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Original Research Article

Green Synthesis of Silver Nanoparticles Using the Leaf Extract of *Pentaclethra macrophylla*: Characterization and Evaluation of Their Antimicrobial Activities

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ABSRTACT

Antimicrobial resistance has become a pervasive medical issue that necessitates immediate attention. Thus, research for better substitutes has become of great importance. Recent studies of the silver nanoparticles (SNP) have demonstrated that they possess promising activities as a bactericidal agent against both Gram-positive and Gram-negative bacteria and also as a fungicidal agent without toxicity to humans. The aim of this current study was determining the antimicrobial property of green synthesized nanoparticles of *Pentaclethra macrophylla* leaf extract. The plant extract was prepared by decoction and green synthesis was carried out. Characterization of the green synthesized silver nanoparticles were carried out using spectroscopic techniques such as UV-Visible spectrometry, Fourier Transform Infrared spectrometer (FT-IR), and Dynamic Light Scattering technique (DLS). Antimicrobial susceptibility testing was done using the agar dilution method with several clinical isolates of the test microorganisms. The UV- Visble spectrum displayed a peak between 418-430 nm. The FT-IR results showed the presence of phytochemicals in *P. macrophylla*. The size distribution histogram of dynamic light scattering (DLS) indicates that the sizes of these silver nanoparticles range from 34.57-134.70 nm with an average of 131.29 nm. Additionally, the anti-microbial sensitivity testing showed an inhibitory effect of silver nanoparticles. The minimum inhibition concentrations (MIC) were at 8 µg/mLfor *Salmonella typhi*, 9 µg/mL for *Escherichia coli*, 5 µg/mL for *Bacillus subtilis*, 8 µg/mL for *Staphylococcus aureus*, 9 µg/mL for *Candida albicans*, and 10 µg/mL for *Aspergillus niger*. These outcomes denotes the promising antimicrobial activity of silver nanoparticles synthesized using the aqueous leaf extract of *P. macrophylla*.

Keywords: Nanotechnology, silver nanoparticles, *Pentaclethra macrophylla*, green synthesis, Anti-microbial activity

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Introduction

Microbial diseases have caused severe illness and death worldwide, accounting for roughly 15 million death cases annually across the globe.[1] There are 4 major types of microbes which include: bacteria, viruses, fungi, and protozoa. These microbes or microorganisms are killed or inhibited by the use of an antimicrobial agent, but in recent times, the successes of these antimicrobial agents are beginning to reduce due to the resistance of the microbes.[2] Resistance of a microbe to an antimicrobial agent can occur by several mechanisms which could be an inherent property of the microorganism or acquired.[3] Several reckless practices in human including therapeutic errors have amplified antimicrobial resistance.[4] Therefore, the problem of resistance to antimicrobial drug is now a major public health issue that has rendered existing antimicrobials less effective or even ineffective. This incidence has raised major therapeutic issues in populations such as serious illnesses, prolonged hospital stay, increase in healthcare costs, and treatment failures.[5]

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Furthermore, current therapies do little to prevent adverse effects. Thus, a continuous search for new and if possible safer drugs with higher efficacy especially from plant sources is necessary.[6]

In a bid to tackle this major public health issue, many have resorted to the use of herbal medicine. Recently, the use of herbal medicines as antimicrobial agents has gained more acceptance in many countries of the world.[7] *Pentaclethra macrophylla* is one of the herbs used for its antimicrobial activity.

Pentaclethra macrophylla is relatively large with average height and belongs to the family Fabaceae.[8] It is the sole member of the genus (pentaclethra) occurring naturally in the humid lowlands of West Africa. The common names in English is 'oil bean tree', in Igbo 'Ugba' and 'Ukana' in Efik.[9] This leguminous woody plant is used traditionally to treat numerous ailments. Extracts from the leaf, stem bark, seed, and fruit pulp have been reported to exhibit anti-inflammatory, anti-helminthic, abortifacient, and analgesic properties.[10] Numerous studies on the nutritional benefits and physicochemical properties of *P. macrophylla* have also been conducted which revealed the presence of proteins, amino acids, fat, riboflavin, fiber, carbohydrates, thiamine, and niacin. [11–15] It also contains some phytochemicals such as flavonoids, saponins, alkaloids, tannins and glycosides.[16]

A nanoparticle is a tiny particle that varies in size from 1 to 100 nanometers. Due to their small size, nanoparticles have a larger surface area compared to other macro-sized materials, which opens up numerous advancements and innovations in the areas of biomedicine and bio-nanotechnology.[17] The application of plants for nanoparticle synthesis can be of more benefit than other biological and chemical processes.[18] It can be scaled up for the large-scale synthesis of nanoparticles. The phytoextracts are rich in biologically complex molecules such as enzymes/proteins, amino acids, polysaccharides,

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flavonoids and vitamins which are well known to prevent diseases.[19] Furthermore, they also act as capping and reducing agents as well as surface enhancers in drug delivery systems.[20] This work is therefore aimed at evaluating the antimicrobial action of green synthesized silver nanoparticles using *P. macrophylla* leaf

Materials and methods

Plant collection and identification

This research work used fresh leaves of Pentaclethra macrophylla collected in December 2020 from Madonna University, Elele, located along Owerri-Port-Hacourt road in Rivers state, Nigeria. The plant was authenticated in the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria Nsukka, with the Voucher number UNN/11775. After collection, the leaves were washed and dried under shade for fourteen days. Following this, a manual grinder was used to reduce the leaves to powder. The powdered leaves were then weighed using an electronic digital weighing balance (model 12051 by Sri Krishna, New Delhi) and stored in an air-tight container.

Preparation of plant extract

The method of extraction used was the decoction method. [21] A 20 g quantity of the powdered leaves of *Pentaclethra macrophlla* samples was weighed and mixed with 400 mL of distilled water and placed in a water bath to boil to 100ºC. At 100ºC, it was still left in the water bath for 30 minutes. The mixture was left to cool and filtered with a funnel plunged with cotton wool. The mixture was further filtered with filter paper to obtain pure filtrate.

Green synthesis of silver nano particles

Silver nitrate (0.169 g) was dissolved in 10 mL of distilled water in a 500 mL beaker. The solution was then transferred to a 1L volumetric flask and diluted to a volume of 1L. This process was carried out to create a 1 mM solution of AgNO3. The silver nitrate solution was used as the source of silver ion for the reaction. 750 mL of the silver nitrate solution was combined simultaneously with 150 mL of plant extract, and the resulting mixture was stored in the dark to prevent any photochemical reaction involving the silver nitrate. The color change of the reaction was closely monitored, and after 1 hour, a cloudy solution was observed, indicating the formation of silver nanoparticles. The obtained solution was centrifuged using a Spectrafuge centrifuge machine (model 6C by Labnet in United States) for 30 minutes at 5000 rpm. The pellet containing silver nanoparticle was dispersed in sterile distilled water and dried in an oven at 115ºC for 20 minutes to evaporate the solvent.

Characterization of silver nanoparticles with UV-visible spectral analysis

The silver nano particles were scanned in the wavelength ranging from 300-800nm using the UV/Vis spectrophotometer (JENWAY 6705, United Kingdom) at intervals of 30 mins for 24 hours.

Fourier-transform infrared spectrophotometric analysis of the silver nanoparticles

FTIR analysis of silver nano particles was also carried out using FTIR-8400S spectrophotometer system (Shimadzu, Japan). Potassium bromide (KBr) pellets were used in formulating the samples for the analysis. The samples and the KBr pellets were mixed thoroughly in a mortar, and then transferred to the spectrophotometer for analysis at a frequency region ranging from of 4000-500 cm^{-1}. The characteristic peaks were detected and were used to deduce functional groups in the extracts. The peak values were recorded.

Analysis of silver nanoparticles using dynamic light scattering

Particle size distribution was determined by Dynamic Light Scattering (DLS) using a Zetasizer nano- ZS instrument (Malvern instruments Ltd, UK) and the various particle size analyzed were recorded.

Antimicrobial assay

This was done using agar dilution method with a stock solution of 100 µg/mL of the nanoparticle. The aqueous plant extract of *P. macrophylla* was reconstituted in DMSO in the ratio of 1:1. The control drugs used were ciprofloxacin and fluconazole. Thirteen (13) agar plates were prepared in Petri dishes and appropriately labeled. Calculated volumes of the silver nanoparticle stock solution were added to agar solution in Petri dishes while the agar was still in liquid form to obtain ten (10) concentrations (ranging in integral values from 1 µg/mL to10 µg/mL) in a final volume of 20 mL of the mixture. The plates were shaken and then allowed to solidify. Each plate was divided into six portions with one for each of the six micro-organisms. The test organisms were inoculated by streaking the surface of the plate with the swap. The plates were incubated at 37ºC for 24 hours. The plant extract was similarly tested against the micro-organisms (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*). Mueller Hinton agar was used for bacteria while Sabouraud agar was for fungi.

Statistical analysis

Data were evaluated using EXCEL software.

Result and Discussion

In this present study, the formation of silver nanoparticles during the reduction process which occurs through the mixture of plant extract of *P. macrophylla* into the beaker with an aqueous solution of silver nitrate is indicated by the color change displayed by the solution from clear brown to cloudy reddish brown (shown in Figure 1) in 1 hour. This is due to the effect of surface plasmon vibrations excitation occurring in silver nanoparticles.[22] Figure 2 gives an extensive details of the ultra violet-visible spectrum of silver nanoparticles. Both the UV-Vis spectra and visual observation confirmed that the silver nanoparticles formation gradually took place over 24 hours. The reaction solution showed no obvious reaction peak until around 7 hours. The achieved curve has a bell-shape, which indicates that the Surface Plasmon Resonance (SPR) is around 420 nm. Again, the UV–Vis absorption spectra of the reaction solution established the formation of silver nanoparticles from silver ions at 418-430 nm. All these observations are supported by the research of Njagi [23] who discovered that this band corresponds to the absorption by colloidal silver nanoparticles in the region (400-450nm) as a result of excitation of surface plasmon vibration. Also, Ezealisiji, [24] recorded the absorption Surface Plasmon peak at the region of 420nm after 8 hours which also corresponds to this band. The broad band observed in the UV-Vis absorption spectra is attributed to the presence of various metabolites from *P. macrophylla* extract within the reactive solution, which were detected within the spectrophotometric range of the investigation.[25] The detailed UV-Vis spectra suggested that bio-reduction was successfully carried out using *P. macrophylla* leaf extract as a reducing agent. Both the UV-Vis spectra and visual observation indicated that the silver nanoparticles formation occurred gradually. The double role of the plant extract as a reducing and capping agent and the presence of some functional groups was confirmed by FTIR analysis of silver nanoparticles. A broad band between 3441-3857 cm^{-1} is attributed to OH stretching vibrations. The band at 1735 cm^{-1} corresponds to C=O stretching, while the peak at 2067 cm^{-1} is associated with the alkyne group present in the phytoconstituents of the extract (as shown in Table 1). The observed peaks at 1041 cm^{-1} indicate COH linkages or COOH bonds. These peaks are primarily attributed to the flavonoids and tannins abundantly present in the plant extract, which have antioxidant properties. The flavonoids in the leaf extract are potent agents, likely contributing to the formation of silver nanoparticles through the reduction of silver nitrate. According to Ezealisiji, [24] presence of steroid, alkaloid, polyphenol, glycosides and flavonoids were recorded in FT-IR analysis using green synthesized silver nanoparticles of *Annona muricata*. Another study also provides evidence of the association of water-soluble flavonoids in the reduction of metal ions using plant extracts.[26]

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Table 1: FTIR Spectrum of the silver nanoparticles obtained from *P macrophylla* leaf extract

Peak no	Absorption frequency (cm^{-1})	Area	Intensity	Possible bond	Possible functional group
1	671.25	1.366	M	C-O	Alcohol, ether, carboxylic acid
2	748.41	0.968	W	ring	ortho, meta, para
3	1041.6	7.965	M	C-O	Alcohol, ether, carboxylic acid
4	1126.47	0.258	M	C-O	Alcohol, ether, carboxylic acid
5	1373.36	4.846	M	C-H	Alkane
6	1465.95	0.294	W	C-H	Alkane
7	1512.24	0.938	W	N-O	Nitro compound
8	1627.97	1.313	M	C=C	Alkene
9	1735.99	0.821	S	C=O	Aldehyde, ketone
10	2067.76	0.419	M	C≡C	Alkyne
11	2399.53	0.138	W	C≡N	Nitrile
12	2947.33	8.613	S	C-H	Alkane
13	3441.12	8.92	S	O-H	Alcohol, carboxylic acid
14	3626.29	0.458	S	O-H	Alcohol, carboxylic acid
15	3695.73	2.175	S	O-H	Alcohol, carboxylic acid
16	3857.76	0.991	W	O-H	Alcohol, carboxylic acid

Table 1 shows possible functional groups at various frequencies in green synthesized silver nanoparticles using leaves extract of *P. macrophylla.* The peaks were observed at 671.25, 748.41, 1041.6, 1126.47, 1373.36, 1465.95, 1512.24, 1627.97, 1735.99, 2067.76, 2399.53, 2947.33, 3441.12, 3626.29, 3695.73 and 3857.76 cm^{-1}. The peaks were analyzed and attributed to corresponding functional groups which revealed the presence of flavonoids, alkaloids, polyphenols, tannins, glycosides and saponin. Consequently, it indicates the double role of *P. macrophylla* both as green reducing agent and stabilizing agent. (S-Strong , M-Medium, W-Weak)

A B

Figure 1: shows the result of color change after mixing $AgN0_3$ and plant extract of *P. macrophylla*. It was observed that the mixture changed from clear brown (A) to cloudy reddish brown (B) after 1 hour

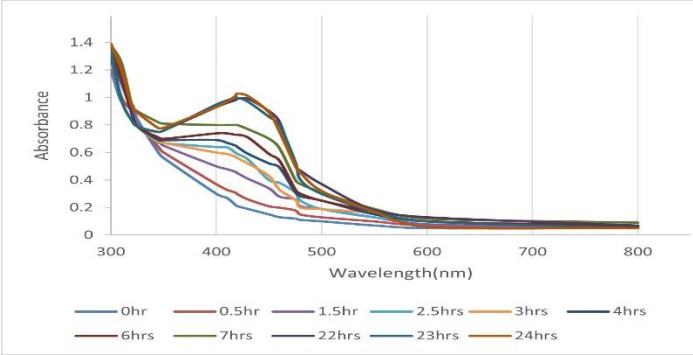

Figure 2: UV-Vis spectrum of the silver nanoparticles at intervals of 30 min

FT-IR spectral analysis

The particle size distribution analysis indicates that the size of these silver nanoparticles ranges between 34.57-134.70 nm in diameter with an average of 131.29 nm (as shown in Table 2). Some distribution at the lower range of particle size indicates that the synthesized particles are also in range of particle size of a nanoparticle. Nanoparticles above 100 nm in this study may be due to particle aggregation. An example is a study conducted by Anandalakshmi,[27] which shows the size of DLS size distribution ranged from10-150 nm. Another cause for the particle size to go above 100 nm could be the extended storage duration during the transportation of samples for study. Figure 3 displays the particle size distribution of silver nanoparticles synthesized using *P. macrophylla* aqueous leaf extract.

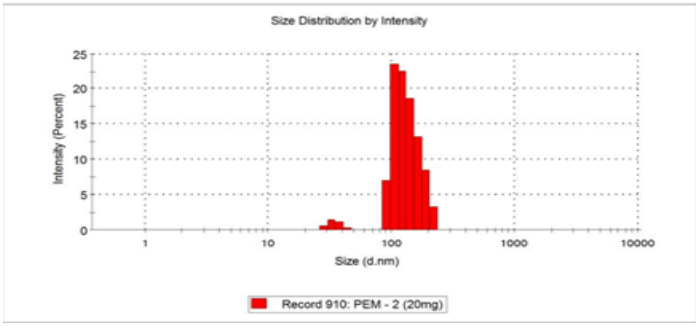

Figure 3: Graphical result of particle size distribution

Table 2: Varied size distribution of synthesized silver nanoparticles

Peaks	Size (nm)	Intensity (%)
Peak 1	134.70	96.6
Peak 2	34.57	3.4
Peak 3	0.00	0.00

z-average (d, nm) 131.29

The minimum inhibitory concentration result (Table 3) showed the minimum inhibition concentration (MIC) of silver nanoparticles at 8ug/mL for *S. typhi*, 9ug/mL for *E. coli*, 5ug/mL for *B. subtilis*, 8ug/mL for *S.aureus*, 9ug/mL for *C.albicans*, and 10ug/mL for *A.niger*. In general, *E. coli* was the most sensitive organism in the antimicrobial susceptibility testing followed by *S.aureus*. This demonstrates the green synthesized silver nanoparticles as effectives for both Gram-positive and Gram-negative bacteria. The most resistant organism in the antimicrobial susceptibility testing was *C.albican*s. In MIC assay, *B. subtilis* was the most sensitive organism and *A.niger* was the most resistant organism. The results show that green synthesized silver nanoparticle of *P. macrophylla* exhibit better anti-bacterial activity than anti-fungal activity.

Table 3: MIC obtained by agar dilution.

Micro organism	*S. typhi*	*E. coli*	*B. subtilis*	*S. aureus*	*C. albicans*	*A. niger*
MIC (µg/mL)	8	9	5	8	9	10
Ciprofloxacin (µg/mL)	10	10	10	10	-	-
Fluconazole (µg/mL)	-	-	-	-	10	10

MIC- Minimum inhibitory concentration

Conclusion

Green synthesis has immense advantages such as its low cost, low toxicity and capacity to produce silver nanoparticles at an ambient temperature. Silver nanoparticle synthesized by green synthesis with *P. macrophylla* leaf has potent antimicrobial activity against some infective bacteria and fungi known for their resistance to some antimicrobial agents. These nanoparticles meet several criteria. Thus, making them potential alternative to current antimicrobial agents after stable dosage forms have been established.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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