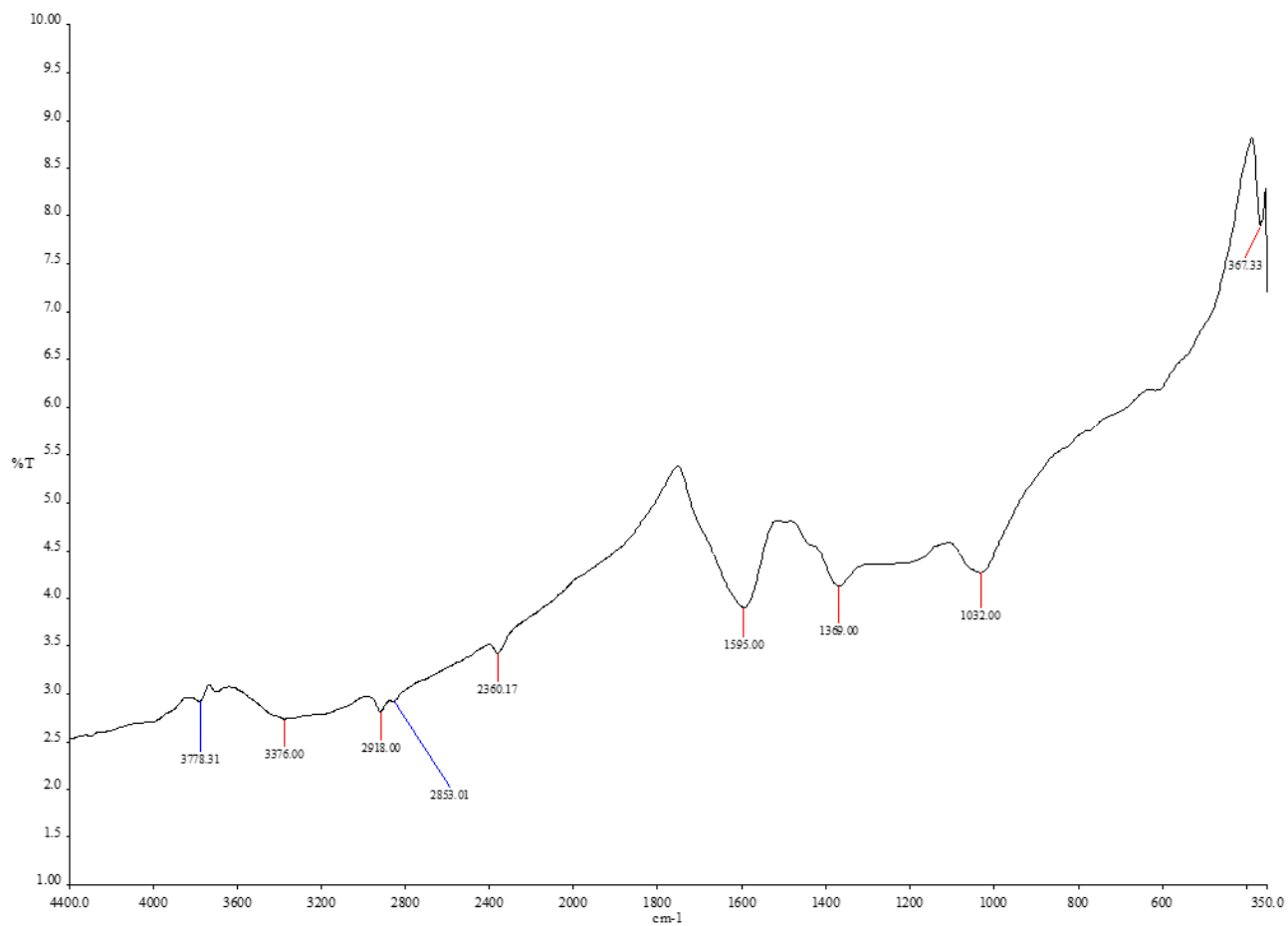


Figure 3

Fourier Transform Infrared Spectrum of the synthesized silver nano particles

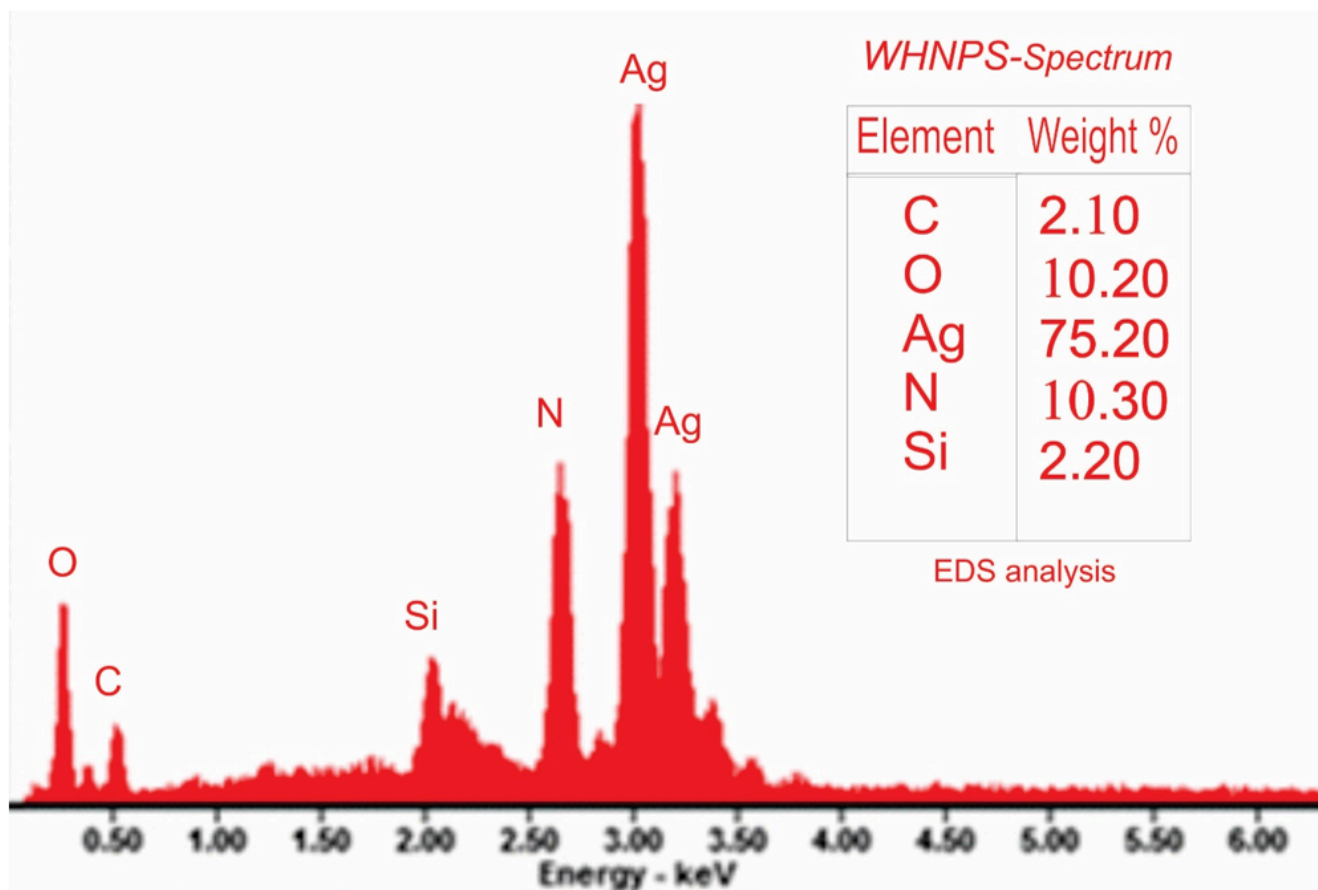


Figure 4

Energy Dispersive X-ray (EDX) spectrum of the biosynthesized silver nanoparticles from African walnut (*Tetracarpidium conophorum*) husk extract.

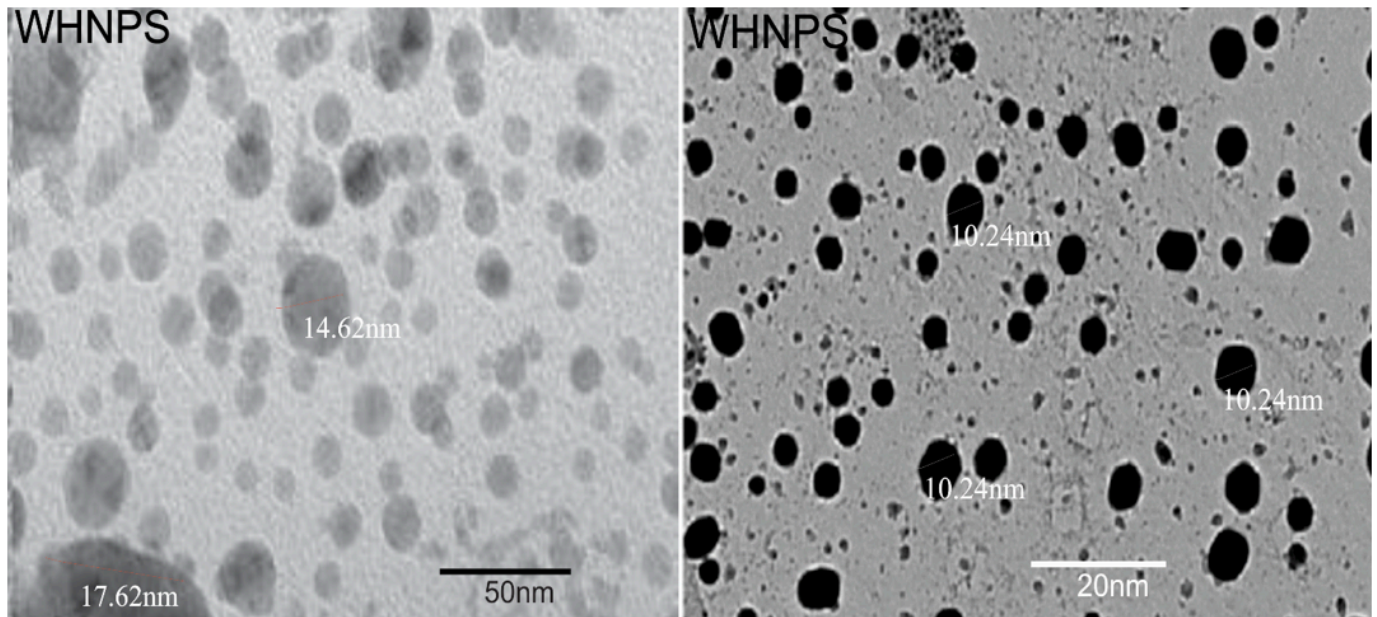


Figure 5

Transmission Electron Microscopy micrograph of the bio-fabricated silver Nanoparticles

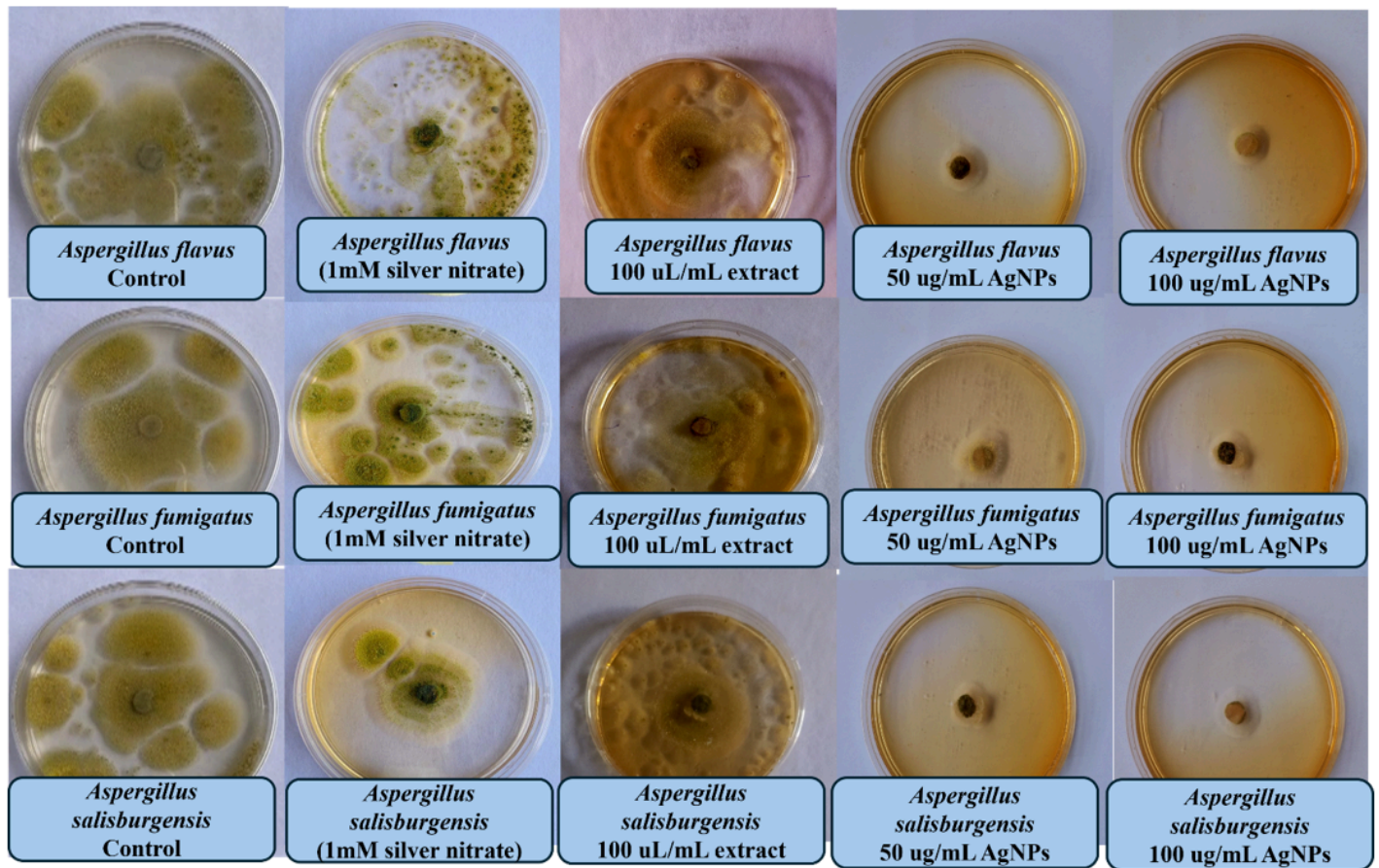


Figure 6

Antifungal activities of the bio-fabricated silver nanoparticles

