Anchor University Journal of Science and Technology (AUJST)
A publication of the Faculty of Science Applied and Health Science, Anchor University Lagos
URL: journal.aul.edu.ng
In AJOL: https://www.ajol.info/index.php/aujst
Vol. 6 No 2, November 2025, Pp. 218-225
ISSN: 2736-0059 (Print); 2736-0067 (Online)

Green Synthesis, Characterization, and Antimicrobial Activity of *Phyllanthus amarus* Silver Nanoparticles

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Submitted 23 June, 2025
Accepted August, 2025

Competing Interests.
The authors declare no competing interests

ABSTRACT

Background: The emergence of antibiotic-resistant microorganisms poses serious concerns to global health, demanding the discovery of novel therapeutic remedies.

Objectives: To investigate the potential of green synthesised silver nanoparticles from Phyllanthus amarus as a novel treatment for combating microbial infections.

Methods: Phyllanthus amarus contains phyto-bioactive compounds such as phenols, alkaloids, and flavonoids, which act as effective reducing and capping agents for the green synthesis of silver nanoparticles, referred to as PA-AgNPs. The biosynthesised nanoparticles were characterised using Ultraviolet-visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, Scanning Electron Microscopy (SEM), and Energy-dispersive X-ray (EDX) analysis. The antimicrobial activity of the nanoparticles was tested against six clinical isolates using the disk diffusion method.

Results: The UV-Vis spectra confirmed the synthesis of PA-AgNPs, with a characteristic absorption peak between 300 - 450 nm. The FTIR spectra indicated a strong interaction between the PA-AgNPs and the plant extract, showing a shift in peak location, reduction of peak intensity, or the formation of new peaks. SEM images showed mostly irregularly shaped and clustered nanoparticles with noticeable porosity. EDX analysis confirmed the presence of silver and elements from the plant extract. The PA-AgNPs displayed significant antimicrobial activity with inhibitory zones against K. pneumoniae (20 mm), E. coli (16 mm), S. aureus (8 mm), S. pneumoniae (4 mm), A. fumigatus (10 mm), but not against C. albicans.

Conclusion: This study successfully demonstrated the green synthesis of silver nanoparticles with promising antimicrobial properties, highlighting their potential for applications in biomedical and pharmaceutical fields.

Keywords. Silver nanoparticles, Phyllanthus amarus, Green synthesis, Antimicrobial

1. Introduction

In 2019, bacterial infections were responsible for over 7.7 million deaths globally, approximately 13.6% of all deaths. Bacterial infections are one of the leading causes of mortality worldwide (Ikuta *et al.*, 2022). The invasion and growth of harmful microorganisms, including fungi, bacteria, viruses, protozoa, and helminths, within the human body is microbial infection. These infections can result in several disorders that impact the skin, lungs, brain, blood, and other body parts (Cleveland Clinic, 2022).

Nanotechnology involves manipulating atoms and molecules to design and produce structures, devices, and systems at the nanoscale (European Commission, n.d.). It has emerged as a promising field with various applications, from electronics to medicine. Nanomaterials such as single-walled carbon nanotubes and metallic nanoparticles like gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have been utilised in nanotechnology.

Silver nanoparticles (AgNPs) are tiny particles ranging from 1 nm to 100 nm, characterised by their small size and large surface-to-volume ratio. This characteristic is advantageous for their application in medicine, such as their ability to exhibit antimicrobial activities in biomedicine (Xu *et al.*, 2020).

The process of synthesising diverse metal

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nanoparticles utilising organic materials, such as plant materials and microorganisms is known as green/biological synthesis (Kumari *et al.*, 2021). The green synthesis of nanoparticles using plant extracts is an environmentally friendly alternative to modern chemical synthesis methods, that employ hazardous chemicals and high energy consumption.

Phyllanthus amarus belongs to the Phyllanthaceae family, which includes roughly 800 species that grow in tropical and subtropical regions of the world (Verma *et al.*, 2014). It is a renowned medicinal plant known for its pharmacological properties, including antimicrobial activities. Using readily available plants like *Phyllanthus amarus* to synthesise AgNPs would encourage sustainable methods in nanomaterial production, minimising environmental effects and improving the viability of large-scale applications.

2. Materials and Methods

Analytical grades of silver nitrate, Mueller-Hinton agar, and distilled water were used. *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus fumigatus*, and *Candida albicans* were collected from the Department of Biological Sciences, Anchor University, Lagos, Nigeria.

2.1. Extraction of Plant

The *Phyllanthus amarus* plant was freshly gathered from the campus of Anchor University in Lagos, Nigeria. The whole plant was washed thoroughly with tap water to remove dust particles and sand. The plant material was air-dried at room temperature. 70 g of the fresh plant material was weighed into a 1000 mL round bottom flask containing 700 mL of distilled water and boiled for 1 hour on the heating mantle. The resulting plant extract was filtered using Whatman filter paper into a 1000 mL conical flask and stored in the refrigerator for further use. 50 mL of the plant extract was concentrated using an oven at a temperature of 80 °C to obtain the crude extract.

2.2. GREEN SYNTHESIS OF *Phyllanthus amarus* SILVER NANOPARTICLES (PA-AgNPs)

100 mL of the prepared plant extract was added to 100 mL of 0.02 M silver nitrate solution in a beaker. The mixture was heated on a hot plate at approximately 70 °C under agitation for 1 hour. The colour change confirmed the reduction of silver nitrate to silver ions. The mixture was centrifuged at 4,000 rpm for 10 minutes to separate the synthesised silver nanoparticles from the reaction mixture. The silver nanoparticles were purified by repeated centrifugation of the reaction mixture. The supernatant was replaced by distilled water each time. The purified suspension was oven-dried at 80 °C for some days to obtain dried pellets. The dried pellets were crushed into fine particles using a mortar and pestle and transferred into a storage bag for further analysis.

2.3. Characterization of PA-AgNPs

The synthesised PA-AgNPs were subjected to four characterisation techniques.

The UV-VIS spectrophotometer UV1200/ UV1200PRO was used to determine the absorption spectrum of the PA-AgNPs. The Pelkin Elmer 3000 MX spectrometer was used to ascertain the organic functional groups in the PA-AgNPs and the plant extract. The Scanning electron microscope (Model: JOEL-JSM 7600F) was used to identify the morphology and size of the PA-AgNPs. This was coupled with the EDX spectroscopy. Energy-dispersive X-ray spectroscopy was used to determine the elemental composition of the PA-AgNPs.

2.4. Antibacterial Activity

The antibacterial activity of the synthesised PA -AgNPs was evaluated on clinical isolates (bacterial species), namely, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. 25 mg/mL of the green-synthesised silver nanoparticles was prepared and placed in a water bath for some minutes to ensure uniform

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dispersion. The pure cultures of bacteria were sub-cultured on Mueller-Hinton agar. Each strain was swabbed uniformly on the individual plates using sterile cotton swabs. Then, a well was made on sterile Petri dishes using a sterile cork borer. The nanoparticle solution was released into the wells on all plates using a micropipette. After incubation at 37 °C for 24 hours, the diameter of zone inhibition was measured in millimetres and was recorded.

2.5. Antifungal Activitiy

The antifungal activity of the synthesised PA-AgNPs was evaluated on clinical isolates (fungi species), namely, *Aspergillus fumigatus* and *Candida albicans*. 25 mg/mL of the green -synthesised silver nanoparticles was prepared and placed in a water bath for some minutes to ensure uniform dispersion. The pure cultures of fungi were sub-cultured on Mueller-Hinton agar. Each strain was swabbed uniformly on the individual plates using sterile cotton swabs. Then, a well was made on sterile Petri dishes using a sterile cork borer. The nanoparticle solution was released into the wells on all plates using a micropipette. After incubation at 27 °C for about 72 hours, the diameter of zone inhibition was measured in millimetres and was recorded.

3. Results and Discussion

3.1. Phytochemical Analysis

Phyto-bioactive compounds such as saponins, tannins, phenols, alkaloids, glycosides, steroids, triterpenoids, and flavonoids are present in the plant extract of *P. amarus* (Table 1). The phyto-bioactive compounds are potential alternatives to synthetic compounds especially antibiotics as therapeutic agents for treating various diseases. This phytochemical's presence is responsible for the plant's thera-peutic significance due to its antioxidant, anticancer, cytotoxic, anti-inflammatory, cardioprotective, hepatoprotective, immunomodulatory, neuroprotective, and antidiabetic activities (Riaz *et al.*, 2023). Saponin has been reported to have hypolipidemic, hypoglycaemic, anti-asthmatic, antioxidant, anti-hypertensive, and anti-microbial cytotoxicity activities (Sharma *et al.*, 2023). Similarly, tannins have significant anticancer, virucides, antioxidant, antimicrobial and anti-inflammatory properties (Pizzi A., 2021). The result is in correlation with the findings of Umar *et al.* (2019) who showed that the aqueous leaf extract of *P. amarus* contains several phytochemical constituents.

Table 1. Phytochemical composition of *Phyllanthus amarus*

Phytochemical	*P. amarus* **extract**
Saponins	+
Tannins	+
Phenols	+
Alkaloids	+
Glycosides	+
Steroids	+
Triterpenoids	+
Flavonoids	+

Key: + = Present; - = Absent

3.2. Absorption Spectra

The plant extract of *P. amarus* caused the aqueous silver ions to be reduced to zero-valent silver nanoparticles, leading to a colour change from pale yellow to dark brown after adding silver nitrate solution in 1 hour. The colour change implies the process known as surface plasmon resonance (SPR) due to the collective excitation of conducting electrons in the metals. The synthesised nanoparticles' spectra ranged from around 300 to 450 nm. AgNPs exhibit spectrophotometric absorption readings in the 400 – 450 nm wavelength region, according to published literature (Ogunsile *et al.*, 2020).

The plant extract showed a high absorbance peak at around 300 – 320 nm. This high absorbance indicates the presence of certain compounds in the extract that absorb strongly in this UV region. These could be flavonoids,

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phenolic compounds, or other phytochemicals commonly found in plants (Ejidike *et al.*, 2023). The intensity of the plant extract peak between 300 – 320 nm was reduced in that of the PA-AgNPs, suggesting an interaction between the plant and Ag ion. PA-AgNPs showed a small peak between 300 to 450 nm which were not found in the plant extract alone.

The UV-Vis analysis shows that silver nanoparticles were successfully synthesised using the plant extract. The initial high absorbance peak of the plant extract, followed by a broad peak around 400 to 450 nm in the PA-AgNPs, confirms the reduction and stabilisation process of silver ions by the plant extract, resulting in the formation of silver nanoparticles (Figure 1).

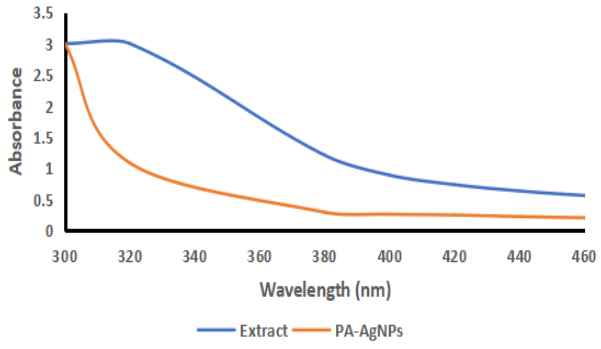

Figure 1. UV-Vis spectra of *P. amarus* extract and PA-AgNPs

3.3. FTIR Spectra

The FTIR spectra of *P. amarus* extract and PA-AgNPs are shown in Figure 2. In the spectrum of *P. amarus* extract, the broad and strong peak around 3461 cm^{-1} can be attributed to the O-H stretching vibrations from alcohols or phenols, indicating the presence of hydroxyl groups in *P. amarus* extract. The strong peaks around 2928 cm^{-1} and 2863 cm^{-1} typically correspond to C-H stretching vibrations from alkanes. The strong peak around 1735 cm^{-1} can be attributed to the C=O stretching vibrations from carbonyl groups. The medium peak around 1452 cm^{-1} is often associated with C=C stretching vibrations in aromatic compounds. The peaks around 834 cm^{-1} and 725 cm^{-1} can be attributed to the =C-H bending vibrations from alkenes, or C-H bending vibrations from aromatic compounds. The peaks in the fingerprint region (below 900 cm^{-1}) can be due to various bending vibrations, out-of-plane C-H bending in aromatics or skeletal vibrations. The FTIR peaks of *P. amarus* extract corroborate the phytochemical result, which shows that *P. amarus* extract contains different bioactive compounds with different OH, COOH, C=O, C=C, and C-C functional groups.

In the spectrum of PA-AgNPs, the strong O-H peak was found at 3405 cm^{-1}, which is different from the O-H peak reported for *P. amarus* extract (3461 cm^{-1}). The slight shift might indicate the interaction of *P. amarus* with silver nanoparticles, suggesting the role of these groups in the reduction and capping process. The medium peak around 1457 cm^{-1} is often associated with C=C stretching vibrations in aromatic compounds. The changes of peaks in the fingerprint region (below 900 cm^{-1}) may indicate the interaction of these bending vibrations with the silver nanoparticles. The changes in peak positions and intensities in the PA-AgNPs spectrum also confirm the successful preparation of the PA-AgNPs, suggesting that these functional groups reduce silver ions and stabilise the formed nanoparticles.

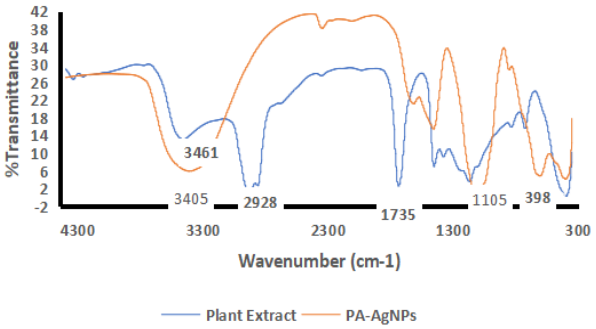

Figure 2. FTIR spectra of *P. amarus* extract and PA-AgNPs

3.4. SEM Analysis

Figure 3 shows the scanning electron micrograph of PA-AgNPs at different magnifications. The particles appeared irregularly shaped and clustered. This irregularity contributed to a unique texture

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texture across the sample. The clusters, composed of numerous interconnected nanoparticles, presented an extensive surface area. The vast surface appeared porous with faceted edges on some of the particles. This suggests that the PA-AgNPs have a crystalline morphology.

3.5. EDX Analysis

The EDX result in Figure 4 confirmed the elemental silver signal energy peak at 2.5 to 2.9 KeV with an abundance of 50.7 %.

Elemental silver (Ag) is the predominant element, suggesting the sample contains a substantial amount of silver nanoparticles. This result validated the successful green synthesis of the PA-AgNPs using phyto-bioactive compounds from the *P. amarus* extract. Elements like oxygen (O), aluminium (Al), and carbon (C) are also significant, which might indicate the presence of oxides, aluminates, or organic materials from the *P. amarus* extract. Other elements such as magnesium (Mg), iron (Fe), calcium (Ca), manganese (Mn), potassium (K), and sodium (Na) are present in smaller amounts as trace elements found in *P. amarus* extract. The direct use of the synthesised PA-AgNPs from the native reaction solution without further purification is probably the cause of the presence of signals from other trace elements (Ogunsile *et al.*, 2020).

3.6. Antibacterial Activity

The antibacterial activity of the PA-AgNPs was evaluated using the disk-diffusion method against three clinical bacterial isolates, and the results are presented in Table 2 and Figure 5. The PA-AgNPs exhibited the highest antimicrobial activity against *K. pneumoniae* (20 mm), followed by *E. coli* (16 mm) and *S. aureus* (8 mm), with the lowest activity observed against *S. pneumoniae* (4 mm) as shown in Figure 5. The PA-AgNPs exhibited superior antibacterial activity against *K. pneumoniae* and *E. coli*; however, *P. amarus* extract showed greater antimicrobial activity against *S. aureus* and *S. pneumoniae*.

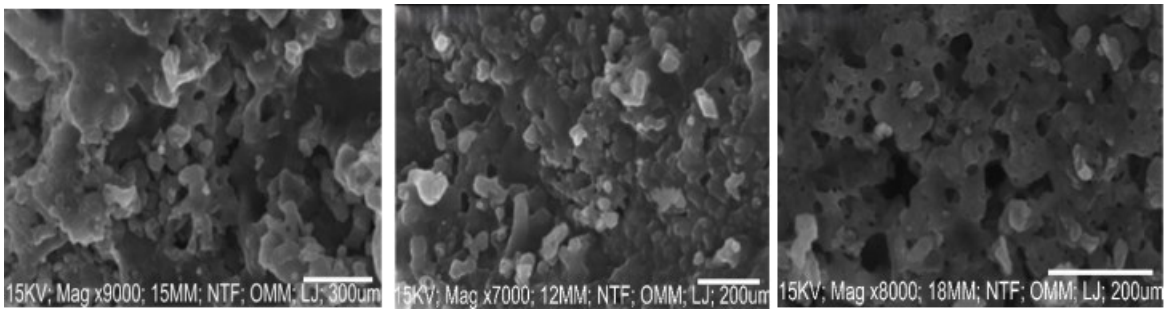

Figure 3. SEM images of PA-AgNPs at different magnifications

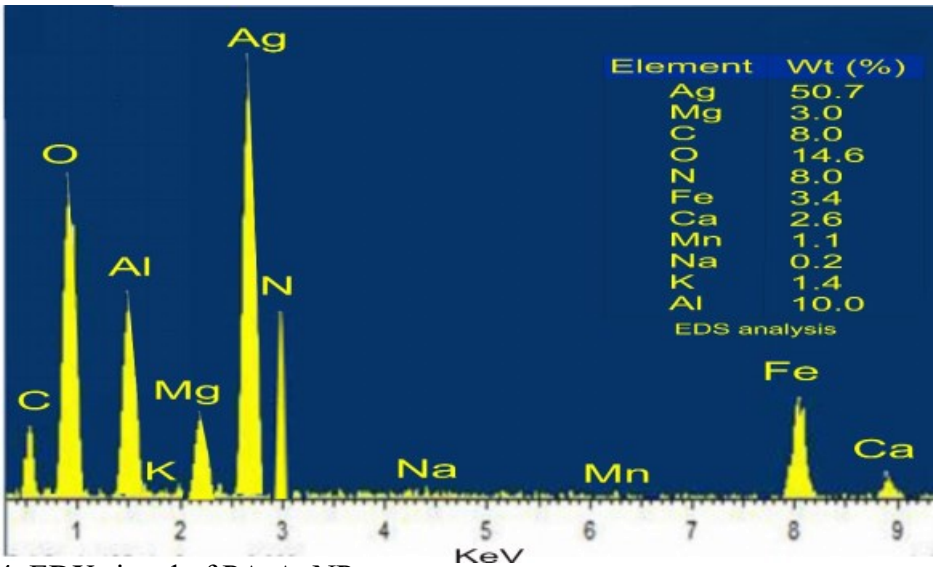

Figure 4. EDX signal of PA-AgNPs

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The antibacterial activity of silver nanoparticles is often attributed to the release of silver ions (Ag^+), which can interact with bacterial proteins, DNA, and cell membranes. The DNA loses its ability to replicate, resulting in the inactivation of ribosomal subunit proteins, and other essential cellular proteins and enzymes required for ATP production (Shenava, 2013). The efficacy can vary based on the rate and amount of silver ion release. Gram-negative bacteria, such as *K. pneumoniae* and *E. coli*, have a thin peptidoglycan layer and an outer membrane containing lipopolysaccharides. This composition might aid in the interaction and penetration of silver nanoparticles. On the other hand, Gram-positive bacteria, such as *S. aureus* and *S. pneumoniae*, have a thicker peptidoglycan layer, which could impede the effectiveness of the nanoparticles (Xu *et al.*, 2020).

3.7. Antifungal Activity

The antifungal activity of the PA-AgNPs was evaluated using the disk-diffusion method against two clinical fungal isolates, and the results are presented in Table 2 and Figure 6. The PA-AgNPs showed greater antifungal activity against *A. fumigatus* (10 mm) and no inhibitory effect against *C. albicans* as shown in Figure 6. The *P. amarus* extract exhibited greater antifungal activity against the fungal isolates than PA-AgNPs.

The structural differences between the cell walls of moulds (filamentous fungi) and yeasts may play a significant role in their varying susceptibilities to the PA-AgNPs. *C. albicans* may have inherent or acquired mechanisms that protect them from the antifungal effects of silver nanoparticles, which limit nanoparticle penetration or express proteins that neutralise silver ions.

Table 2. Antimicrobial activity of *P. amarus* extract and PA-AgNPs against clinical isolates

	Zone of inhibition (mm)			
Bacteria				
Sample/Isolate	*S. pneumoniae*	*K. pneumoniae*	*S. aureus*	*E. coli*
P. amarus extract	16	10	14	12
PA-AgNPs	4	20	8	16
Control	35	30	28	28
Fungi				
	A. fumigatus		*C. albicans*	
P. amarus extract	18		14	
PA-AgNPs	10		-	
Control	40		41	

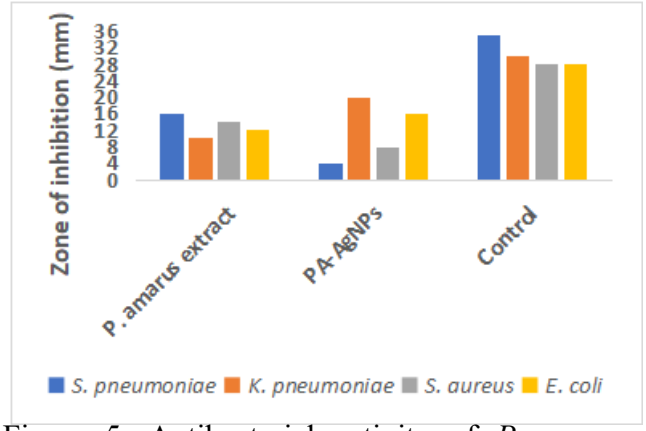

Figure 5. Antibacterial activity of *P. amarus* extract and PA-AgNPs against clinical bacterial isolates

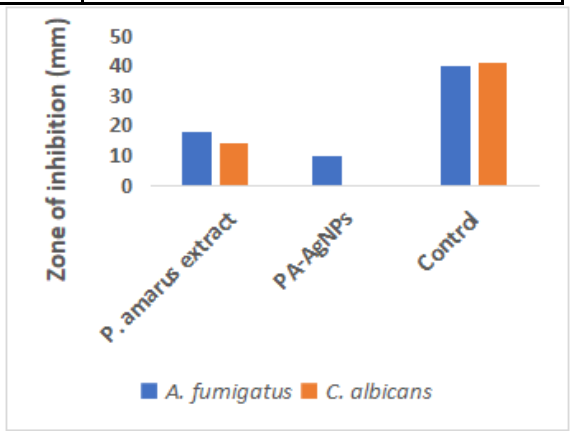

Figure 6. Antifungal activity of *P. amarus* extract and PA-AgNPs against clinical fungal isolates

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4. Conclusion

This study successfully demonstrated the green synthesis of silver nanoparticles using *P. amarus* plant extract. The comprehensive UV-Vis, FTIR, SEM, and EDX characterisations confirmed the formation, stability, and composition of the nanoparticles. The biosynthesised nanoparticles have demonstrated significant antimicrobial properties, particularly against bacterial pathogens compared to fungal pathogens, indicating their potential for use in the biomedical and pharmaceutical fields as antimicrobial agents. However, further research is warranted to explore the practical applications of these nanoparticles.

References

Cleveland Clinic. (2022, September 20). *Bacterial Infection*. https://my.clevelandclinic.org/health/diseases/24189-bacterial-infection.

Ejidike, I., Ijimdiya, R., Emmanuel-Akerele, H., Emmanuel, G., Ejidike, O., Bamigboye, M., Seyinde, D., Olaleru, A., Tanimowo, W., & Awolope, R. (2023). Biosynthesis, Characterization, and Antimicrobial Assessment of Metal Nanoparticles from Dryopteris manniana (Hook.) C. Chr Leaf Extract. *Bull. Pharm. Sci., Assiut University*, *46*(102), 225–238. http://bpsa.journals.ekb.eg/.

European Commission. (n.d.). *Nanotechnologies: 1. What is nanotechnology?* Retrieved April 11, 2024 from https://ec.europa.eu/health/scientificcommittees/opinionslayman/en/nanotechnologies/1-2/1.

Ikuta, K. S., Swetschinski, L. R., Aguilar, G. R., Sharara, F., Mestrovic, T., Gray, A. P., Weaver, N. D., Wool, E. E., Han, C., Hayoon, A. G., Aali, A., Abate, S. M., Abbasi-Kangevari, M., Abbasi-Kangevari, Z., Abd-Elsalam, S., Abebe, G., Abedi, A., Abhari, A. P., Abidi, H., … Naghavi, M. (2022). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet*, *400*(10369), 2221–2248. https://doi.org/10.1016/S0140-6736(22)02185-7.

Kumari, S. C., Dhand, V., & Padma, P. N. (2021). Green synthesis of metallic nanoparticles: a review. *Elsevier EBooks*, 259–281. https://doi.org/10.1016/b978-0-12-822401-4.00022-2.

Ogunsile, B. O., Seyinde, D. O., & Salako, B. A. (2020). Green synthesis of silver nanoparticles from leaf extract of Tetrapleura tetraptera and its antimicrobial activity. *IOP Conference Series: Materials Science and Engineering*, *805*(1). https://doi.org/10.1088/1757-899X/805/1/012032.

Pizzi A. (2021). Tannins medical/pharmacological and related applications: A critical review. *Sustainable Chemistry and Pharmacy*, *22*, 100481. https://doi.org/10.1016/j.scp.2021.100481.

Riaz, M., Khalid, R., Afzal, M., Anjum, F., Fatima, H., Zia, S., Rasool, G., Egbuna, C., Mtewa, A. G., Uche, C. Z., & Aslam, M. A. (2023). Phytobioactive compounds as therapeutic agents for human diseases: A review. *Food Science and Nutrition*, *11*(6), 2500–2529. https://doi.org/10.1002/fsn3.3308.

Sharma, K., Kaur, R., Kumar, S., Saini, R. K., Sharma, S., Pawde, S. V., & Kumar, V. (2023). Saponins: A concise review on food-related aspects, applications and health implications. *Food Chemistry Advances*, *2*. https://doi.org/10.1016/j.focha.2023.100191.

Shenava, A. (2013). Synthesis of silver nanoparticles by chemical reduction method and their antifungal activity. *International research journal of pharmacy*, *4*(10), 111–113. https://doi.org/10.7897/2230-8407.041024.

Umar, I. S., Yusuf, A. A., Alawode, A. R., Obiekezie, C. I., Okunlola, B. M., Abdulra

-zaq, O. M., Ariyeloye, S. D., & Lawal, B. (2019). Phytochemical compositions and biochemical effect of *Phyllanthus amarus* in albino rat. *GSC Biological and Pharmaceutical Sciences*, *8*(1), 128–133. https://doi.org/10.30574/gscbps.2019.8.1.0125.

Verma, S., Sharma, H., & Garg, M. (2014). *Phyllanthus Amarus*: A Review. ~ *18* ~ *Journal of Pharmacognosy and Phytochemistry*, *3*(2), 18–22.

Xu, L., Wang, Y. Y., Huang, J., Chen, C. Y., Wang, Z. X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics*, *10*(20), 8996–9031. https://doi.org/10.7150/thno.45413.