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Green synthesis, characterization and preliminary antimicrobial study of silver nanoparticles from *Parquetina nigrescens* (Afzel)

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Abstract

The recent geometrical increase in the applications of nanosized materials in almost all areas of human endeavors has continued to draw keen interest of scientists to the investigations on nanoscience and nanotechnology. To meet the increasing demands for commercial nanoparticles, green synthesis method, which is simple and eco-friendly is the widely preferred option of production of nanoparticles at laboratory and industrial scales. This study reports the synthesis of silver nanoparticles using *Parquetina nigrescens* (Afzel) extract and its characterization using ultra violet visible spectroscopy, Fourier Transform Infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Four (4ml) of the aqueous bark extract was mixed with 36ml of silver nitrate and shaken continuously for 1hour until a deep brown colour was observed. The electron microscopic study carried out on the synthesized AgNPs shows that the synthesised AgNPs were spherical, with sizes ranging from 0.092 to 0.375 µm as shown by SEM analysis. The UV-Visible spectroscopy of the synthesized *Parquetina nigrescens* silver nanoparticles shows maximum absorbance at 436.92nm. The FTIR spectrum shows strong peaks at 3334.23, 2872.60, 2095.63 and 1625.94 cm-1. The synthesised AgNPs shows inhibition against the growth of *Pseudomonas*, *Aspergillus flavus* (76%), *Aspergillus niger* (81.17%) and *Aspergillus fumigatus* (44.87%) fungi . This study has shown that the green synthesised AgNPs from *parquetina nigrescens* has a strong antimicrobial activity and potential biomedical applications.

1. Introduction

The utilization of nanotechnology for constructing nanoscale products in research and development divisions is growing over the years. Nanotechnology has gained huge attention over time with its fundamental component being nanoparticles [1]. Among the several noble metal nanoparticles, silver nanoparticles (AgNPs) have attracted much attentions due to their special properties, which include favourable electrical conductivity, chemical stability, catalytic and antibacterial activity [2]. The green synthesis of AgNPs has been accomplished using plants, microorgan-

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isms and other biopolymers. Biologically synthesized silver nanoparticles with antimicrobial, antioxidant, and anticancer properties are possible through the collaboration of different natural science sectors. These nanotechnologies may provide novel resources for the evaluation and development of newer, safer, and effective drug formulations [3]. In Nigeria, as in most developing countries, 80% of the population still use traditional (herbal) medicines for treatment of wide range of diseases including gastro-intestinal disorders. *Parquetina nigrescens* is one of the herbs commonly used for this purpose in South Western part of Nigeria. *P. nigrescens* is an herbaceous plant belonging to the family *Asclepiadaceae*. It is usually woody at the base and measures between 7 and 8 m in length. The plant is commonly found growing on ant-hills across the African regions specifically from Senegal to Nigeria, and over the Congo basin down to south tropical Africa [4]. The beneficial properties and medicinal potentials of *P. nigrescens* have been documented in literature. For instance, it has been shown to ameliorate haemorrhagic anemia [5]. The analgesic, anti-inflammatory and antipyretic effects of *P. nigrescens* leaf extract have been documented [6]. Currently, the biosynthesis of AgNPs with *P. nigrescens* is being exploited because there is dearth of reports on the green synthesis and characterization of silver nanoparticles from the plant as well as the antimicrobial study, hence, the drive for this study.

Fig. 1: A typical *Parquetina Nigrescens* plant

2. Materials and Methods

2.1 Sample Collection and Preparation

The leaf of *P. nigrescens* plant was collected locally at a farm at Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria farm (8°10′ 19″ N; 4°16′ 13″ E) and were identified at the Department of Pure and Applied Biology, LAUTECH, Ogbomoso. The plant was rinsed thoroughly with distilled water to remove all dusts and unwanted visible particles, after which they were cut into small pieces and air dried at room temperature. It was later pulverized into powdery form.

2.2 Green synthesis and Characterization of AgNPs

The pulverized plant (5 g) was weighed out separately and soaked in 150 ml of distilled water, stirred continuously and thereafter left for five hours. The extract was obtained through filtration using Whatmann No.1 filter paper. The filtrate (4 ml) was added to 36 ml of 5 mM $AgNO_3$ solution in a 250 ml conical flask [7]. A colour change from pale yellow to reddish brown was observed, and gradual shaking led to the deepening of the brown colour after shaking for 1 hour. The formation of AgNPs was monitored through visual observation of the change of colour and thereafter characterized using UV-visible spectrophotometer, Fourier Transformed Infra-

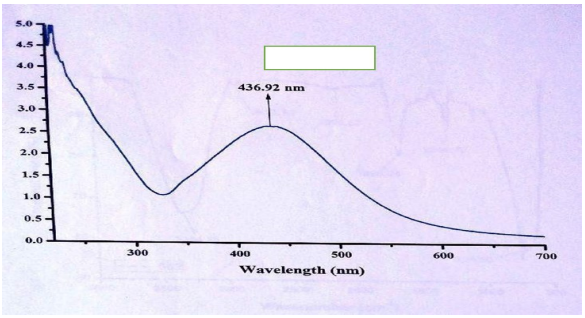

Fig. 2: UV-Visible spectrum of the synthesized silver nanoparticles

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red (FTIR), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) [8].

2.3 Characterization of synthesized AgNPs

2.3.1 UV-Visible Spectroscopy analysis

The bio reduction of silver ions in aqueous solution was monitored by periodic sampling of the mixture and subsequently measuring UV-Vis Spectra of the solution. A UV-visible (UV1-Thermo Electronic Corporation, Merck) spectrum of this extract was monitored as a function of time of reaction.

2.3.2 Fourier Transformed Infrared (FTIR)

The FTIR spectroscopic analysis was carried out using IR Affinity-IS Spectrometer on the powder sample of AgNPs [9]. The AgNP solution was centrifuged at 10000 rpm for 20 min. The solid residue obtained was then dried at room temperature and the powder obtained was used for FTIR measurement using KBr pellets.

2.3.3 Scanning Electron Microscopy (SEM)

The morphological properties (size and shape) of the AgNPs were examined with (SEM) scanning electron microscope JOEL JSM 6380 LA. The dried AgNPs sample was sprayed on a carbon tape and it was pasted on the sample holder of the SEM. The sample holder was transferred to the glass chamber and a completed sputtering occurred after 2s. It was then fixed with the sample holder and analyzed at room temperature.

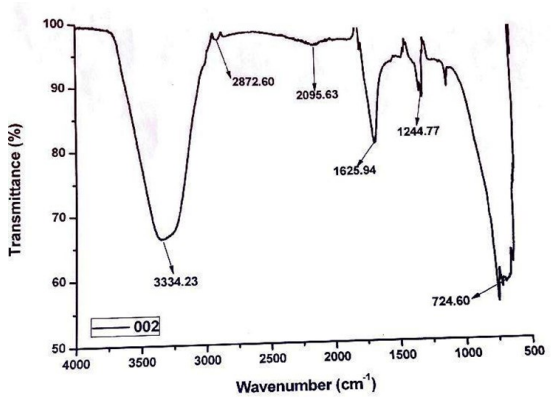

Fig. 3: FTIR Spectrum of the plant extract

2.4 Antifungal activity of synthesized AgNPs

The antifungal activities of the *P. nigrescens* were evaluated using Mycelial Inhibition method [10, 11] by incorporating lowest graded concentration used in the study (250 µl/ml) of *P. nigrescens* into potato dextrose agar plates, which were then inoculated with agar plug of 6 mm of 48 hr old cultures of *A spergillus niger, A spergillus flavus and Aspergillus fumigatus.* In the control experiments, fungal plugs were inoculated on PDA plates without the incorporation of *P. nigrescens*. All the plates were incubated at 28±2 °C for 72 h. The diameters of fungal growths in all the plates were measured and used to determine the percentage growth of inhibition as follows:

$$(Dcontrol - Dtest)/Dcontrol\ X\ 100$$

Where D is the diameter of fungal growth on the PDA plates.

2.5 Antimicrobial activities of synthesized AgNPs

The antimicrobial property of the synthesized AgNPs against some clinical isolates was also investigated using the agar-diffusion method. Clinical isolates of *Klebsiella oxitoca* and *Pseudomonas*

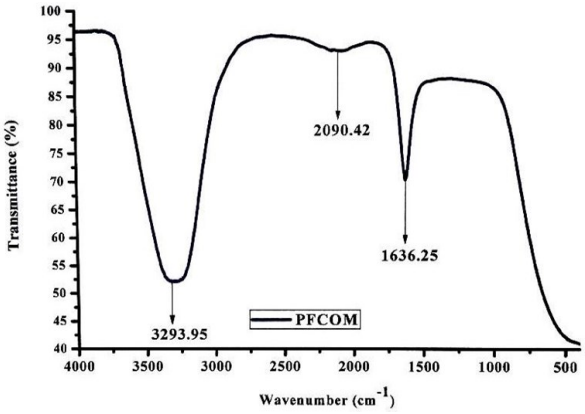

Fig. 4: FTIR Spectrum of the synthesized AgNPs

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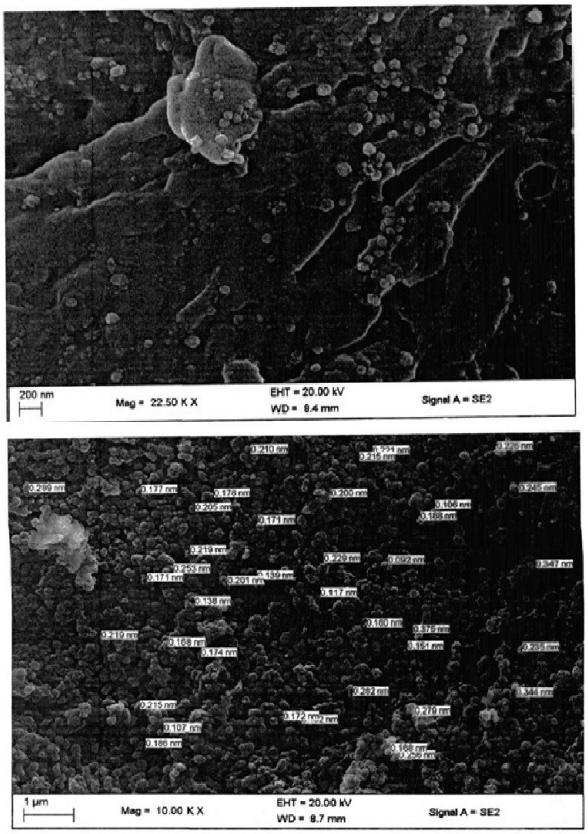

Plate 1: SEM image of synthesized nanoparticles at 200nm

aeruginosa were obtained from Bowen Teaching Hospital, Ogbomoso were used as test organisms. Each bacterium was grown overnight in peptone water and 18-h culture was used to seed the plates of Mueller-Hinton Agar (Lab M Ltd.). Thereafter, the plates were then bored using cork borer (7mm) to create wells to which 100µl of graded concentrations (250-100 µl/ml) of *P. nigrescens* prepared by dispersion in sterile distilled water were added. The plates incubated at 37 °C for 24 hr for the examination of zones of inhibition, which were measured in mm.

3. Results and Discussion

Silver nanoparticles exhibit yellowish-brown colour in water [12]. The formation of AgNPs by reduction of the aqueous Ag^+ during exposure to the aqueous extract of *P. nigrescens* shows yellowish brown colour, which suggests the formation of AgNPs in solution. The color arises due to excitation of surface plasmon vibrations in silver nanoparticles as reported by some previous authors [12, 13].

3.1 Biosynthesis and characterization of AgNPs

The biosynthesized AgNPs was subjected to analysis by using the UV-Vis Spectrophotometer which displayed maximum absorbance at the wavelength of 436.92 nm which falls within the reported range of 391-440 nm for AgNPs [14-17]. The FTIR absorption spectrum of the synthesized nanoparticle shows distinct peaks at 3334.23, 2872.60, 2095.63, 1625.94, 1244.77 and 724.60cm^{-1}. 3334.23cm^{-1} corresponds to N-H stretching vibration which indicates the presence of amine group, 2872.60cm^{-1} was assigned to C-H stretching bond which indicates the presence of an alkane group.

Microscopic analysis using TEM at 200nm resolution (plates 1,2) shows that the biosynthesized AgNPs are spherical in shape with no agglomerated particles and this conforms with results earlier reported by several authors [14-17]. SEM analysis of the biosynthesized AgNPs also shows high density of AgNPs and also reveals that AgNPs are spherical shaped, well distributed without aggregation in solution. The SEM micrographs show that most of the silver nanoparticles basically have a smooth surface and well dispersed with an average particle size found within the range of 0.092 to 0.375 µm. This finding is in line with previous study on AgNPs synthesized from plant extracts [16].

Table 1: Mycelia inhibition growth

Microorganisms	MSE NPs (mm)	M.I %
A. flavus	20.5	76
A. fumigatus	27.5	44.87
A. niger	19.5	81.17

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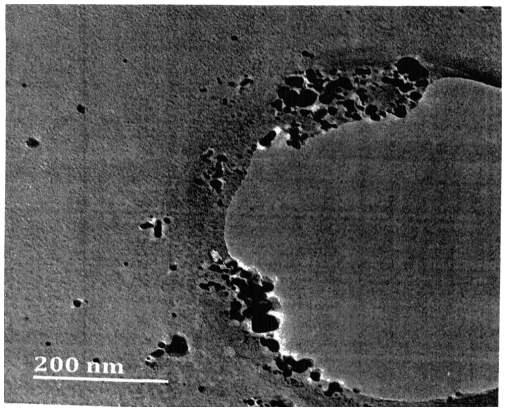

Plate 2: TEM image of the synthesizes AgNPs at magnification 200nm

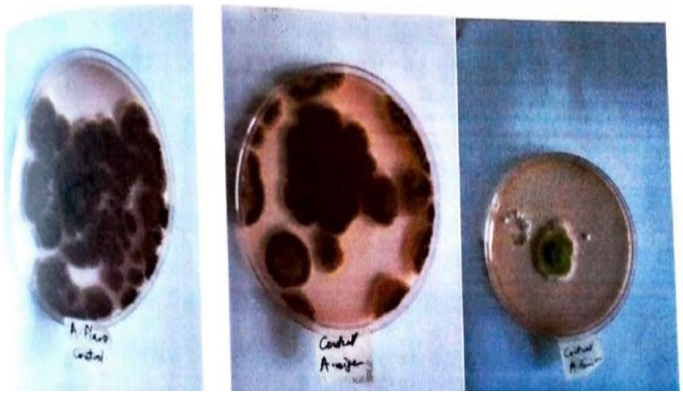

Plate 3: Growth of *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*

3.2 Antimicrobial activities of biosynthesized AgNPs

The biosynthesized AgNPs depicts antibacterial activities in the range of 12-35 nm against multi-drug resistant isolates of *Klebsiella oxytoca* and *Pseudomonoas aeruginosa*. The activities shown by the AgNPs are consistent with several reports on the antibacterial activities of AgNPs [18-20]. The antibacterial activities of AgNPs have been linked to the interaction of AgNPs with sulfur and phosphorus containing constituents of the bacterial cell to initiate cell killing by attacking the respiratory chain and cell division [21]. The potency of the AgNPs can enhance its application in combating drug-resistant bacteria.

The biosynthesized AgNPs also shows a significant mycelia inhibition effect on the growth of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus*. AgNPs synthesized from *P. nigrescens* shows inhibitions of 81.17% against *Aspergillus niger*, 76% against *Aspergillus flavus* and 44.87% against *Aspergillus fumigatus* as shown in the table 1. Several authors have also reported the antimicrobial activity of biosynthesized nanoparticles against fungal isolates [17, 22-28].

4. Conclusion

In this study, the green synthesis of AgNPs using *P. nigrescens* extract was successfully carried out. The particles are spherical in shape and displayed good antibacterial and antifungal activities against multidrug resistant strains of bacteria. Therefore, this research work has showcased an environmentally friendly approach of synthesizing AgNPs using *P. nigrescens* and the synthesized AgNPs has potential for biomedical applications.

Conflict of Interest

The authors declare that the manuscript has no conflict of interest

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