Faculty of Natural and Applied Sciences Journal of Applied and Physical Sciences
Print ISSN: 3026-9857
www.fnasjournals.com
Volume 2; Issue 4; December 2025; Page No. 23-31.
DOI: https://doi.org/10.63561/japs.v2i4.1027

Biosynthesis, Characterization, and Antimicrobial Activity of Silver Nanoparticles Derived from the Stem Bark Extract of *Persea Americana*

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Abstract
The increasing resistance of disease-causing bacteria to the traditional antibiotics is a strong factor indicating the necessity of the environmentally friendly alternatives, and silver nanoparticles (AgNPs) produced using the plant extracts are a potential remedy. Silver nanoparticles (AgNPs) in this paper were produced through the biosynthesis process of aqueous stem bark extract of *Persea Americana* (avocado) and their properties measured through UV- Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and X ray diffraction (XRD). Phytochemical screening assay identified alkaloids, tannins, saponins and cardiac glycosides whereas quantitative analysis identified 27 major bioactive compounds, most of them flavonoids and phenolic acids. Flavone (17.797 µg/mL), flavon 3 ol (17.092 µg/mL) as well as ellagic acid (15.596 µg/mL) and gentisic acid (11.397 µg/mL) were also identified, as well as daidzin, luteolin and myricetin. These plant-based constituents were reducing and stabilizing in the formation of nanoparticles. Strong surface Plasmon resonance at 425 nm was observed in the UV- Vis, FTIR validated the presence of functional groups (-OH, C=O, C -O), and XRD identified crystalline AgNPs with face centered cubic planes. Antimicrobial assays showed that AgNPs had better inhibitory effects on Escherichia coli (29.26 mm), Salmonella typhi (27.01 mm) and Staphylococcus aureus (24.18 mm), that were in most cases better than the crude extract and comparable to chloramphenicol. Such results point to P. Americana mediated AgNPs as a viable and sustainable antimicrobial approach with great biomedical and pharmaceutical prospects

Keywords: Biosynthesis, Antimicrobial, Nanoparticles, Stem bark, Characterization.

Introduction

The rising burden of diseases across the globe and the constraint of the conventional drugs, such as side effects and the culminating antimicrobial resistance, underscores the necessity of finding safer and cheaper therapeutic options. A viable option is through the use of medicinal plants because secondary bioactive metabolites are abundant in them and have been utilized by different cultures over the centuries in the prevention and treatment of diseases (David et al., 2016). These kinds of phytochemicals include alkaloids, flavonoids, terpenoids, tannins and steroids, which are playing a crucial role in boosting the immune system and are the lead compound in the creation of new pharmaceuticals (Doughari et al., 2008; Ochor et al., 2024). In the discovery of drugs, the screening of natural extracts is frequently the first step in finding active pharmacologically active compounds (Pandey et al., 2014). The fact that medicinal plants are easily available is especially appealing, and they often have fewer side effects, and are also deeply ingrained into the systems of traditional medicines, particularly in developing countries where a large part of their population depends on herbal cures (Okocha et al., 2023). Their therapeutic potential is also supported by ethnobotanical knowledge because most of the indigenous plants have a variety of bioactive components, with proven medicinal effects (Nna et al., 2020; Peteros & Uy, 2010). In spite of this potential, thus far a small percentage of the estimated 250,000 -500,000 plant species all over the world have been given systematic analysis concerning their phytochemical make-up (Prabhu et al., 2010; Okocha et al., 2023). In response to this shortcoming, pharmaceutical companies are dedicating more efforts to research natural products to produce affordable therapeutic agents that are affordable to a larger group of people (Jayachandran et al., 2023). The knowledge of the chemical structure of plants not only makes it easy to produce complex compounds but also serves as a basis to topple the discoveries of new drugs. Medicinal plants, therefore, are an unexploited but invaluable resource in drug development in modern times.

Cite this article as:
Nna, P. J., Ogacha, G. A., & Legborsi, J. (2025). Biosynthesis, Characterization, and Antimicrobial Activity of Silver Nanoparticles Derived from the Stem Bark Extract of Persea Americana, *2*(4), 23-31. https://doi.org/10.63561/japs.v2i4.1027

Aim and Objectives of the study

This research paper intends to examine the biosynthesis, characterization and antimicrobial properties of silver nanoparticles that are produced by using the ethanol extract of the stem bark of *Persea Americana*. In particular, the proposed study aims at identifying the phytochemical composition of the ethanol stem bark extract and synthesizing silver nanoparticles with the plant extract as a green reducing and stabilizing agent and the characterization of the synthesized silver oxide nanoparticles with the help of X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, ultraviolet-visible (UV-VIS) spectroscopy, and scanning electron microscopy (SEM). Moreover, the antimicrobial ability of the crude stem bark extract and the synthesized silver oxide nanoparticles is analyzed and antimicrobial activity of the nanoparticles is compared to the one of a standard antimicrobial drug.

Materials and Methods

Sample collection and Preparation

The stem barks of *Persea Americana* plants were collected from Iwofe community in Obio/Akpor local government in Rivers State, and they underwent a two-week air-drying process. Subsequently, the stem barks were finely powdered using a ceramic mortar and pestle. Five grams of powdered stem bark were placed in a beaker and mixed with 100 milliliters of ethanol. The mixture was stirred continuously at 700 revolutions per minute using a magnetic stirrer for 30 minutes. Following this, the mixture was heated to a temperature of 60 °C. The solution was then filtered using Whatman filter paper, and the resulting filtrate was collected and reserved for subsequent procedures.

Phytochemical characterization of stem bark extract of *Persea Americana* plant

Flavonoid

Each crude extract was combined with 4.0 mL of a 4% sodium hydroxide (NaOH) solution. This mixture produced an intense yellow color. Upon the addition of a few drops of diluted hydrochloric acid (HCl), the yellow color disappeared. This change indicated the presence of flavonoids in the sample.

Cardiac glycosides (Keller-kilani test)

A combination of Acetic acid glacial (3.0mL) with 3 drops of 3% $FeCl_3$ solution was added to the each of the plant extract and drops of concentrated H_2 SO_4 in addition. An observation of brown ring at the interface indicated the presence of cardiac glycosides.

Alkaloids (Mayer's reagent)

About 2.0mL each of the plant extract, 4.0mL of Mayer's reagent (potassium mercuric iodide solution) was added. The formation of a dull white precipitate serves as an indication of the presence of alkaloids.

Phenols and tannins:

Each of plant extract was mixed with 2.0mL of 2% solution of $FeCl_3$. An observation of blue -green or black coloration indicated the presence of phenols and tannins.

Steroids (Lieberman-Burchard's test)

The crude extract was dissolved in 3 mL of chloroform. Subsequently, 15 drops of acetic acid and 8 drops of concentrated sulfuric acid (H_2SO_4) were added to the solution and mixed thoroughly. The observation of a color change from red, passing through blue, and finally to green served as an indicator of the presence of steroids.

Saponins

About 3.0mL crude extract of each was mixed with 7.0mL of distilled water in a test tube and it was shaken vigorously. An observation of stable foam was an indication for the presence of saponins.

Synthesis of Silver-Nanoparticles of the plant

The synthesis procedure followed the method described by Mbagwu et al. (2022), with minor modifications. Initially, 200 mL of a 0.01 M silver nitrate ($AgNO_3$) solution was prepared in the laboratory and stored in a volumetric flask. For the synthesis, 50 mL of the $AgNO_3$ solution was combined with 50 mL of the stem bark extract filtrate of *Persea americana* in a conical flask. The mixture was gently heated to facilitate the reduction of Ag^+ ions to metallic silver (Ag^0).

After heating, the solution was left to stand overnight in a dark cabinet. It was then stored at a temperature of -4.0 °C for further experimentation. The initial color change from light pale yellow to brown upon the addition of $AgNO_3$ to the culture filtrate served as a primary indication of the formation of silver nanoparticles.

X-ray Diffraction Silver-Nanoparticles of stem bark extract of *Persea Americana* The shape and distribution of the AgNPs were analyzed using the X-ray diffraction (XRD) method. The sample of material was cut into sections and submerged in a sterile water solution before being centrifuged three times. Hermetic sealing and subsequent drying were applied to the pellet. An X-ray diffraction (XRD) lattice retained the tiny particles released from the section. The XRD

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spectrum was obtained using a Shimadzu XRD-7000 instrument, operating at 35 kV and 30 mA, and utilizing K alpha and K beta radiation. The decrease in potency was observed over a temperature range of 10 to 70 °C, as measured at various 2 theta angles.

Scanning Electron Microscopy (SEM) of Silver-Nanoparticles of stem bark extract of *Persea Americana*

The JEOL 5800LV scanning electron microscope was used to examine the morphological features of the Ag-NPs. The size of silver nanoparticles was measured using Scanning electron microscope analysis. This was carried out by transporting the synthesized silver-nanoparticles to the University of Zaria, Department of Chemistry. The stem bark extract of *Persea Americana* extract used to synthesize silver nanoparticles was allowed to dry entirely and grounded to powder for this technique. Complete dryness is a standard requirement for the SEM specimen since the specimen is at a high vacuum

UV-Vis of Silver-Nanoparticles of stem bark extract of *Persea Americana* plant

The Ag-NPs) were characterized using a wide range of techniques. The UV-Visible Spectrophotometer (Pg instrument) T60 was used to perform a spectral study spanning 250 to 600 nm.

Quantitative phytochemical screening by GC-FID

The phytochemicals in the extract were measured using BUCK M910 Gas Chromatography (BUCK Scientific, USA). A RESTEK 15-m MKT1 column (15m x 20m x 0.15um) and a flame ionization detector were used with the gas chromatography. A 20cm split less injection of the sample was made at a temperature and velocity of 280ºC and 30cm/s, respectively. The carrier gas utilized was helium (5.0pa), flowing at a rate of 40 ml/min. 200ºC will be the first oven temperature. The detector was run at 320ºC while the oven was heated at a rate of 3ºC/min until a temperature of 330 ºC was reached. Phytochemicals were identified by comparing the area and mass of the internal standard to the area of the newly found phytochemicals.

Collection of Microorganisms

The Microbiology Laboratory at the University of Port Harcourt Teaching Hospital in Port Harcourt, Rivers State, Nigeria, provided the clinical isolates of *Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis*, and *Salmonella typhi*. In order to positively identify the samples, the Microforms laboratory ran Gram staining and other specialized biochemical assays.

Preparationof Ag-NPs and Plant Extract

30 mg of the extract/ Ag-NPs were dissolved in 5.0 mL of DMSO (20%v/v) to create a stock solution of each biosynthesized product. Other concentrations of the antimicrobial test agents (25 mg/mL, 20mg/mL, 15 mg/mL, 10mg/mL) were generated using the serial dilution procedure with DMSO contained in various sterile test tubes and labelled accordingly. The stock solutions were serially diluted using the formula.

$$V_s = \frac{C_d V_d}{C_s}$$

Antimicrobial Activities

Clinical isolates of *Proteus mirabilis, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis*, and *Salmonella typhi* were used to test the antibiotic activity of the extracts in a well-in-agar diffusion assay (Nna et al., 2020). After 30 minutes of suspension in new agar media, dormant strains of the potentially hazardous bacteria were brought back to life. The number of infectious germs was increased to 10^8 cells, or a marching 0.5 McFarland standard. The medium was sterilized after being drilled into a cork borer. The wells then received varying concentrations of the synthesized compounds. For 24 hours, the contaminated medium was stored at 37 ºC. At the end, the plates were analyzed, and the millimeter (mm) dimensions of the inhibition zones around the wells were determined.

Results

Table 1: Phytochemical screening of stem bark extract of *Persea Americana*

Phytochemical	Result
Alkaloid	+
Cardiac Glycoside	+
Steroids	-
Anthraquinone	-
Tannins	+
Saponin	+
Flavonoids	-

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Reducing sugar	+

Table 2: Quantitative Phytochemical Screening of steam back of *Persea Americana*

	Compounds	Conc.(µg/mL)	Functional groups	Family
1	Daidzin	5.79720	-OH, C=O, Glucoside (-O-glucose)	flavonoid
2	Butein	4.79777	-OH, Ketone (C=O), Phenyl (-C_6H_5	flavonoid
3	Naringenin	0.237282	-OH, C=O, Benzopyran	flavonoid
4	Luteolin	4.51648	-OH, C=O, Benzopyran	flavonoids
5	Kaempferol	0.305119	-OH,C=O, Benzopyran	flavonoids
6	Epicatechin	0.822314	-OH, Benzopyran	flavonoid
7	Epigallocatechin	0.471428	-OH, Benzopyran	flavonoid
8	Quercetin	0.487359	- OH), Ketone (C=O), Benzopyran	flavonoid
9	Gallocatechin 3 gallate	0.173139	-OH), Ester (-COO- from gallate), Benzopyran	flavonoid
10	Robinetin	0.213070	-OH, C=O	flavonoid,
11	Myricetin	3.35567	-OH, C=O	flavonoids
12	Nobiletin	2.39335	-OCH_3, C=O	flavonoids
13	Baicalin	0.474066	-OH), Glucuronide (-COO-glucose), Ketone (C=O)	flavonoids
14	Tangeretin	0.318264	-OCH_3), Ketone (C=O	flavonoids
15	Artemetin	2.42077	-OCH_3), Hydroxyl (-OH), Ketone (C=O	flavonoids
16	Naringin	0.407388	ydroxyl (-OH), Ketone (C=O), Rhamnoglucoside	Flavanone glycoside
17	Lunamarin	0. 656105	Ketone (C=O),	Flavonoids
18	Cinnamic acid	0. 465011	Hydroxyl (-OH), Benzopyran	Phenolic Acids
19	Vinnillic acid	2.37956	-OH), Carboxyl (-COOH), Methoxy (-OCH_3	Phenolic Acids
20	Coumaric acid	0. 85394	-OH), Carboxyl (-COOH), Phenyl (-C_6H_5)	Phenolic Acids
21	Ferrulic acid	0.812418	-OH), Carboxyl (-COOH), Methoxy (-OCH_3	Phenolic Acids
22	Piperic acid	12.46763	-COOH), Methylenedioxy (-O-CH_2-O), Alkene (-C=C	Phenolic Acids
23	Ellagic acid	15.59621	-COO-), Hydroxyl (-OH), Benzophenone	Phenolic Acids
24	Flavone	17.79720	Ketone (C=O), Benzopyran	flavonoids
25	Flavon-3-ol	17.09166	Hydroxyl (-OH), Benzopyran	flavonoids
26	Gentisic acid	11.39680	Hydroxyl (-OH), Carboxyl (-COOH)	Phenolic Acids
27	Cinnamic acid	2.93940		Phenolic Acids

Table 3: Antimicrobial activity of stem bark extract of *Persea Americana*

Bacteria	**Concentration (mg/ml stem bark extract of *Persea Americana* of inhibition (mm)**				**Chloramphenical**
	30 mg/ml	25 mg/ml	20 mg/ml	10 mg/ml	10mg/ml)
B. subtilis	14.70	12.40	5.12	0	18.0
S. aureus	15.10	10.10	6.10	0	25.0
E. coli	18.20	16.15	6.31	0	29.0
K. pneumonia	16.50	9.80	4.15	0	12.0
Salmonella typhi	17.31	12.60	4.60	0	23.0

Table 4: Silver -Nanoparticles of stem bark extract of *Persea Americana* synthesized (Ag-NPs)

Bacteria	**Concentration (mg/ml stem bark extract of *Persea Americana* of inhibition (mm)**				**Chloramphenical**
	30 mg/ml	25 mg/ml	20 mg/ml	10 mg/ml	10mg/ml)
B. subtilis	20.17	16.10	10.12	8.12	18.0
S. aureus	24.18	19.16	12.23	10.90	25.0
E. coli	29.26	23.19	14.30	12.19	29.0
K. pneumonia	19.50	15.80	11.15	10.7	12.0
Salmonella typhi	27.01	20.16	14.60	13.10	23.0

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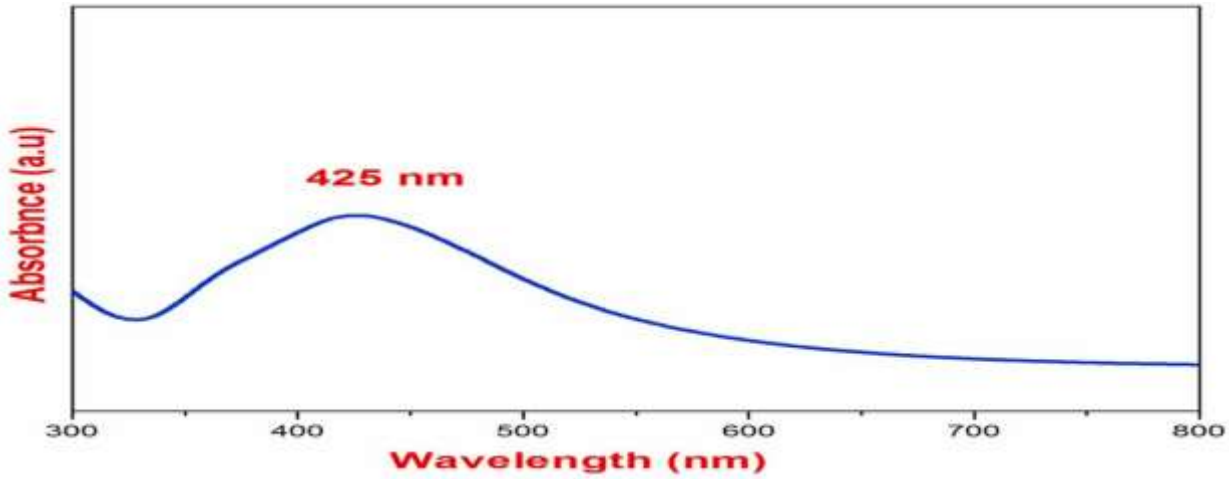

Figure 1: UV-VIS spectrum of AgNPs using **stem bark extract of *Persea Americana***

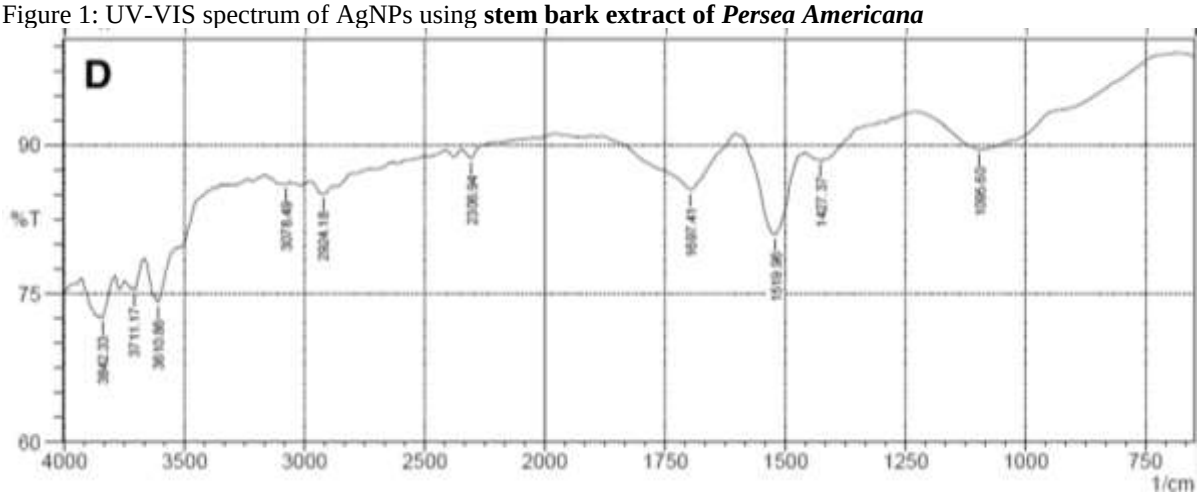

Figure 2: FTIR spectrum of AgNPs using **stem bark extract of *Persea Americana***

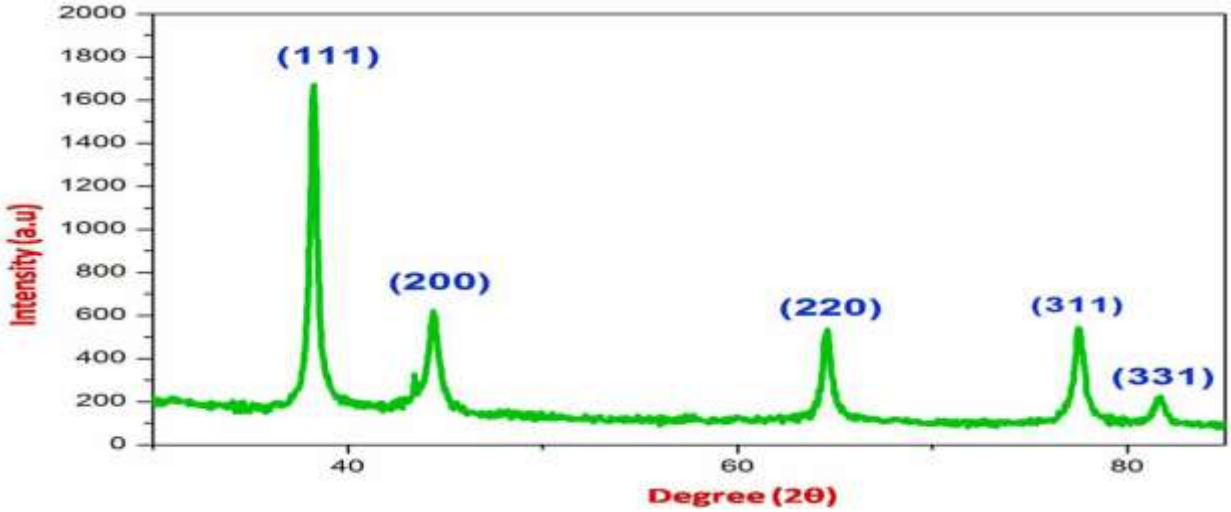

Figure 3: X-ray diffractogram of AgNPs using stem bark extract of *Persea Americana*

Discussion

In a screening of *Persea americana* in photochemistry, table 1 showed that alkaloids, cardiac glycosides, tannins, saponins and reducing sugars are present in the phytochemical extracts of the *Persea americana* stem bark extract which has various pharmacological activities that justify its usage as a traditional medicine. Antimalarial, antimicrobial, and analgesic, alkaloids are also expected to be detected in the extract, and this effect is mostly explained by their capacity to disrupt the process of microbial DNA replication (Njerua et al., 2013). On the same note, other than their known use in the regulation of cardiac activity by moderating sodium potassium ion levels, cardiac glycosides have also been found to have

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antimicrobial and anticancer properties (Sodipo et al., 2000). Tannins are yet another indication of the therapeutic value of the extract since they are antioxidants and antimicrobials with implications rooted in their precipitation of proteins and destabilization of microbial membranes (Gul et al., 2017). Also identified are saponins, which are immunomodulatory, antifungal, antibacterial, and anti-inflammatory, and have other possible applications as vaccine adjuvants (Sparg et al., 2004). The decrease in the level of sugars, although not directly therapeutic, could be related to the antioxidant activity and could help to form glycosides or flavonoid conjugates with an increased bioactivity (Akinmoladun et al., 2007). Interestingly, the extract was negative to flavonoids, steroids and anthraquinones. Failure to produce flavonoids in the qualitative assay is opposed by subsequent quantitative analyses which revealed their presence which may indicate possible limitations of the detection technique, low concentrations of the compound or effects of solvent polarity (Harborne, 1998). This mismatch brings out the significance of qualitative and quantitative complementary methodology in phytochemical analysis.

The quantitative phytochemical analysis of the stem bark of *Persea americana* in table 2 revealed the presence of flavonoid and phenolic acid as the main components, which proved that the plant has a therapeutic potential and justifies its traditional medicinal use in the treatment of infections, inflammation, and oxidative stress. The most common were the flavonoids which were identified as flavone (17.797 µg/mL), flavon-3-ol (17.092 µg/mL), and then ellagic acid (15.596 µg/mL) which is a potent antioxidant and antiprolliferation compound (Umesalma & Sudhandiran, 2010). Hydroxyl and carbonyl groups of flavonoids are the contributors of the free radical scavenging effect, which lowers the oxidative stress and cell damages (Panche et al., 2016). The other interesting flavonoids comprised luteolin, butein, and myricetin and are related to anti-inflammatory, antibacterial, and antidiabetic (Kumar & Pandey, 2013; Mirza et al., 2023). Naringenin is also known to have hepatoprotective and cardioprotective effects albeit in lower concentration (Mulvihill & Huff, 2012). Glycosylated flavonoids as naringin and daidzin are detected, which indicates improved solubility, stability and bioavailability since these compounds are soluble enough to be hydrolyzed in vivo to liberate the active aglycones (Hollman et al., 1999). The phenolic acids such as gentisic (11.397 µg/mL), piperic (12.468 µg/mL), cinnamic, ferulic and vinillic acids also play a role in the antioxidant and anti-inflammatory properties of the extract. They are found to prevent chronic conditions like cancer and atherosclerosis by their capacity to chelate metals and scavenge radicals as well as inhibit lipid peroxidation (Balasundram et al., 2006). The functional groups were also found to be functional groups, including, but not limited to, -OH, -C=O, -COOH, and -OCH3, whereby the methoxy bond provided lipophilicity and cellular penetration, and glucuronide bond provided solubility and immunomodulatory properties (Yao et al., 2004). Interestingly, the observed high flavonoid content is opposite to the negative results obtained after qualitative analyses. This difference can also be due to the inability of the colorimetric tests to detect low levels or in a glycosidic form, and the sophisticated methods of HPLC and LCMS are more effective (Sasidharan et al., 2011). In summary, the prevalence of flavonoids and phenolic acids indicates the pharmacological importance of P. americana and justifies the use of P. americana as a source of bioactive compounds to develop drugs.

As shown in table 3, Persea americana stem bark extract had a broad-spectrum antibacterial activity which confirmed its use in traditional medicine against infections. The extract exhibited significant inhibition zones at 30 mg/mL with the greatest sensitivity being observed in E. coli (18.20 mm), S. typhi (17.31 mm), K. pneumoniae (16.50 mm), S. aureus (15.10 mm) and B. subtilis (14.70 mm). No activity was seen at 10 mg/mL, which is a concentration-dependent effect in line with other reports in the literature on plant-derived antimicrobials (Cowan, 1999; Nair et al., 2005). Though not as effective as chloramphenicol, the high activity of the extract at higher concentrations is an indication that it can be used in complementary or adjunctive therapy especially where antibiotic-resistant organisms are omnipresent and drugs are scarce (Barbour et al., 2004). Sensitivity decrease of K. pneumoniae and B. subtilis could be related to structural defenses, efflux mechanisms, and multidrug resistance of K. pneumoniae is consistent with the previous results (Borges et al., 2016; Podschun & Ullmann, 1998).

Table 4 showed that the Persea americana stem bark extract converted into Silver nanoparticles (AgNPs) exhibited greater antimicrobial activity as compared to the crude extract. AgNPs gave large inhibition zones against E. coli (29.26 mm), S. typhi (27.01 mm), and S. aureus (24.18 mm) at 30 mg/mL, and the inhibition zone was even larger than chloramphenicol in some cases. In contrast to the crude extract, AgNPs were active at 10 to 20 mg/mL, and this indicates a dose-sparing effect (Franci et al., 2015; Rai et al., 2012). The most sensitive was E. coli and S. typhi, which is consistent with the Gram-negative bacteria permeability of silver ions. AgNPs were also found to have a superior outcome over chloramphenicol against K. pneumoniae (19.50 mm vs. 12.0 mm) and this indicates their ability to resist resistant pathogens (Lara et al., 2020; Loo et al., 2018) as well. These results validate the synergetic usefulness of photochemistry and nanotechnology, and green-synthesized AgNPs can be used as substitutes or supplements to traditional antibiotics. The future research must focus on toxicity, mechanisms and clinical uses.

Figure 1 revealed that the UV-Vis spectrum of the silver nanoparticles (AgNPs) produced with the help of *Persea americana* stem bark extract had a sharp absorption peak at 425 nm, which is the surface plasmon resonance (SPR) band of AgNPs (Sharma et al., 2022). It is a normal range of 400-450 nm of wavelength that fulfills the formation of small and spherical well-dispersed nanoparticles (Iravani et al., 2014). The sharpness of the peak implies concentration of the nanoparticles that has minimal aggregation and absence of the broadening implies homogeneity of the nanoparticles. To detect a change in particle size or aggregation, changes in SPR are additionally known to be redshifted with larger particles, and blue shifted with smaller particles (Kora & Rastogi, 2021). The SPR band can also be considered a fast-determined

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indicator of the efficiency of the reduction process of the nanoparticles and their stability during green synthesis, as in the case of plant extracts (Ahmed et al., 2016). The obtained spectrum, then, indicates that P. americana extract biosynthesized stable AgNPs.

According to figure 2, the FTIR analysis revealed that a number of categories of phytochemicals were used in the synthesis of silver nanoparticles by the stem bark extract of *Persea americana*. It observed the OH stretch of phenols and flavonoids that were broad and at 3842 - 3711 cm $^{-1}$, meaning that it participated in the reduction of Ag + to Ag 0 (Raji et al., 2023). Minus the peak, C-H stretching of terpenoids and saponins were at 2924 and 3078 cm, likely as a result of the stabilization of the nanoparticles (Moteriya & Chanda, 2020). The capping agent was protein or enzyme which had C=O strong absorptions at 1697 and 1519 cm -1 and C-O strong absorptions at 1427 cm and 1065 cm -1 respectively as indicative of aromatic C=C and C-O groups respectively which was typical of polysaccharides and esters respectively (Noman et al., 2023). These findings reveal that phytochemicals are dual reducing/stabilizing agents, which explains the complex bio reduction/capping mechanisms involved in the synthesis of green nanoparticles (Rautela et al., 2021).

The analysis through X ray diffraction (XRD) revealed that the silver nanoparticles prepared with the help of *Persea americana* stem bark extract were crystalline in nature as it appeared in figure 3. The visible sharp peaks of 2 that 38.1, 44.3, 64.5, 77.4 and 81.6 are (111), (200), (220), (311) and (331) planes characteristic of face centered cubic (fcc), structure of metallic silver (Baranwal et al., 2018). The highest value of the reflection at 38.1 indicates that the nanoparticles are preferentially grown along the (111) plane which is characteristic of the biosynthesized AgNPs due to its low surface energy and stability in structure (Logaranjan et al., 2020). The sharp and narrow diffraction peaks show a high degree of crystallinity which is requisite in optical, electrical and catalytic properties of the nanoparticles. It is noteworthy that no other peaks besides those of the metallic silver, show that purity of the phase, silver oxide and other impurities are not detected. It indicates that the green synthesis method is good in reducing Ag + ions to stable and single phase AgNPs (Kumar et al., 2021; Singh et al., 2022).

Conclusion

Through this paper, it has been demonstrated that silver nanoparticle (AgNPs) were prepared in a green way using *Persea Americana* stem bark extract. The extract contained significant phytochemicals including alkaloids, tannins, saponins, cardiac glycosides and reducing sugars that were involved in the formation of nanoparticles as well as supported their biological activity. Quantitative analysis also revealed that flavonoids and phenolic acids are highly heterogeneous (in particular flavones, flavon 3 ol, ellagic acid, gentisic acid) and, therefore, underline their roles as reducing and stabilizing agents. The UV-vis, FTIR, and XRD characterizations allowed concluding that the stable and crystalline face centered cubic AgNPs had been formed. The antimicrobial tests indicated that the biosynthesized AgNPs were much more effective compared to the crude extract and the most sensitive were E. coli, S. typhi and S. aureus. It was demonstrated at least once that AgNPs were several times more effective than the traditional antibiotic, chloramphenicol, against K. pneumoniae and S. typhi. On the whole, the crude extract and AgNPs had dose dependent antibacterial ability but increased efficiency of AgNPs indicates its future uses as environment friendly therapeutic agents in the treatment of resistant bacterium strain.

Recommendations

Based on this paper findings, it is proposed that future research needs to concentrate on the study of the extraction of the stem bark of *Persea americana* using binary solvent systems to enhance and further determine the quantity of extractable bioactive and mineral substance. One can also recommend the use of the extract of the stem bark of *Persea americana* when preparing nanoparticles with other metal oxides in order to increase its potential use and compare the physicochemical and biological characteristics. In addition to that, the chemical composition, crystalline nature and structural aspects of the isolated nanoparticles are stated to be further elaborated through the assistance of more qualitative and intricate analytical tools, respectively. Finally, the antimicrobial action of the crude extract of the stem bark and the generated silver nanoparticles against a wider range of the clinically important bacterial organisms should be evaluated to further define their therapeutic potential.

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Cite this article as:
Nna, P. J., Ogacha, G. A., & Legborsi, J. (2025). Biosynthesis, Characterization, and Antimicrobial Activity of Silver Nanoparticles Derived from the Stem Bark Extract of Persea Americana, *2*(4), 23-31. https://doi.org/10.63561/japs.v2i4.1027

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