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COMPARATIVE STUDY OF ANTIMICROBIAL ACTIVITY OF THE BIOACTIVE FRACTION AND PHYTOFABRICATED SILVER NANOPARTICLES OF *PTEROCARPUS SANTALINOIDES* L'HERIT EX DC

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Abstract

Background: Recently, the use of medicinal plants for therapeutic effects has gained attention, especially in the fight against drug ineffectiveness and antimicrobial resistance. Many studies have investigated the antimicrobial activities of various parts of *Ptercarpus santaliniodes* but there is limited knowledge about the activity of phytofabricated silver nanoparticles of the leaf extract. Therefore, this study is aimed at comparing the antimicrobial activities of the most active fraction of *P. santaliniodes* L'Herit ex DC. and its phytofabricated silver nanoparticles.
Materials and Methods: 500 g of pulverized dried leaves were extracted, fractionated, and investigated for antimicrobial activities using the agar diffusion method. Silver nanoparticles were phytofabricated and characterized using colour change, UV-Vis spectroscopy, and Fourier transform infrared. The percent inhibition, minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs),and minimum fungicidal concentrations (MFCs) of silver nanoparticles and the most active fraction were determined.
Results: Phytochemical screening showed the presence of secondary metabolites. The inhibition zone diameters of fractions were in the range of 8.5-18 mm with fraction 5 exhibiting the highest antimicrobial activity than the standard, ciprofloxacin. The brown colour and UV-Vis spectroscopy at 318 nm confirmed the formation of silver ions and O-H, O=C=O,C=H,C=O, C=C, and N-H functional groups. The phytofabricated silver nanoparticles had a higher percent inhibition compared to fraction 5, thus, a more significant antimicrobial activity. The MICs of both samples on three organisms are 0.16 mg/mL and MBC/MFCs are >1.25 mg/mL.
Conclusion: The phytofabricated silver nanoparticles of *Pterocarpus santaliniodes* could be a template in antimicrobial drug discovery and development.

Keywords: *Pterocarpus santalinoides*, phytofabricated nanoparticles, *Candida albicans*

List of abbreviations: Ultraviolet Visible (UV-Vis), Fourier transform infrared (FTI), Minimum Inhibitory Concentrations (MICs), Minimum Bactericidal Concentrations (MBCs), Minimum Fungicidal Concentrations (MFCs), *Ampicilin- Resistant Escherichia coli* (AREC), *Ampicilin- Resistant Pseudomonas aeruginosa* (ARPA) *and* , *Ampicilin-Resistant Staphylococcus aureus* (ARSA), Energy Dispersive X-ray (EDX).

Introduction

Nature has been a great source of natural products and the supply from plants, animals, or microorganisms is unlimited. Plants, on the other hand, have exhibited unmatched usefulness to man right from the time of old (Borges *et al*., 2016). They have contributed immeasurably to the healthcare system both at local and international levels. According to Sánchez *et al*. (2020) more than 80% of developing countries patronize herbal medicines/products or

make use of plant and plant parts in the treatment of their various ailments. Some of the prescription drugs are gotten directly from plants and they also serve as a template for the production of some semi-synthetic and synthetic drugs. Currently, some countries have integrated the use of clinically approved herbal products into their medical system (Salmerón-Manzano, Garrido-Cardenas & Manzano-Agugliaro, 2020).

Increasing resistance of bacteria to traditional antibiotics i.e. the orthodox antibiotics has shifted the paradigm of research to herbal sources. Antibiotic resistance is a significant and growing challenge in the healthcare system, potentially turning minor injuries or surgeries into serious threats(Spellberg & Gilbert, 2014). This resistance could drive the next pandemic, making common infections and surgical recoveries difficult to manage. This resistance has been reported to be due to irrational drug use or overuse of antibiotics, use of antibiotics in animal farms, inappropriate disposal of drug waste or expired by pharmaceutical companies, farmers, contamination of water beds with antibiotics medication, bacteria gene mutation (Ayukekbong et al., 2017; Habtamu Endale et al., 2023). Another concern, according to US Centers for Disease Control, (CDC, 2019), is the incorrect prescription of antibiotics. This is a result of an inappropriate diagnosis. Lack of use of correct diagnostic tools and a lot of assumptions of similar cases have resulted to use of antibiotics to treat infections which ordinarily would not have been used. And this could lead to cross-resistance (Gilchrist *et al.*, 2017).

Nanoscience is an emerging area of science that offers more promising ways of treatment of diseases (Afzal et al., 2022). Positive reports on disease treatment have been documented but more research in drug development and search for better ways to reduce toxicity to humans and the environment i.e. ecofriendly methods of synthesis are encouraged (Shilpa Borehalli Mayegowda et al., 2023). The nanomaterials are divided into organic and inorganic materials. Metallic nanoparticles are classified as inorganic nanomaterials and silver nanoparticles are a type of metallic nanoparticles (Harish et al., 2022). The use of silver nanoparticles as a carrier in the treatment of bacterial infection has been widely applied. Some of the cited studies are; in the treatment of *Staphylococcus aureus, Methicillin Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* (MRSA), *Klebsiella pnuemoniae, Salmonella Typhimurium, Streptococcus pneumoniae, Bacillus subtilis, Ampicilin- Resistant Escherichia coli* (AREC), *Ampicilin- Resistant Pseudomonas aeruginosa* (ARPA) *and, Ampicilin- Resistant Staphylococcus aureus* (ARSA) infections (Acharya *et al*., 2018; Brown *et al*., 2012; Esmaeillou *et al*., 2017; Lara *et al*., 2010; Otari *et al*., 2013 & Saeb *et al*., 2014).

The plant *Pterocarpus santalinoides* L'Herit ex DC is commonly known in English as Red Sandal wood (Anowi *et al*., 2012). It is from the family Leguminosae, currently known as Fabaceae. There are known 35 species of tree of the genus Pterocarpus and are woody climbers. It grows in tropical Africa and South America, but is native to Nigeria, Cameroon, Ghana, Senegal, and Brazil (AgroforestryTree Database, 2011; Keay, 1989 & Osuagwu and Akomas, 2013). It flowers from December to March, with fruit ripening between March and April. The Nigerian species are trees with light yellow flowers and they usually have alternate leaflets. *P. santalinoides* being an indigenous Nigerian plant, have local names and they include: in Igbo, nturukpa; in Yoruba, gbengbe; in Hausa, gunduru, gyadar, or kurmi; in Edo, akumeze; in Nupe, nja and maganchi; kereke in Tiv (Anowi *et al*., 2012). Ethnobotanically, according to Nwokorie, *et al*.,(2019), reported that the grated root of *P. santalinoides when* mixed with tobacco are smoked to treat cough. It is a plant commonly known as a vegetable plant, especially in the southeastern part of Nigeria, where the leaves are used to treat candidiasis, eczema, elephantiasis, liver disease, diarrhea, acne, and tooth and mouth diseases while the stem bark is used for cough, sore belly, and diabetes (Anowi *et al*, 2012, Bothon *et al*., 2014; Ama 2010, Osuagwu *et al*., 2013; Okwuosa *et al*., 2011 & Igoli *et al*., 2005). A survey showed that the stem bark of the plant is used in the treatment of hypertension in Cameroon. (Tsabang *et al*., 2017).

Having confirmed the antimicrobial properties of *P. santalinoides* leaves through evidence-based studies by Ayena *et al*. (2021), Njokuocha & Ewenike (2020), and Bothon *et al*. (2014), and considering the innovative use of nanoparticles for enhanced delivery systems (Afzal et al., 2022) to counter potential bioactivity resistance. Furthermore, many studies have investigated the antimicrobial activities of various parts of *Ptercarpus santaliniodes* but there is limited knowledge about the activity of phytofabricated silver nanoparticles of the leaf extract. Therefore, this study is aimed at comparing the antimicrobial activities of the most active fraction of *P. santaliniodes* L'Herit ex DC. and its phytofabricated silver nanoparticles

Materials and Methods

Plant collection and extraction

Fresh leaves of *Pterocarpus santalinoides* L'Herit ex DC family Fabaceae were collected in July 2023 from the Botanic Garden, University of Nigeria, Nsukka, Enugu State, Nigeria. The plant authentication was done by Mr Felix Nwafor of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka and assigned voucher specimen number PCG/UNN/0036. The leaves were washed and dried under shade (21 ^{0}C – 30 ^{0}C) until constant weight was obtained. The dried material was pulverized and extracted with n-hexane solvent using a Soxhlet extractor. The extract was concentrated with a rotary evaporator at 40ºC and stored in the refrigerator at 4ºC till needed.

Phytochemical Analysis

The preliminary phytochemical screening of active secondary metabolites in the n-hexane crude extract of *Pterocarpus santaliniodes* L'Herit ex DC was carried out using standard methods (Harbourne, 1984; Trease and Evans 1994).

Purification of n-hexane extract with column chromatography

Using a modified method described by Kulkarni *et al.,* (2004). The sintered column (450 x 10 mm) was packed with silica gel (60-200 mesh) in n-hexane. A weighted quantity of the n-hexane extract, 1.6 g was dissolved in 16 mL of n-hexane solvent and loaded onto the column and n-hexane was used as the eluting solvent. A total of 38 fractions were collected and pooled into 9 groups based on their R_f values. The fractions were pooled together based on how close their Rf values were.

Thin layer chromatography (TLC)

Micro drops of column fractions were spotted on preparative TLC plates (10 x 20 cm) using a micro pipette at a 1/2 inch apart and above the lower edge of the TLC plates. The plates were developed in n-hexane as a mobile phase. The plates were removed from the chamber, and air dried and the solvent front marked with a pencil. The spots on the plates were detected visually, under UV light (254 nm) and in the iodine chamber and the Retardation factors (R_f) of the spots were calculated using this formula as early reported (Kulkarni *et al*., 2004).:

$$Rf = distance\ travelled\ by\ sample\,(cm)/distance\ travelled\ by\ solvent.$$

Antimicrobial screening

Test Organisms: Clinical isolates of *Escherichia coli, Salmonella typhi,* and *Candida albicans* gotten from Department of Microbiology Central Research and Diagnostic Laboratory, Tanke, Ilorin, Kwara State.

Preparation of cultural media

The culture media (Muller Hinton Agar, and Sabouraud Dextrose Agar) were prepared according to the manufacturer's instructions in aseptic condition. And all experiments carried out in aseptic conditions as well.

Preparation of Test Inoculum

The test bacteria and fungus from the agar slant were sub-cultured on nutrient agar by stocking and incubated at 37 ^{0}C for 18-24 and 48 h respectively. Different suspensions of the organisms were made using the 18-24 h and 48 h culture in 2 mL of sterile distilled water; its density was adjusted to match the 0.5 McFarland density.

Antimicrobial Activity of the Pooled Fractions of *P. santalinoides* (PSH)

Using the method described by Balouiri *et al.,* 2016, the antibacterial and fungal activities of the grouped isolates were determined only on sensitive micro-organisms (*S.typhi, E.coli,* and *C.albicans*) using the Agar well diffusion method. A concentration of 2.5 mg/mL of the pooled fractions was prepared by dissolving 25 mg in 10 mL of 10% tween 80. A 20 µL volume of the standardized organisms was spread on already solidified Mueller Hinton and Sabouraud Dextrose Agar surface using a sterile cotton swab. The surfaces of the media were allowed to dry for 3 minutes and a sterile 6 mm cork borer was used to bore holes each on the agar plates. A 20 µL volume of each of the pooled fractions and standards; ciprofloxacin (25 mg/mL) and fluconazole (75 mg/mL) dispensed into designated holes. The plates were kept undisturbed for about 15 minutes before they were incubated at 37 ^{0}C for 24 h and 48 h respectively. This was done in triplicate and the diameters of zones of inhibition were measured in millimeters using a transparent ruler and the result was presented in the mean and standard error of the mean.

Phytofabrication of Silver Nanoparticles (SNPs)

The most active of the pooled fractions (fraction PSH 5) was used for the phytofabrication of silver nanoparticles. This experiment was carried out by a modified method described by Adebayo-tayo *et al*., (2016). A Volume of 25 mL of 1 mM of the aqueous solution of silver nitrate ($AgNO_3$) was prepared in 250 mL Erlenmeyer flasks and 10 mL of 3.8 mg/mL of fraction 5 was added into the flasks for the bio-reduction of the $AgNO_3$ to silver

(Ag+) ions. This composite was placed in an incubator for the complete bio-reduction at a temperature of 37 ^{0}C for 24 - 72 h and color change observed after 24 h intervals. Colour change was observed visually and UV-Vis spectrophotometry was employed to confirm the formation of silver nanoparticles. The mixture was centrifuged, freeze-dried and stored in an air-tight container for further analysis.

Characterization of the phyto-fabricated silver nanoparticles

These analyses were done by the method described by (Adebayo-tayo *et al*., 2016; Adebayo-Tayo *et al.*, 2020)

Visual observation of phytofabricated silver nanoparticles

Gradual color change of the incubated mixture of the isolates and the silver nitrate was observed visually at 24, 48, and 72 h of incubation.

UV-visible spectroscopy analysis of phytofabricated silver nanoparticles

UV-visible spectroscopy was used to determine the reduction and stable formation of the phyofabricated silver nanoparticles by reading off the absorbance spectra of a small volume of the mixture in the range of 200-800 nm and spectra recorded at 24 h.

Scanning electron microscopy analysis

This analysis was done to determine the shape and the size of the phytofabricated silver nanoparticles using Scanning electron microscopy (Model No: JSM-7900F; JEOL, USA). A small quantity of the dried nanoparticles was loaded on an aluminum holder stub and coated with carbon. Afterward, the sample holder was tightly mounted on the chamber and the sample was viewed at different magnifications after a high vacuum of <5 x 10^{-5} Pa was achieved. Information on the size, voltage, and magnification were inscribed on the image.

Fourier-transform infrared spectroscopy analysis

A small quantity of the phytofabricated nanoparticles was ground with potassium bromide salt and put in a mold to form a pellet at 25 ^{0}C. A Nicolet 800 FTIR spectrometer (Nicolet, Madison, WI, USA) was used to determine the biomolecules and the spectra recorded at a wave range of 900-4000 cm^{-1}.

Energy-dispersive X-ray spectroscopy analysis

The purity and elemental analysis of the Phytofabricated silver nanoparticles ($PsAgNO_{3)}$ was determined by energy-dispersive X-ray spectroscopy by subjecting small dried particles to rays and the elements determined over a specified time, 31 seconds at an accelerated voltage of 5.3 ekV.

Determination of MIC, MBC, and MFC of fraction PSH 5 and phytofabricated silver nanoparticles

The assay was carried out using a slightly modified micro broth dilution method described by (Rahman *et al*., 2014), a method of the National Committee for Clinical Laboratory Standard (2012 and 2000). All test organisms were standardized to match 0.5 McFarland after incubation. ~~A~~ 100 µL of Mueller Hinton Broth was added to the microtiter plates. A 100 µL of 2.5 mg/mL of the sample (SPH fraction 5) was added to the first row of a 96-well micro titer plate to produce an initial concentration of 1.25 mg/mL. Using a two-fold serial dilution method, a total of four different concentrations (1.25 - 0.16 mg/mL) of the plant extracts were obtained. Two columns were used as sterility control (no cultures added) and growth control (extracts replaced by blank solvent). The 96-well microtiter plates were sealed in plastic bags, homogenized, and incubated at 37 ^{0}C for 18 h in a 100% humidified incubator. After incubation, the plates were homogenized, and the MIC was determined visually by the presence or absence of growth and further confirmed spectrophotometrically with a Diateh microtitre plate spectrophotometer at a wavelength of 600 nm. The percentage of inhibition was calculated using a formula and the MIC was determined as the lowest concentration at which the percentage is in the positive range.

% of inhibition = $[(A_{control} - A_{test}) / A_{control}]$ x 100

Where A control = Optical Density of growth control,

A test= Optical Density of the tested sample

The Minimum bacterial/fungicidal concentrations were determined by the method described by (Odeh and Tor-Anyiin, 2014) by inoculating a loopful of the MIC onto the nutrient agar plate and the plate incubated at 37 ^{0}C for 24 and 48 h. After incubation, the plates were visually observed for the presence or absence of growth and minimal bacterial or fungicidal concentration was the lowest concentration that showed no bacterial or fungal growth.

NB: These same procedures were also used to determine the MIC, MBC, and MFC of the phytofabricated silver nanoparticles

Results and Discussion

The n-Hexane extract showed the presence of 7 out of 13 secondary metabolites that were screened in this study as shown in Table 1. The phytochemical screening of the leaf of *P. santalinoides* reveals the presence of phytochemicals like alkaloids, terpenoids, saponins, tannins, fats and oils, resins, acidic, and steroids. The qualitative study of the phytochemicals present in various plant parts is very crucial while investigating the pharmacological attributes of plants and their parts. The presence or absence of secondary metabolites is also dependent on the extractive solvent while the pharmacological activities of plants are attributable to these secondary metabolites and the amount therein (Ayena *et al.*, 2021 & Hussein & El-Anssary, 2019).

Table 1: Qualitative phytochemical screening of crude leaf extracts of *P. santalinoides*

Secondary metabolites	n-Hexane extract
Alkaloids	+
Terpenoids	+
Saponins	+
Flavonoids	-
Tannins	+
Proteins	-
Glycosides	-
Carbohydrates	-
Reducing sugars	-
Resins	+
Steroids	+
Acidic compounds	-
Fats and oil	+

Key; (-) – absent, (+) - present

The fractions of the extract were pooled together based on the retardation factor as monitored by thin-layer chromatography as shown in Table 2.

Table 2: Range of retardation factors of pooled fractions

Fractions	R_f Range	Fractions
1.	0.20-0.24	3, 28
2.	0.33	11, 12, 13, 14,
3.	0.37-0.41	1, 4, 5, 7, 8, 9, 10
4.	0.46-0.49	23, 18
5.	0.52-0.56	16, 17, 19, 21, 22
6.	0.63	29
7.	0.70-0.75	2, 20, 32
8.	0.94-0.95	30/31, 38
9.	-	6, 15, 24, 25, 26, 27, 33, 34, 35, 36, 37

Key: - = no spot

Table 3 shows that the zones of inhibition recorded by the pooled fractions ranged from 8.5-18 mm on the candida and the bacterial isolates. This range is similar to the IZD (Inhibition Zone diameter) that Ukwueze et al. recorded (2018). PSH Fraction 7 recorded the highest (18 mm) and the lowest (8.5 mm) inhibition zones for *E. coli* and *C. albicans* respectively. PSH Fractions 4 and 6 have activity against *E. coli* and *C. albicans* at IZD of 17 and 9 mm

and 12.5 and 9.5 mm respectively. PSH Faction 2 has activity for *C. albican* only at IZD of 11.5 mm, which is higher than that recorded for Fluconazole, a standard antifungal drug. PSH Fraction 5 is the only fraction with activity against *S.typhi* at an IZD of 11.5 mm and an IZD of 17.5 mm was recorded against *E. coli*. While PSH Fractions 1, 3, 8, and 9 have no activity against the microbial agents. PSH Fraction 5 was then used for further studies.

Table 3: Inhibition Zone Diameter(mm) of the pooled fractions at 2.5mg/mL

Pooled Fractions	***E. coli***	***S. typhi***	***C. albicans***
PSH 1	-	-	-
PSH 2	-	-	11.5±0.29
PSH 3	-	-	-
PSH 4	17.0±0.58	-	9.0±0.57
PSH 5	17.5±0.29	11.5±0.00	-
PSH 6	12.5±0.28	-	9.5±0.00
PSH 7	18±0.50	-	8.5±0.28
PSH 8	-	-	-
PSH 9	-	-	-
C+	21.0±0.00	23.0 ± 0.00	11.2 ± 0.17
C-	-	-	-

Key: - = no activity, C+ = positive control (ciprofloxacin and fluconazole), C- = negative control (10% tween 80)

Visual observation and UV-Vis spectra

A gradual color change of the mixture was observed at 24 h intervals during the incubation time of silver nanoparticle phytofabrication. The colour of the sample changed to light brown and tiny pellets were also formed. The slight colour change in Fig.1. is due to a lower concentration of nanoparticles used. The UV absorption spectrum of phytofabricated nanoparticles was recorded within the range of 200-800 nm as shown in Fig 2. As expected, all metals have free electrons and yield surface plasmon resonance (SPR) absorption band (peak) when their vibrations resonate with light waves.

Visual observation and UV-Vis spectra Fig. 2. showed that the absorption peak of the phytofabricated silver nanoparticles was 318 nm after 24 h of incubation which is lower than 363 nm reported by Elhawary et al., in 2020. The characteristic deep colour change and higher absorption wavelength of phytofabricated nanoparticles were not observed in the PsAgNP because of the less amount of the fraction used for the synthesis. This agrees with a previous studies where the amount and concentration of reducing agent and other phytochemicals in the fraction of plant extract were minute leading to less relative activity in silver nitrate reduction, as they are necessities for increased rate of reaction, amount of nanoparticles produced and production of characteristic colour (Agarwal et al., 2017; Saeb et al., 2014)

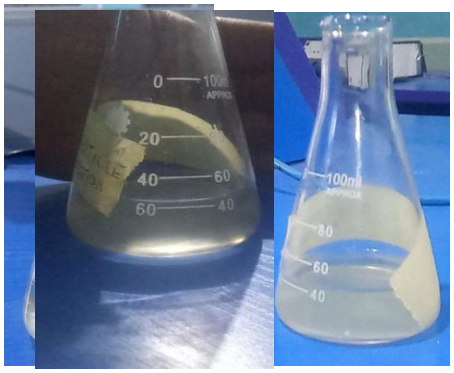

Figure 1: Colour change of silver nanoparticle at A: 24h, B: 48h, C:72h

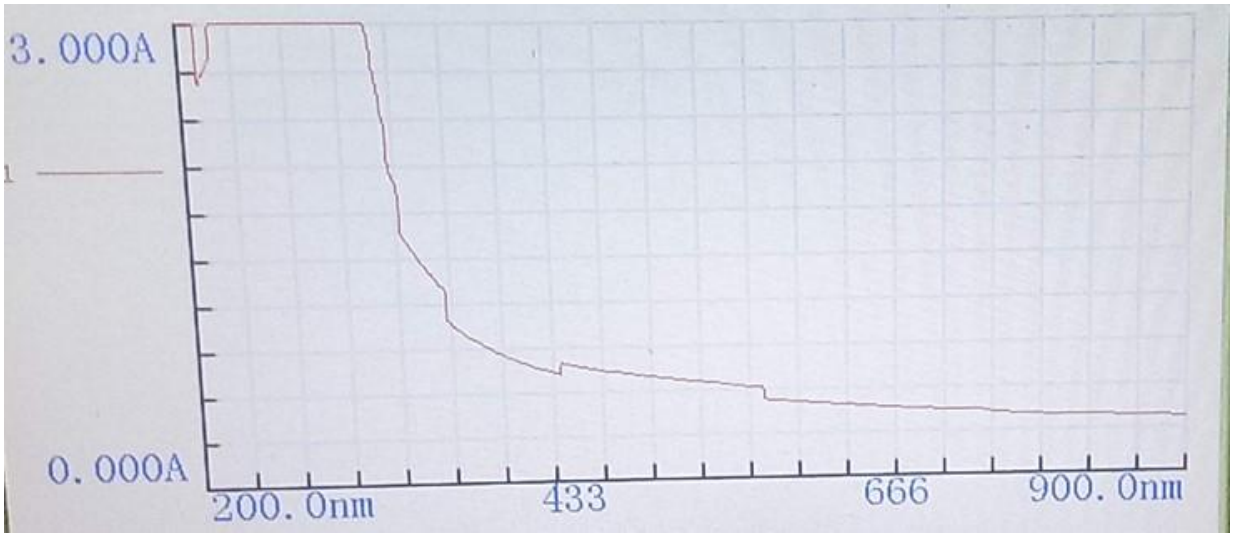

Figure 2:UV-Vis Spectrum of PsAgNPs

Scanning Electron Microscopy

The morphology and the structure of the PsAgNPs were observed with Scanning Electron Microscopy (SEM) as shown in Fig. 3. below. The particles are monodispersed with visible boundaries between each particle. They are spherical and measure 100 um in diameter at a magnification of 500.

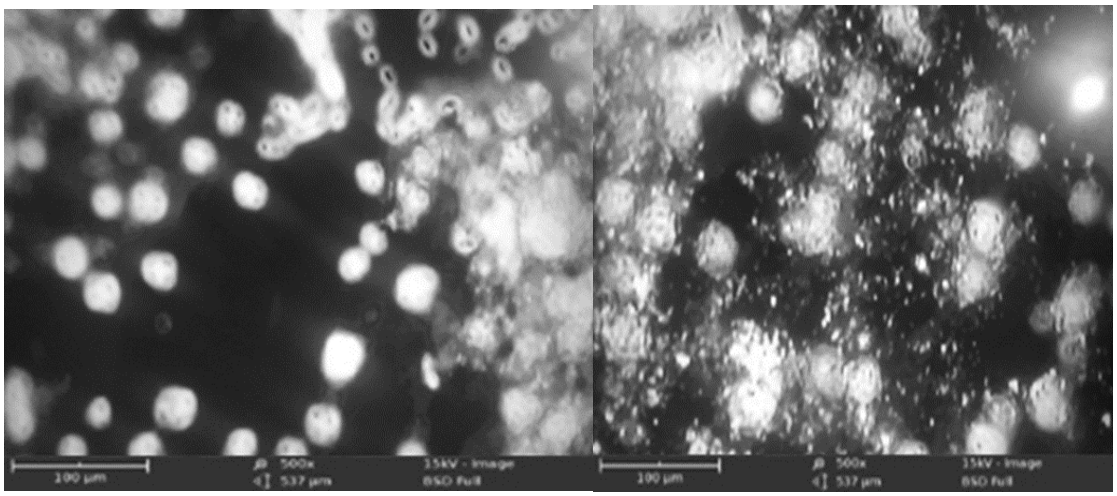

Figure 3: Scanning Electron Microscopy image of PsAgNP

Fourier-transform infrared spectroscopy

FTIR is used as a tool for characterizing samples by determining the functionl groups present, the chemical bond, and even the complexities of compounds as seen in Fig. 4. FTIR spectrum of five peaks or less are said to be simple organic compounds while that with five and above are said to be complex compounds (Nandiyanto & Okitiani, 2019). A total of 11 peaks were observed from the PsAgNPs spectrum at 3926.84, 3577.05, 3545.71, 2481.53, 1724.79, 1590.98, 1510.94, 1402.59, 1020.09, 995.42 and 973.13 cm^{-1}. The Peaks at 3926.84 cm^{-1}, 3577.05 cm^{-1}, and 3545.71 cm^{-1} show weak O-H stretching. The band at 2481.533 cm^{-1} indicates the presence of O=C=O stretching. The small band at 1724.79 cm^{-1} indicates the presence of C=O stretching of carboxylic acid or ketone. A C=C stretch of an aromatic compound is indicated by the peak at 1590.98 cm^{-1} while the peak at 1510.94 cm^{-1} indicates the presence of the N-H stretch of an aromatic compound. A C=H stretch of a methylene group with a peak at 1402.59 cm^{-1} and C=O of an alcohol indicated by a small absorption band at 1020.09 cm^{-1}. Peaks 995.42 cm^{-1} and 973.13 cm^{-1} indicate the presence of a C=C bend of the alkene group.

These functional groups highlights the presence of secondary metabolites like phenols, proteins, and carbohydrates in the plants and they also contribute to the reduction process in the Phytofabrication of silver nanoparticles and the antimicrobial activities of the nanoparticle (Ukwueze *et al*., 2018)

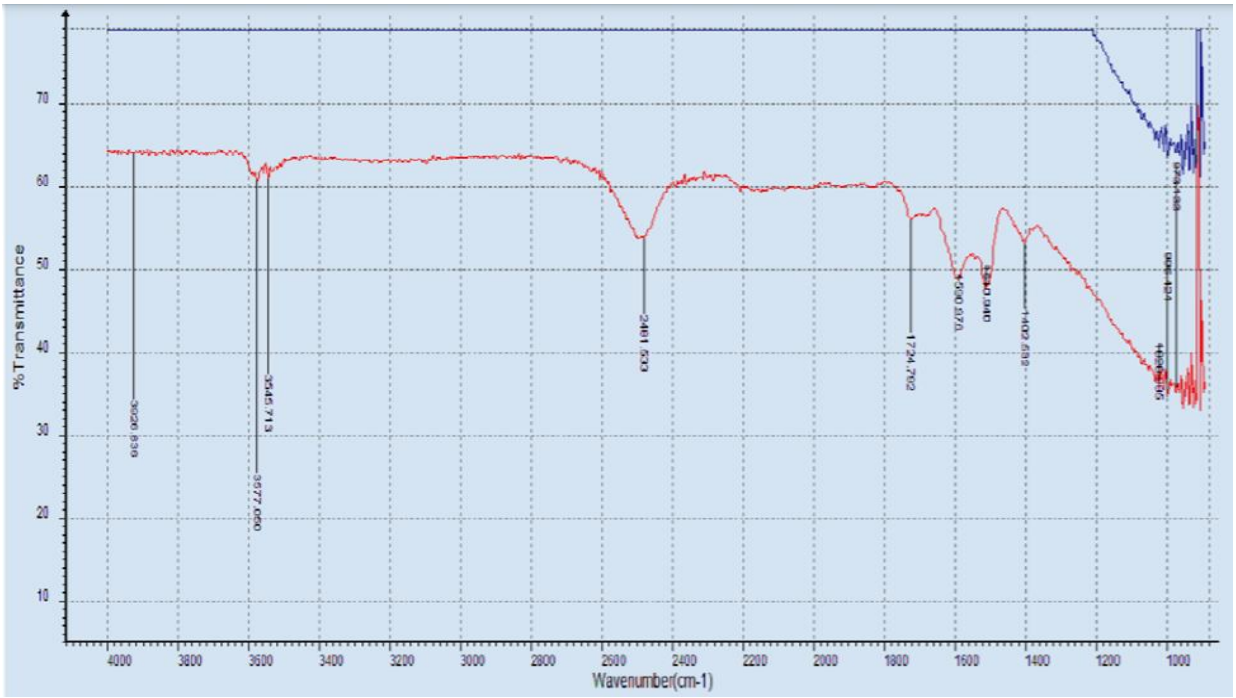

Figure 4: FTIR spectrum of PsAgNPs

Energy Dispersive X-ray of Phytofabricated nanoparticles

The spectrum in Fig. 5. shows that the major peak is silver at 70.41%. Other elements present in minimal amounts serve as stabilizing agents (Dada et al., 2018) and are usually bound to the surface of the silver nanoparticle. Presents are Iron, Fe (0.96%); Calcium, Ca (1.69%); Chromium, Cr (0.85%); Manganese, Mn (0.32%); Palladium, Pd (0.64%); Magnesium, Mg (1.164%); Zinc, Zn (0.42%); Phosphorus, P (0.81%); Sodium, Na (2.67%); Potassium, K (0.48%); Rhodium, Rh (0.04%); Ruthenium, Ru (0.16%) and Nickel, Ni (0.06%).

Reading No	Duration	Units	Sequence	SAMPLE	Fe	Ca	Cr	Mn	Pd	Ag	Mg	Zn	P	Sn	Na	K
ED-XRF	31.13	ppm	Final	Nanoparticle	0.961	1.687	0.852	0.318	0.636	70.413	1.164	0.421	0.807	Nil	2.673	0.477

Si	Rh	Ru	Ni	Cd	Pb	Zn
Nil	0.038	0.157	0.064	Nil	Nil	Nil

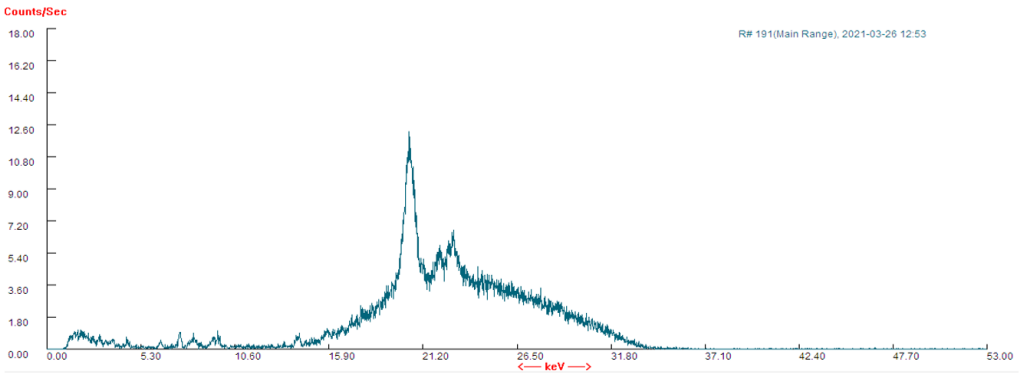

Figure 5: EDX spectrum of Phytofabricated nanoparticle

MIC of PsAgNPs and PSH Fraction 5

From the bar chart above, Fig. 6., all the concentrations are in the positive range and this shows that all the concentrations of the PsAgNPs and the fraction inhibited the visible growth of the bacteria and fungus. Both the silver nanoparticles and PSH fraction 5 showed the highest % inhibition at the highest concentration, 1.25 mg/mL. This shows the activities of the samples are dose-dependent except for the *C. albicans* where higher % inhibition was observed at 0.1563 mg/mL than 0.3125 mg/mL for the PsAgNPs and the same was observed between 0.3125 mg/mL and 0.625 mg/mL for the fraction.

The Minimum Inhibitory Concentration for silver nanoparticles and PSH fraction 5 on three organisms is 0.16 mg/mL. The % inhibition of PsAgNPs and fraction 5 on *E. coli, S.typhi,* and *C. albicans* at various concentrations are; at 1.25 mg/mL; 74.75%, 76.98%, and 67.64% for PsAgNPs respectively while those of fraction 5 are 49.66%, 24.72 and 26.8% respectively. At 0.63 mg/mL, the % inhibition of PsAgNPs is 41.95%, 43.01%, and 34.36%, and fraction 5, 25.46%, 22.83%, and 10.58% on the organisms respectively. At 0.31 mg/mL, 13.83%, 31.42%, and 27.57% for PsAgNPs and 22.22%, 19.91%, and 10.73% for the fraction of the organisms accordingly. At the lowest concentration, 0.16 mg/mL, the % inhibition of PsAgNPs are 5.30%, 2.83%, and 30.81% while that of the fraction is 11.41%, 11.035, and 7.18% on the organism respectively. The analysis of the MIC and MBC of the silver nanoparticles and PSH fraction 5 showed a dose-dependent activity which agrees with a previous study by Gopinath et al., 2013.

Silver nanoparticles have been shown to have antimicrobial dose-dependent activity by other researchers (Suganya *et al.*, 2015) which agrees with this study. Comparatively, the %inhibition of PsAgNPs at all concentrations is higher than that of fraction 5 and thus, has more activity than PSH fraction 5 which may be due to their mechanisms of Action especially the generation of radicals, their sizes, and the increased surface area leading to increased bioavailability (Singh *et al*., 2016 & Wang *et al*., 2017). The MIC for both samples on three organisms was in tandem with the MIC of aqueous and ethanol leaf extract of *Pterocarpus santalinoides* on *E. coli and S. typhi* to be 3.125 mg/mL and 1.563 mg/mL respectively. In 2014, Odeh and Tor-Anyinn also determined the minimum inhibitory concentrations of the leaf extract of *Pterocarpus santalinoides* on *E. coli, S. typhi,* and *C. albicans* to be 5 mg/mL for both ethanol and butanol extract and 10 mg/mL for aqueous extract. The MBC/MFC are >1.25 mg/mL, because the organisms showed traces of growth after incubation at all the concentrations.

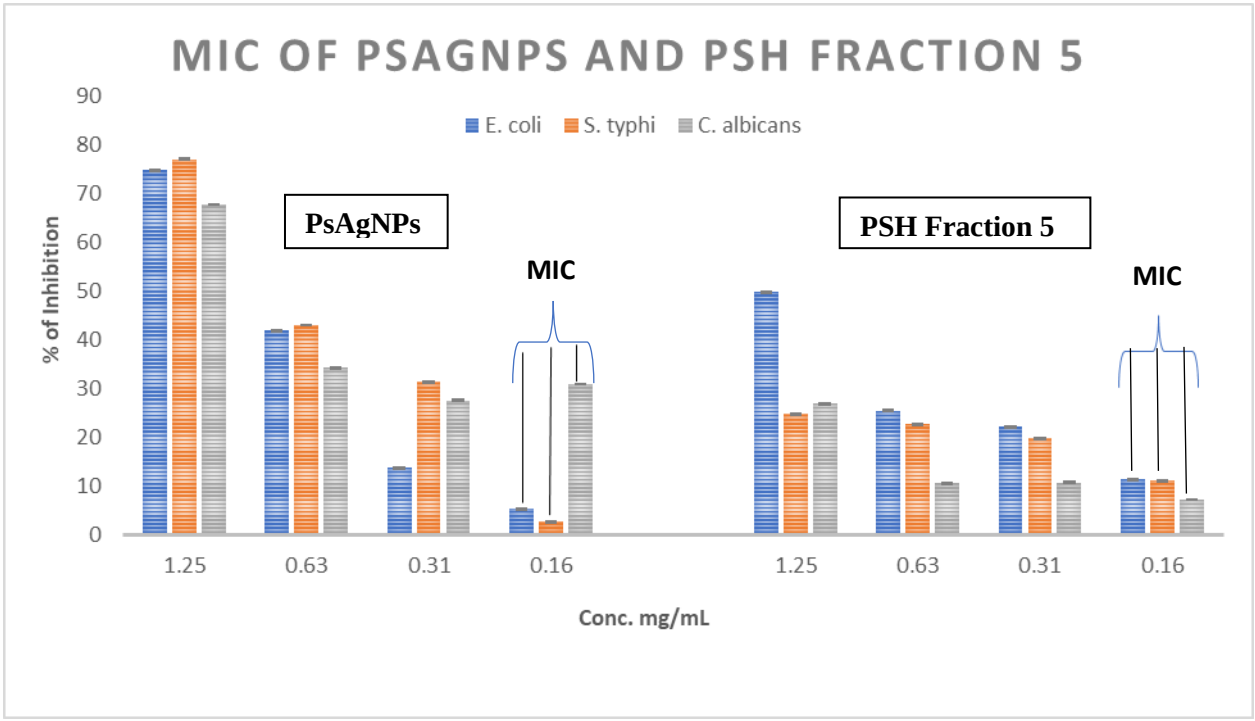

Figure 6: Represents the percentage inhibition and MIC of PsAgNPs and PSH Fraction 5

Conclusion

This novel study has demonstrated and confirmed that the phytofabricated silver nanoparticles of n-hexane fraction of leaf extract of *Pterocarpus santalinoides* L'Herit ex DC has a more significant *in vitro* antimicrobial activity compared to the fraction and may be a potential resistance-free template in future antimicrobial drug discovery.

Conflict of interest

The Authors declare that there is no conflict of interest associated with this study

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