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## In-Vitro Evaluation of Antimicrobial Activity of Silver Nanoparticles of *Plectranthus neochilus* Leaf Extract

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### Abstract

Infectious diseases have continued to be problematic due to globalization, an increasing number of patients affected, increasing cost of treating bacterial infections, adverse and side effects associated with antibacterial drugs, as well as resistance associated with antibacterial drugs. The study is aimed at assessing the antimicrobial activity of silver nanoparticles (AgNPs) synthesized using the aqueous extract of *Plectranthus neochilus* Schltr leaf. Silver nanoparticles were synthesized by mixing the aqueous extract prepared from leaves of *P. neochilus* and 1mM silver nitrate solution under controlled conditions. The silver nanoparticles were then analyzed through spectroscopic methods (Fourier transform infrared (FTIR) and Ultraviolet (UV)-visible spectroscopy) as well as physical characterization (particle size distribution) and antimicrobial activity. Results from FTIR analysis showed that silver nanoparticles of *P. neochilus* leaf contain flavonoids. In the UV analysis, absorbance spectra of the AgNPs of *P. neochilus* leaf extract indicated higher peaks at longer time intervals. Particle size distribution analysis of the AgNPs of *P. neochilus* leaf extract had a good size distribution ranging from 13.88 - 82.37nm. The antimicrobial assay showed minimum inhibitory concentrations of *Staphylococcus aureus* and *Escherichia coli* being 10 and 9µg/mL, respectively, and the highest zones of inhibition for the AgNPs at the concentrations of 50 and 100 µg ranged from 6 to 8 mm. There were higher zone diameters with the gram-positive organisms (*S. aureus*, 8 mm; *B. subtilis*, 7mm) relative to the gram-negative organisms (*P. aeruginosa*, 7 mm; *E. coli*, 7 mm). AgNPs synthesized from *P. neochilus* leaf extract were stable with good size distribution, thereby increasing the bioavailability of the particle, and may be useful in the treatment of gram-negative and gram-positive organisms as well as fungal organisms.

**Key words:** Silver nanoparticles, antimicrobial activity, Synthesis, *Plectranthus neochilus* Schltr.

### Introduction

*Plectranthus neochilus* Schltr is an important member of the *Plectranthus* genus's therapeutic species. The plant is used to treat dyspepsia and hepatic insufficiency. Its fresh leaves are used medicinally as an aqueous extract or infusion; they have a distinct smell (Duarte, 2007). The plant has other

traditional applications such as the use of vermicide, antiseptic, use for digestive disturbances (as a purgative and for nausea), pain relief, oedema, skin infections, and respiratory ailments (Borges et al., 2016).

The major components in the essential oils present in *P. neochilus* are terpenoids and polyphenolic compounds, and

phytosterols (Pereria et al., 2015; Matias et al., 2019). *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* are among the bacteria against which the essential oil of *P. neochilus* is thought to have antibacterial activities. It also exhibits anti-schistosomal and insecticidal properties. (Crevelin et al., 2015). Activities have also been reported against *Klebsiella pneumoniae*, *Salmonella typhi*, and *Candida albicans* (Itelima, 2017), and also some cariogenic bacteria (Dias et al., 2017). Other biological activities reported for *P. neochilus* include cytotoxic effect, anti-inflammatory and analgesic effects (Borges et al., 2016), blood glucose levels reduction of the alloxan-induced diabetic rats (Itelima, 2017), antioxidant properties, and antifungal activity (Aguiar et al., 2018).

In all fields of science, including the medical sciences, nanotechnology has emerged as one of the highest significant technological advancements. Nanoparticles (NPs) are a wide range of materials with dimensions below 100 nm, which can be used in various applications due to their multiple properties (Khan et al., 2019). NPs can be found amongst others as nano-capsules, nano-emulsions, metallic NPs, ceramic NPs, and polymer NPs (Castillo-Henríguez et al., 2020). The outstanding properties of metallic NPs (high dispersion in solution, high surface-to-volume ratio, surface potential, surface energy, crystallinity, catalytic effect, magnetic effect) have resulted in the development of different methods for their synthesis. In particular, gold (Au) and silver (Ag) NPs prepared from plant extracts have elicited much research interest in the attempt to develop suitable antimicrobial agents. This interest is premised on the fact that silver being a nontoxic and safe inorganic antibacterial agent capable of killing close to 650 types of pathogenic microorganisms. Thus, the increasing interest in silver nanoparticles is because of the antimicrobial properties that they display (Jeong et al., 2005; Choi et al., 2008).

The purpose of this study was to evaluate the antimicrobial activity of silver nanoparticles synthesized using the aqueous extract of *Plectranthus neochilus* Schltr leaf. By synthesizing and assessing the silver nanoparticle synthesized using the aqueous extract of *Plectranthus neochilus* Schltr leaf, insights would be gained into their potential use in the treatment of some microbial infections, considering the antibiotic resistance, the cost of treatment of infections, and the eco-friendly nature of the product. It is also important to harness available resources to supplement health care.

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## Materials and methods

### Samples and reagents

Fresh *P. neochilus* leaves, silver nitrate (analytical grade), distilled water, Bacterial and fungal isolates (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Staphylococcus aureus*, *Salmonella typhi*, *Aspergillus niger*) procured from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Madonna University, Nigeria. Nutrient agar plates, Mueller Hinton medium, gentamycin and ofloxacin, ciprofloxacin, fluconazole, 50% Dimethyl sulfoxide (DMSO), and PLN-8 were used.

### Plant extract preparation

*P. neochilus* leaves were harvested from the Lamingo golf course location in July 2020, in Jos North Local Government Area of Plateau State, Nigeria. After being thoroughly washed with clean water, the fresh plant material was separated from the short stems and chopped into little pieces and dried in a drying cabinet (Leec, Nottingham, UK), maintained at a temperature range between 35 - 40 °C. A 300 ml of distilled water and 10 g of *Plectranthus neochilus* Schltr leaf powder were mixed, and heated at 100 °C for 30 minutes. The extraction mixture was cooled and filtered with cotton cloth and Whatman No. 1 filter paper.

### Preparation of silver nanoparticles

A solution of 1 mM silver nitrate in double-distilled water was used. A 5:1 ratio was used to mix the leaf extract with the silver nitrate solution, and heated at 100 °C for 1 hour in a water bath. Reddish-brown coloration formed within one hour signified the production of silver nanoparticles. The resulting suspension was centrifuged using *D-78532*, Hettich Zentrifugen, (Germany) at 6,000 rpm for 30 minutes. Sample tubes containing the nanoparticles were rinsed with distilled water to remove silver ions. To obtain the silver nanoparticle crystals, the suspension was heated to dryness.

### Ultraviolet-visible Spectroscopy (UV-Vis)

The UV-VIS spectrophotometer (*Jenway 6705*) was used to scan the AgNPs' absorption spectra every 30 minutes for two hours, with a wavelength ranging between 300–800 nm.

### Determination of the Particle Size

Dynamic Light Scattering (DLS) was used to determine average particle size, distribution of particle size by volume and intensity, and polydispersity index (PDI) of the silver nanoparticles utilizing a zetasizer nano-ZS instrument (*Nano ZS7.01*, Malvern Instruments Limited, UK).

### Fourier Transformed Infrared Spectroscopy (FTIR)

The Shimadzu FTIR spectrophotometer (*FTIR 8400S*) was used for the Fourier Transform Infrared spectroscopy (FT-IR) investigations.

### Antimicrobial Analysis

The broth dilution and standard disc diffusion methods were utilized to investigate the antimicrobial activity of silver nanoparticles. Following the seeding of the test microorganisms (*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*) in the nutrient agar plates, 6- 5 mm paper discs were saturated with silver nitrate solution as

well as 5 and 10 µg/mL AgNPs aqueous solution for the standard disc diffusion method. The standard controls were gentamycin and ofloxacin. After setting the paper discs on the solidified agar plates, the bacterium and yeast cultures were incubated for 24 hours and 48 hours, respectively, at 37 °C, preceded by inhibitory zone diameter measurement.

For the broth dilution method, a series of tubes were set using the Mueller-Hinton medium. The antimicrobial agents were introduced in serial concentrations ranging from 1 to 10 µg/mL. A standardized suspension of test organisms (*Aspergillus niger*, *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*) were added to the tubes for inoculation. Four (4) control solutions (ciprofloxacin, fluconazole, 50% DMSO, and PLN-8:50% DMSO in 19 ml) were used for 24 hours and 48 hours, respectively. The test tubes containing the bacteria and yeast cultures were incubated at 37 °C and the MIC was determined.

## RESULTS

### UV-visible Spectroscopy

The color of the silver nitrate solution and *P. neochilus* aqueous extract changed from pale yellow to reddish brown. Figure 1 displays the combined UV-visible spectroscopy graph of the reaction from zero to 4 hours.

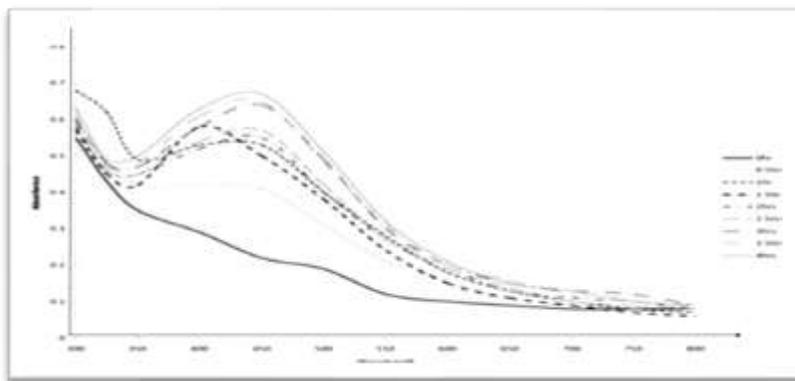


Figure 1: UV absorption spectrum at 4 hours and the combined charts

Figure 2 shows the synthesized AgNPs of *Plectranthus neochilus*'s particle size properties. *Plectranthus neochilus* synthesized AgNPs were found to be of good quality, as

evidenced by their Z average of 75.9 and polydispersity index (PDI) of 0.197 and good size distribution ranging from 13.88 - 82.37nm

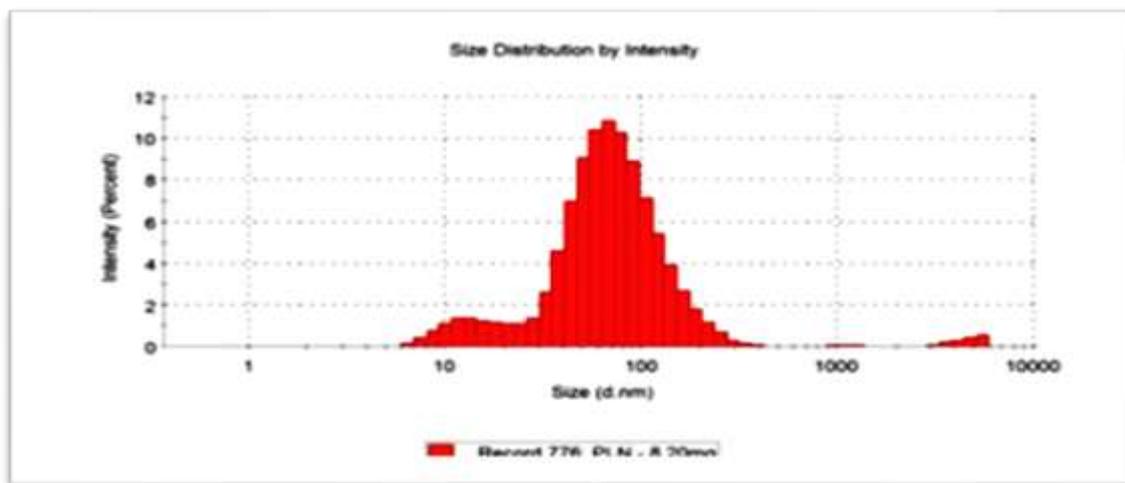


Figure 2: AgNPs of *Plectranthus neochilus*'s particle size properties

**FTIR Analysis**

The shows activities within the specific absorption frequency of about 550 to 3900. Within this range, nineteen (19) peaks were observed and the intensities for the representative functional groupings in the sample ranged from weak (thiols), medium (alkane, alkene, alcohol) to strong (alcohol, alkene, carboxylic, ether, halo compounds sulfoxide). Ether, carboxylic acid, alkene, alcohol and sulfoxide with the absorption frequencies of 1134.18 to 1273.06, 1759.14, 1635.69, 3518.28 and 3711.17, 1033.88 respectively showed strong intensities.

**Antimicrobial Analysis**

Table 1 and Table 2 show the zones of inhibition and the minimum inhibitory concentrations for the microbial organisms used in the study. Table 1 shows that the highest zone of inhibition for the AgNPs of *P. neochilus* at the concentration of 100 µg were obtained mostly with the Gram-positive organisms (*S. aureus* = 8 mm; *B. subtilis* = 7mm) relative to the Gram-negative organisms (*P. aeruginosa* = 7 mm; *E. coli* = 7 mm). No inhibition was observed with silver nitrate, gentamicin and ofloxacin, which were used as controls. The specific minimum inhibitory concentrations for particular organisms are as recorded in Table 3.

Table 1: Various inhibition zone diameters

Treatments	<i>Staphylococcus aureus</i> (Gram-positive)	<i>Pseudomonas aeruginosa</i> (Gram-negative)	<i>Bacillus subtilis</i> (Gram-positive)	<i>Escherichia coli</i> (Gram-negative)	<i>Candida albicans</i> (Fungus)
AgNPs100 µg	8	7	7	7	0
AgNPs50 µg	0	6	7	0	0
Silver nitrate	6	0	0	6	0
Gentamycin	20	10	12	14	0
Ofloxacin	24	0	22	20	0

Values are zones of inhibition in millimeters (mm)

Table 2: Table of Minimum Inhibitory Concentration

Concentration (µg/ml)	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Aspergillusniger</i>
10	-	-	-	-	-	-
9	-	-	-	+	-	+
8	-	+	-	+	-	+
7	+	+	-	+	-	+
6	+	+	-	+	+	+
5	+	+	-	+	+	+
4	+	+	+	+	+	+
3	+	+	+	+	+	+
2	+	+	+	+	+	+
1	+	+	+	+	+	+
PLN-8 (1 IN 19ML)	+	+	+	+	+	+
50% diluted DMSO	+	+	+	+	+	+
Cyprofloxacin 10 µg/ml	-	-	-	-	+	+
Fluconazole 10µg/ml	+	+	+	+	-	-

+ (Growth); - (No Growth); DMSO (Dimethylsulphoxide); PLN-8 (1 IN 19ML), *P. neochilus* extract 1 in 19 mL

Table 3: Various Minimum inhibitory concentrations

Micro organisms	Concentration(µg/ml)
<i>Salmonella typhi</i>	8
<i>Escherichia coli</i>	9
<i>Bacillus subtilis</i>	5
<i>Staphylococcus aureus</i>	10
<i>Candida albicans</i>	7
<i>Aspergillus niger</i>	10
PLN-8 (1 IN 19ML)	Control
50% diluted DMSO	Control
Cyprofloxacin 10ug/ml	Control
Fluconazole 10ug/ml	Control

## Discussion

Additional physico-chemical properties, such as color change, are conferred on the nanoparticle interface by the adsorption of plant moieties onto the nanocrystals. As a result, the color of the silver nitrate solution and *P. neochilus* aqueous extract changed from pale yellow to reddish brown in a single step reaction. This change in color demonstrated how the nanoparticle was synthesized from the plant extract (Ezealisiji et al., 2017). The color may be as a result of surface plasmon vibration, an optical characteristic specific to noble metals (Ibrahim, 2015) that results from free surface electrons moving when they align with both the wavelength of irradiation light and in resonance.

Using Fourier transform infrared and UV-visible spectroscopy, the formation of AgNPs was further verified. The maximum absorbance was obtained between 400 and 450nm, with peak around 420 nm over 1.5 to four hours. Literature sources report that absorbance at this range is a

property of silver nanoparticles (Rautela et al., 2019). Figures 1 show an increase in absorbance as the extract and silver nitrate are incubated for longer periods of time (from baseline to four hours).

FTIR was used to identify the main functional groups in the aqueous extract and their possible role in the production and stabilization of AgNPs. The C–Br and C–Cl stretches were present at lower wavelengths of 500 cm<sup>-1</sup>, which indicates the existence of alkyl halides, also known as halo compounds. At 3318 cm<sup>-1</sup>, an O–H stretch related to alcohol or phenol was also noted. These are thought to be hydroxyl functional groups of flavonoids, polyphenols, and alcohols' –OH stretching vibration, as reported by Ezealisiji (2017). Different functional groups of the plant extract were likely involved in the formation of AgNPs, and they play a significant role in the capping and stabilization, which may help to prevent AgNPs from agglomerating, as indicated by the relative shift of these peak positions and intensity distribution in the spectra (Kavaz et al., 2018). Studies show that phytochemical components of *Plectranthus spp.* are mainly composed of mono- and sesquiterpenes and phytosterols in addition to polyphenolic compounds (Pereira et al., 2015; Matias et al., 2019).

The distribution of particle size analysis showed that AgNPs of *P. neochilus* leaf had a good size distribution ranging from 13.88 - 82.37nm and a PDI of 0.197. This increases antibacterial activities because of the larger surface area of the smaller nanoparticles (Erci et al., 2018; Vanlalveni, 2021).

Silver nanoparticles (AgNPs) are thought to be one of the most effective metal nanoparticles for antibacterial purposes, since they can effectively combat a wide variety of bacteria, fungi, and protozoa. Prokaryotes are more

susceptible to the toxicity of silver than are eukaryotic cells. The combined effect of plant extracts and nanoparticles can be considered as a substitute for traditional antimicrobial treatments (Nayak et al., 2019).

The antibacterial evaluation showed the various organisms susceptible to the synthesized AgNPs and their various minimum inhibitory concentration (MICs). The MICs of *Staphylococcus aureus* and *Escherichia coli* are 10 and 9 µg/mL. A closer look at Table 1 highest zones of inhibition for the AgNPs at the concentrations of 50 and 100 µg ranged from 6 to 8 mm. However, of note is the fact that the results showed that there were higher zone diameters with the Gram-positive organisms (*S. aureus* = 8 mm; *B. subtilis*, 7mm) relative to the Gram-negative organisms (*P. aeruginosa*, 7 mm; *E. coli*, 7 mm).

There has been no consensus as to what actually is the reactive species responsible for the activity. Thus, opinions have been divided as to whether it is the AgNPs or the released Ag<sup>+</sup> that is responsible. However, several pathways have been proposed for the bactericidal activity of AgNPs. These have been listed (Castillo-Henríquez et al., 2020, Vanlaveni et al., 2021) to include the generation of reactive oxygen species, free radicals derived from the surface of AgNPs, silver ion stress, coating agents and various interactions with the bacterial cell. Acting together, these processes are thought to cause depletion of intracellular ATP level and damage in respiratory enzymes, interruption in electron transport systems, cell wall leakage, interaction with enzymes and DNA bases as well as ribosomal destabilization. It is not clear why there appeared to be relative differences in sensitivities between the Gram-positive and Gram-negative bacteria used in the current study. However, it may be possible that these differences reflect the differences in the cell wall structures between the two. This is more so as the postulated mechanics of activity have to do with the ease of uptake of generated reactive oxygen species by the cells. Thus, the difficulties that are likely to be encountered by the reactive species in penetrating the Gram-negative bacterial cell may be responsible for the perceived lower sensitivities in these organisms. Several reports show that Gram-positive bacteria, such as *S. aureus*, due to their thick cell wall of peptidoglycan layer are less susceptible to AgNPs than Gram-negative bacteria, such as *E. coli*, with cell wall consisting of lipopolysaccharides at the exterior, followed underneath by layer of peptidoglycan (Erci et al., 2018). However, this is not the case everywhere (Erci et al., 2018, Tormena et al., 2020) and especially as reflected in this study wherein the Gram-positive bacteria showed greater

sensitivity. Thus, it could be possible that the lipopolysaccharides in Gram-negative bacteria might have acted as a shield against some AgNPs and also the synergetic effect of AgNPs and biomolecules that act as capping might have altered the mode of interaction of the AgNPs with the cell wall. Moreover, it is also thought that the electrostatic repulsion between the nanoparticles and Gram negative bacteria hinders particle attachment and penetration into the cell (Erci et al., 2018) and thus the lower sensitivity.

## CONCLUSION

The study demonstrated that the silver nanoparticles produced from the *P. neochilus* Schltr leaves' aqueous extract were stable and with good size distribution, which will help increase the bioavailability of the particles if appropriately formulated. The aqueous extract of *Plectranthus neochilus* Schltr leaves was used to create silver nanoparticles, which demonstrated a reasonable level of antibacterial activity. These nanoparticles can be applied to the treatment of fungal organisms, Gram-negative and Gram-positive pathogens.

## Conflict of Interest

No conflict of interest

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