

## Biosynthesis and Characterization of Silver Nano-Composites of Enantia chlorantha Extracts

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ABSTRACT: Biosynthesized nanoparticles are receiving attention because of naturally existing secondary metabolites from plants that support green synthesis and their biological applications. Hence, the Objective of this paper as to biosynthesize and characterize silver nano-composites of Enantia chlorantha extracts using appropriate standard techniques. The results of the FTIR research revealed significant heterogeneity in chemical shifts among the functional groups, indicating changes in chemical surroundings. Furthermore, the GC-MS analysis showed that the extract and Ec-AgNPs had different numbers of compound peaks: the extract had 28 peaks, while Ec-AgNPs had 32 peaks. Ec-AgNPs showed more antibacterial activity than the extract, although having equal phytochemical contents, suggesting their potential for medicinal uses. The importance of using plant extracts in green synthesis techniques to produce nanoparticles and their potential biological uses is highlighted by this study.

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Nanotechnology has emerged as а significant, intriguing field of study due to its unique properties and wide-ranging applications in food, agriculture, and biomedicine (Ghidan and Antary, 2020; Biswas et al., 2022). The optical, magnetic, chemical, mechanical, and small size of nanoparticles, as well as their significant surface area to volume ratio, make them desirable possibilities for novel biomedical applications (Joudeh and Linke, 2022; Yusuf et al., 2023; Harish et al., 2022). These are well-documented uses that span from serving as antioxidants, anticancer agents, and antibiotics (Modi et al., 2022; Sofi et al., 2022). Concerning biomedical applications, noble metal nanoparticlessuch as those of silver, gold, platinum, copper, zinc, titanium, and magnesiumhave attracted a lot of interest because of their

multifunctional theranostic properties (Pandit et al., 2022; Hossain et al., 2023). While chemical and physical methods are still commonly used to synthesize nanoparticles, they frequently involve potentially toxic chemicals (Hossain et al., 2023). Nevertheless, a growing alternative is the synthesis of metal nanoparticles through plant-mediated means (Gopalakrishnan and Muniraj, 2021). The method's environmental friendliness and lack of use of dangerous chemicals make it more acceptable. Plantmediated synthesis is an appealing option in the rapidly developing field of nanotechnology research because of its ecological advantages and minimal environmental impact (Alvarez-Chimal and Arenas-Alatorre, 2023; Radulescu et al., 2023). Numerous research conducted in the past 20 years have

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emphasized the special biological, chemical, and physical characteristics of silver (Hemlata et al., 2020). The Research suggests that a lower concentration of AgNO<sub>3</sub> shows improved chemical stability, catalytic activity, biocompatibility, and inherent therapeutic potential despite its toxicity at greater concentrations (Hemlata et al., 2020; Arshad et al., 2023). The importance of silver nanoparticles is further highlighted by their claimed antibacterial and anticancer properties (Oves et al., 2018). Amongst the many advantages these nanoparticles have over bulk metals and their salts is the tardy and controlled release of silver (Hemlata et al., 2020). The guiding concept of the modern bio-nano formulation is to embrace the combination of traditional medicine and nanotechnology (Zhang et al., 2021). The green synthesis of AgNPs from plant leaves has been the subject of many investigations. Still, little research has been done on biosynthesis from wild and native species. even though these species can potentially display vital biological activities.One member of the Annonaceae family is Enantia chlorantha(Adebiyi and Abatan, 2013; Akinwale et al., 2022). The plant is found mostly on the shores of West and Central Africa, it grows well in Nigeria's forests. Reaching as high as 30 meters, this ornamental tree is distinguished by its vivid yellow slash and unique black fruits (Akinwale et al., 2022). Due to its therapeutic qualities, rural populations in Nigeria place a high value on Enantia chlorantha (Adebiyi and Abatan, 2013). Although E. chlorantha has been traditionally thought to have medicinal properties, little scientific research has been done on the plant. However, research indicates that it may have the ability to treat ailments like jaundice, typhoid fever, cough, wounds, and rickettsia fever(Sarbadhikary and George, 2022). Aqueous extract from the plant has shown analgesic, antipyretic, antibacterial, and antimalarial properties. These plant's extracts, rich in water-soluble alkaloids, exhibit healing properties for wounds, leprous patches, and ulcers (Akinwale et al., 2022). This study also emphasizes the possible use of leaf extract from Enantia chlorantha in biomedical nanotechnology-driven applications. Information about synthesizing and characterizing AgNPs derived from E. chlorantha is currently scarce. Hence, the Objective of this paper as to biosynthesize and characterize silver nanocomposites of Enantia chlorantha extracts

### **MATERIALS AND METHODS**

*Plant Collection and Authentication*: The stems of *Enantia chlorantha* were collected in Ijebu Itele, Ogun State, in the southwest region of Nigeria on November 25<sup>th</sup> 2022. The plant samples were taken to the Forestry Research Institute of Nigeria (FRIN),

Medicinal Plant Section, Jericho, Ibadan, Oyo State for identification and authentication.

*Plant Sample Preparation:* The stem of the plant material was carefully washed with distilled water, air dried for 40 days, and ground into a fine powder using an electric Kinelco blender. The powder was kept in an air tight container for studies

*Reagents Used:* The analytical grade chemicals used were purchased from Bristol Scientific Company, an authorized Sigma-Aldrich distributor in Nigeria

*Plant Sample Extraction:* A complete maceration using 4000 mL of ethanol was utilized on 500g of finely ground plant material. A thorough extraction and dissolution of compounds was ensured by agitation with an ultrasonic sonicator for an hour at room temperature (27 <sup>o</sup>C).

Green Synthesis of Silver Nanoparticles: We employed the ethanolic extract of E. chlorantha that was made in the previous stage to create silver nanoparticles in a green manner. For this, 90 mL of a 1 mM aqueous silver nitrate solution and 10 mL of the extract were combined, and the mixture was heated to 80 0C for three hours while stirring continuously. The color shift from yellow to dark brown served as a preliminary indicator of the AgNPs' development. For twenty minutes, the green-synthesised nanoparticles were separated by centrifugation at 15,000 × g. To remove the free silver linked to Ec-AgNPs, this procedure was carried out three times. Ec-AgNPs, the final green-synthesised silver nanoparticles, were freeze-dried and kept at 4 °C until needed again.

Qualitative Phytochemical Screening of Plant extracts: A standardized methodology outlined by Shaikh and Patil (2020) was used to assess the phytochemical components of *Enantia chlorantha* extracts and the synthesized nanoparticle from the plant extract. Various phytochemicals were investigated, including phenol, anthraquinone, reducing sugar, alkaloids, glycosides flavonoids, tannins, steroids, and terpenoids.

*Alkaloids:* A solution of 1% hydrochloric acid (HCl) in water was used to acidify 4 mL of test extracts in a steam bath. After adding a drop of Meyer's reagent, each test tube was filled with 1 mL of the acidified solution. A creamy white precipitate's development confirmed the presence of alkaloids.

*Tannins:* Ferric chloride (FeCl<sub>3</sub>) was added to each 2 mL test solution in five drops. Subsequently, a murky green precipitate was seen, indicating tannins' presence.

Anthraquinones: In a separate investigation, five (5) millilitres of each test solution were boiled in a water bath with a 10% hydrochloric acid (HCl) solution for ten minutes at 100°C. The filtrate was cooled and mixed with an equal volume of trichloromethane (CHCl<sub>3</sub>). The combination was then heated, and 10% ammonia (NH<sub>3</sub>) solution was added. The emergence of a rose-pink solution provided evidence of the existence of anthraquinones.

*Glycosides:* A 50% sulfuric acid concentration (5 mL) was added to each test extract in each test tube. Then, heat the mixture in boiling water for fifteen minutes. After adding Fehling's reagent, the resulting mixture was heated until it reached boiling point. It was determined that glycosides were present by examining a brick-red precipitate.

*Reducing Sugars:* Each extract was mixed with distilled water in a test tube, agitated vigorously, and filtered. Fehling's solutions A and B were then added, and the filtrate was allowed to boil for ten minutes. Reducing sugars were verified to exist when an orange-red precipitate formed.

*Saponins:* Five (5) mL of distilled water were mixed with two (2) mL of the extracts, agitated well, and heated to boiling. The presence of saponins was verified by the appearance of foaming, which looked like creamy milk with tiny air bubbles.

*Flavonoids:* Four (4) milliliters of sodium hydroxide solution (NaOH) in water were added to each of the about two milliliter extracts in separate test tubes. The presence of flavonoids was confirmed by a yellow solution that turned colorless when hydrochloric acid (HCl) was added.

*Phlobatanins:* Five (5) millilitres of the extracts were mixed with distilled water and filtered. The resulting filtrate was boiled with a 2% hydrochloric acid (HCl) solution. The presence of phlobatanin was confirmed by observing a crimson residue in the mixture.

*Steroids*: The presence of steroids was confirmed by the brownish-to-red colour that appeared when five drops of concentrated sulfuric acid  $(H_2SO_4)$  were added to each milliliter of individual test extract solutions.

*Terpenoids (Salkowski test):* Each 2 mL extract was mixed with 2 mL of chloroform (CHCl<sub>3</sub>) and 3 mL of concentrated sulfuric acid ( $H_2SO_4$ ) to make a layered solution. The interface began taking on a reddishbrown colour, confirm the presence of terpenoids.

*Phenolic:* Phenols were confirmed by developing a bluish-black colouration in 5 millilitres of extracts when precisely four drops of ferric chloride were added.

#### Antimicrobial assays

*Collection and maintenance of test microbes:* In this study, fungi (*Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus*) was utilized. The microorganisms were sourced from the Department of Microbiology, LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Nigeria, originating from established stock cultures. Maintenance involved agar slants in Mc-Cartrey bottles stored in a refrigerator.

Antifungal Activity of the Synthesized Nanoparticle and Crude Extracts of E. chlorantha extract: Using the Mycelial Inhibition Method (Lateef et al., 2017), the antifungal activities of the plant extract and nanoparticles were assessed. Potato dextrose agar plates were inoculated with a 6 mm agar plug containing 48-hour-old cultures of Aspergillus fumigatus, Aspergillus flavus, and Aspergillus niger. The graded concentrations of the synthesized nanoparticles were added to the plates. Fungal plugs were inoculated on PDA plates in the control trials without the addition of plant extract or silver nanoparticles. For 72 hours, all of the plates were incubated at  $28 \pm 2$  C. The % growth inhibitions were calculated using the measured diameters of the fungal growth in each plate. Where D represents the fungal growth's diameter on the PDA plates

### Characterization of the synthesized AgNPs

*UV-visible spectroscopy:* A UV-Vis spectrophotometer (Shimadzu UV-1601) operating at a resolution of 10 nm in the 250–900 nm range was used to determine the synthesis of AgNPs. A blank reference was used to do the spectrophotometer's baseline correction. By periodically sampling the aqueous component and evaluating the solution's UV-Vis spectra, the decrease of Ag<sup>+</sup> ions in the solution was monitored.

*Fourier Transform Infrared analysis:* The infrared (IR) spectra were obtained using a Fourier transform infrared spectrophotometer (FT-IR) equipped with a universal attenuated total reflector (ATR) sampling accessory, manufactured by Perkin Elmer Spectrum 100. Fourier transform infrared spectroscopy (FTIR) was utilized to determine the functional groups in each fraction of separated samples utilizing the Spectra 100 apparatus manufactured by Perkin Elmer in the United States.

*Transmission electron microscopy (TEM) analysis:* TEM analysis was used to visualize the green

synthetic AuNPs' size and shape. Water that had been double-distilled was utilized to distribute the sample. A thin dispersion droplet was put to a "staining mat." The carbon-coated copper grid was inserted into the drop with its coated side facing upward. After removing the grid and letting it air dry for about ten minutes, a JEOL JEM 2100 Transmission Electron Microscope (JOEL Corp, Tokyo, Japan) was used to screen it.

Gas Chromatography-Mass Spectrometry analysis: The analysis made use of gas chromatography-mass spectrometry (GC-MS) with a special apparatus (QP-2010 Ultra Shimadzu, Japan) and a capillary column (DB-5MS) with dimensions of 30 m in length, 0.25 m in internal diameter, and 0.25 m in film thickness that operated in SCAN mode. Helium was used as the carrier gas, with a purge flow of 3.0 mL/min and a flow rate of 0.72 mL/min, resulting in a total flow of 31.8 mL/sec with a specified linear velocity. Temperatures of 250 °C and 300 °C, respectively, were maintained for the injection port and detector. The oven temperature program started at 60 °C for 0 minutes. It then increased the temperature gradually to 300 °C at a rate of 10 °C/min. Finally, there was a 16-minute stabilization phase. A 1 L injection volume was used in a splitless injection mode.

### **RESULTS AND DISCUSSION**

Qualitative Phytochemical Analysis Result: The various phytochemical elements included in the ethanolic bark extract of *E. chlorantha* that are in charge of capping and reducing silver nanoparticles are examined. Ec-AgNPs and the ethanolic bark extract of *E. chlorantha* underwent phytochemical screening, as shown in Table 1, and the results showed that they were a great source of secondary metabolites. Alkaloids, glycosides, flavonoids, tannins, saponins, terpenoids, phenols, steroids, anthraquinones, reducing sugar, and phlobatannins are among the

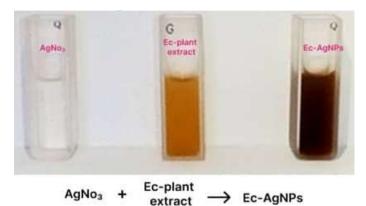
similar phytoconstituents discovered in the phytochemical analysis. According to Hemlata et al. (2020), these phytochemicals may reduce silver and act as a capping agent to maintain the stability of the nanoparticles and stop them from aggregating. The majority of phytochemicals that are extracted using polar solvents are polar substances that are crucial for the creation of nanoparticles. 2020; Nawez et al. This result is in line with studies conducted by Ohemu et al. (2018), who discovered similar phytoconstituents in methanol-extracted extracts of E. chlorantha stem bark.

 
Table 1: Phytochemical composition of Ethanolic extract and synthesized AgNPs of *E. chlorantha*

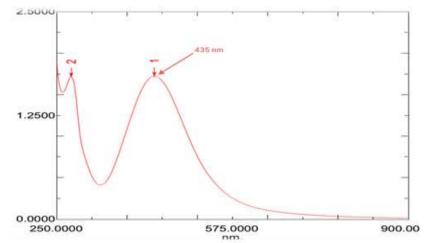
Phytoconstituents	EtOH extract	Ec-AgNPs	
Alkaloids	+	+	
Glycosides	+	+	
Flavonoids	+	+	
Tannins	+	+	
Saponins	+	+	
Terpenoids	+	+	
Phenols	+	+	
Steroids	+	+	
Anthraquinones	+	+	
Reducing Sugar	+	+	
Phlobatanins	+	+	
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Key: + represent (present)

Synthesis and Characterization of Ec-AgNPs Results: Synthesis of Ec-AgNPs Results: After three hours, the solution's color changed from pale yellow to dark brown due to the reduction of Ag+ to Ag° nanoparticles in the ethanolic bark extract of E. chlorantha, which showed the creation of Ec-AgNPs (Figure 1). The UV-vis spectra of the Ec-AgNPs exhibit a prominent peak at 435 nm following three hours of incubation (Figure 2). UV-visible spectroscopy is one of the most widely used techniques for functionally characterizing nanoparticles. At roughly 435 nm, an absorbance peak shows the emergence of AgNPs in the solution.



**Fig 1:** The effect of AgNO<sub>3</sub> salt on the biosynthesis of *E. chlorantha* silver nanoparticles and UV–vis absorption spectra of the Ec-AgNPs by using *E. chlorantha*.



**Fig 2:** The effect of AgNO<sub>3</sub> salt on the biosynthesis of *E. chlorantha* silver nanoparticles and UV–vis absorption spectra of the Ec-AgNPs by using *E. chlorantha*.

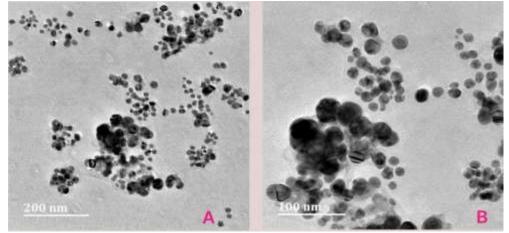


Fig 3:TEM images of (A and B) of AgNPs at different magnifications

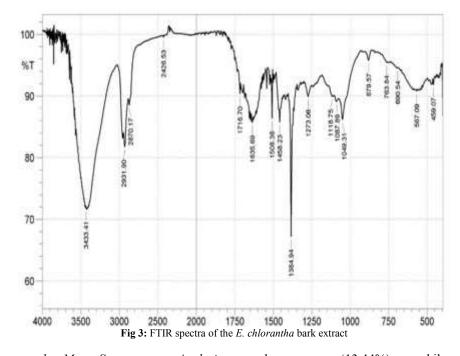
Transmission Electron Microscopy (TEM) Results Comprehensive insights into the shape, particle size, and crystalline nature of the produced AgNPs were obtained through investigation using Transmission Electron Microscopy (TEM). Figure 3(A–B) shows TEM images taken at different magnifications, making detailed characterization easier. The AgNPs' size distribution, which varied from 12 to 29 nm and had an average diameter of 19.45 nm, demonstrated the uniformity of the synthesis process. In addition, the AgNPs primarily displayed spherical, triangle, and asymmetrical patterns, suggesting a variety of configurations crystalline and production circumstances.

*Fourier-transform infrared (FTIR) Result:* Exciting insights into the chemical transformations taking place during the synthesis process are shown by the Fourier-transform infrared (FTIR) spectra of the produced nanoparticle (Figure 4) and the ethanolic extract

(Figure 3) of Enantia chlorantha. Characteristic absorption bands at different wavenumbers are visible in the ethanolic extract's spectrum, indicating the presence of particular functional groups in the extract. The presence of hydroxyl groups, common in organic compounds comprising alcohols or phenols, is indicated by a peak at 3433.41cm<sup>-1</sup> corresponding to O-H stretching (Hamidu et al., 2020). C-H stretching frequently present vibrations. in aliphatic hydrocarbons, are shown by the 2931.90 cm<sup>-1</sup> and 2870.17 cm<sup>-1</sup> peaks (Westbrook et al., 2022). The absorption at 1716.70 cm<sup>-1</sup>, which may have originated from carbonyl groups found in the extract's components, shows C=O stretching. The peak 1635.69cm<sup>-1</sup> represents C=C stretching, frequently linked to aromatic compounds. Various functional groups, such as aromatic rings or alkyl chains, may be represented by the peaks at 1508.38 cm<sup>-1</sup>, 1458.23 cm<sup>-</sup> <sup>1</sup>, and 1364.94 cm<sup>-1</sup>.

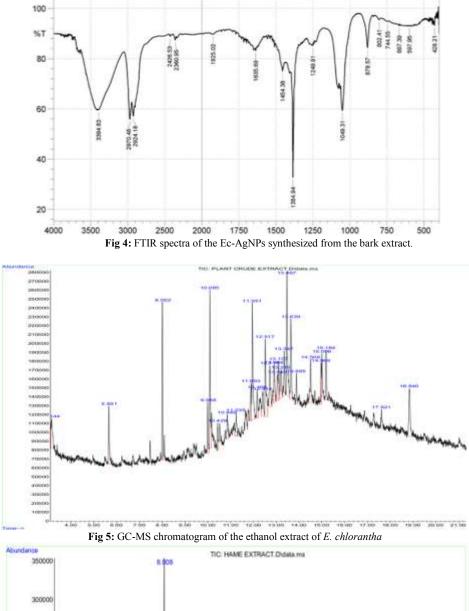
Significant changes in the FTIR spectrum are seen once the extract has been processed into nanoparticles. New peaks appear with considerable shifts, yet some peaks, such as the C-O stretching at 1049.31 cm<sup>-1</sup> and the C=C stretching at 1635.69 cm<sup>-1</sup>, are still consistent with the extract. The O-H stretching peak undergo redshifts from 3433.41 cm<sup>-1</sup> to 3394.83 cm<sup>-1</sup>, which may indicate altered hydrogen bonding interactions or hydroxyl group encapsulation in the nanoparticles. The C-H stretching peaks have shifted from 2931.90 cm<sup>-1</sup> and 2870.17 cm<sup>-1</sup> to 2970.48 cm<sup>-1</sup> and 2924.18 cm<sup>-1</sup>, respectively. These changes suggest that the synthesis process altered the alkyl groups. Furthermore, the emergence of new peaks at C=C

stretching of 2360.95 cm<sup>-1</sup> and C=O stretching of 1925.02 cm<sup>-1</sup> indicates that alkynes and ketones are involved in the production of nanoparticles. These modifications show significant chemical changes that occurred during the synthesis, possibly as a result of interactions between the constituents of the extract and the synthesis condition. Intricate changes in chemical bonding are shown by comparing FTIR spectra, which illustrates the production of unique nanoparticles with modified functional groups and possible uses in a variety of industries, including materials science and medicine. These results are in line with the phytochemical study that was done on *N. odorata* (Gudimalla *et al.*, 2020).



Gas Chromatography Mass Spectrometry Analysis Results: The GC-MS analysis was conducted on both the ethanol extract of Enantia chlorantha stem bark and the synthesized nanoparticles (Ec-AgNPs). In the extract. 28 compounds were detected, as shown in Figure 4, while 32 compounds were detected in the Ec-AgNPs, as shown in Figure 5. The compound with the highest peak area in the extract was 1, 2-Benzenedicarboxylic acid dipropyl ester (8.56%), whereas the lowest peak area was observed for 1, 5-Heptadiyne (1.47%). The main compounds identified in the extract were 7-Hexadecene, 9-Eicosene, 1, 5-Heptadiyne, 1-Octadecene, Caryophyllene oxide, 1, 2-Benzenedicarboxylic acid dipropyl ester, and Cyclotetradecane. Among the compounds found in the Ec-AgNPs, 4-Tetradecene (E) showed the highest

3-Amino-5area (13.44%),while peak trifluoromethylbenzoic acid and methoxyacetic acid, 2-tetradecyl ester, had the lowest peak area (0.70%). The major compounds identified in the Ec-AgNPs were 2-Dodecene (Z), 4-Tetradecene (E), 3-Amino-5trifluoromethylbenzoic acid, 9-Octadecene (E), 1-Nonadecene, Dibutylphthalate, 1-Octadecanol, and Bis (2-ethylhexyl) phthalate. The comparison between the Enantia chlorantha stem bark extract and the synthesized Ec-AgNPs highlights the diverse chemical composition of the latter, potentially contributing to its high potency. The unique compounds and altered peak areas in Ec-AgNPs may enhance bioactivity through synergistic effects or increased chemical reactivity from nanoparticle synthesis.



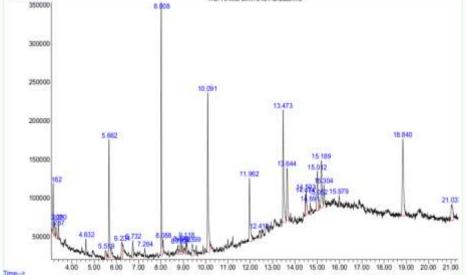


Fig 6: GC-MS chromatogram of the Ec-AgNPs

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Antifungal Activity of the Synthesized Nanoparticle and Crude Extracts of E. chlorantha extract Results: The antimicrobial potency of both Enantia chlorantha extract and Ec-AgNPs was examined against flavus, Aspergillus Aspergillus *fumigatus* and Aspergillus niger(Fig.5). The results showed the extent of inhibition of the examined organisms Table 2. As shown in the result, the silver nanoparticles synthesized from E. chlorantha extract(Ec-AgNPs) inhibited the growth of Aspergillus flavus, Aspergillus fumgatus and Aspergillus niger by 87.07%, 86.63% and 85.57% respectively which were more potent when compared to that of the E. chlorantha extract activities against the same organisms by 83.94%, 72.65% and 83.92% at 150  $\mu$ l/ml, this is in accordance with Lateef et al. (2017) who reported the mycelial silver inhibition of nanoparticles from Synsepalumdulcificum against Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger at the range of 73 to 75% at 100  $\mu$ g/ml respectively. These mycelial inhibitory activities could be as a result of silver nanoparticles interaction with the cell

membrane of fungalmycelia and disrupting the integrity of the cell membrane (Jian et al., 2022). Furthermore, it has been reported by Михайлова (2020) and Jian et al. (2022) that silver nanoparticles have the ability of generating reactive oxygen species such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and superoxide radicals (O<sup>2-</sup>) when they come in contact with fungal cells, also, silver nanoparticles can interfere with the activity of essential enzymes within Aspergillus mycelia thus disrupting normal metabolic pathways, impairing energy production and finally leading to growth inhibition. To lend more credence to the mycelial inhibitory activities of the silver nanoparticles, it has been reported that silver nanoparticles interact with fungal DNA leading to structural damage or inhibiting DNA replication and transcription processes (Михайлова, 2020; Jian et al. (2022). On the evidence of these facts, the silver nanoparticles synthesized from E. chloranthaextract have proven to be a reliable agent in combating mycotoxigenic molds.

Table 2: Antifungal activities of greens plant extract and synthesized silver nanoparticles (Ec-AgNPs)

Test Organism	Inhibition of control	Extract inhibition	Ec-AgNPs inhibition
Aspergillus fumigates	67.3	72.65	86.63
Aspergillus niger	72.8	83.92	85.57

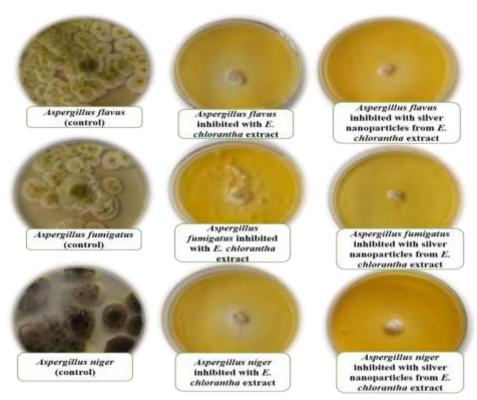


Plate 1: Antifungal activities of *E. chlorantha*extract and its silver nanoparticles *ADEYENI, E. G; AYODELE, E. T; OLAWOORE, I. T; ADEYENI, R. A* 

One of the greatest breakthroughs in combating mycotoxigenic molds in this fourth industrial revolution is nanotechnology. The menace molds have wrecked on farm produce and stored grains are beyond measure; from the reduction in market value to pollution through mycotoxin secretions (Zahra et al., 2023). Thanks to nanotechnology for eco-friendly approach in combating these molds. Aspergillus sp., are one of the moulds that have been reported to Ochratoxin, secrete Aflatoxins, Patulin and Sterigmatocystin, these are known carcinogens and could also lead to acute poisoning and chronic health effects including increased risk of liver cancer (Bennett and Klich, 2003). These are the feat of information that encouraged the choice of test organisms used in this research antifungal assay.

*Conclusions:* The results of this study highlight the higher antibacterial activity and spectrum analysis of E. chlorantha silver nanoparticles (Ec-AgNPs) compared to the *E. chlorantha* extract. The FTIR, GC-MS methods and Ec-AgNPs showed unique functional groups and a more varied profile of bioactive substances than the extract. Additionally, Ec-AgNPs showed better antibacterial activity than the extract in the antimicrobial studies, indicating their increased potential for use in nanomedicine and related fields. These results support the intriguing potential of Ec-AgNPs as effective antibacterial agents and emphasize the need to synthesize them with environmentally acceptable and sustainable methods.

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