Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles from Aqueous extract of Elephant grass (*Pennisetum purpureum*)

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ABSTRACT

This paper assessed the use of aqueous extract of Elephant grass (*P. Purpureum)* in the synthesis of silver nanoparticles (AgNPs). The synthesis of AgNPs was confirmed by the measurement of the Surface Plasmon Resonance (SPR) at 480 nm using UV-visible spectrophotometry. Fourier Transform Infrared (FTIR) spectroscopic analysis of the nanoparticles indicated the presence of biomolecules as capping agents for the synthesized AgNPs which increased the stability of the nanoparticles thus synthesized. In addition, the antibacterial activity of the synthesized nanoparticles was tested as potential inhibitory activity against two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*).The results showed that the AgNPs have appreciable inhibition and antimicrobial effects on the two test organisms.

Keywords: Silver nanoparticles; antibacterial activity; green synthesis, elephant grass; *S. aureus; B. Subtilis*.

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INTRODUCTION

Nanotechnology, a field of research which centres on synthesis of particle with structures ranging from 1-100 nm has grown in the last decade to include the synthesis of metal nanoparticles (MNPs) with various chemical compositions, sizes and morphologies (Iravani *et al.*, 2014). MNPs have found wide applications in differs spheres of life including bimolecular detection, catalysis and plasmonic and antimicrobials (Elechiguerra *et al.*, 2005; Khlebtsov & Dykman, 2010; Saxena *et al.*, 2012; Veerasamy *et al.*, 2011). Nanoparticles of various metals including that of silver, gold, copper, zinc, titanium, cadmium and iron have been synthesized (Oberdörster *et al.*, 2005; Schabes-Retchkiman *et al.*, 2006; Vankar & Shukla, 2012). Among these metals, silver has been widely used in the synthesis of MNPs. AgNPs possessing less than 100 nm particle size and high- area- to volume ratio have been reported to possess increased reactivity relative to the bulk silver material (Oberdörster *et al.*, 2005). AgNPs been widely reported to possess antimicrobial sensitivity against disease causing organisms such as bacteria (Morones *et al.*, 2005).

AgNPs have been traditionally synthesized via physical or chemical route(Oliveira *et al.*, 2005); this routes involved the use of toxic and/ or expensive chemicals and specialised equipment. These limitations and increasing awareness towards green chemistry and other biological processes have led to the development of cheap and ecofriendly approach to synthesis of AgNPs involving use of either microorganisms such as bacteria, fungi, yeast or plant extracts (Hussain *et al.*, 2011; Udayasoorian *et al.*, 2011). However, the use of plant extracts in the synthesis of AgNPs is on the rise due to a wide range of metabolites/phytochemicals present in plants which hasten the synthesis and stabilize the AgNPs in solution (Franke *et al.*, 2010).

Pennisetum purpureum (Elephant grass), a common weed found in Nigeria is known to be rich in metabolites such as tannins, alkaloids, flavonoids, saponins, cyanogenic glycosides and oxalates, which are known to have **antimicrobial properties (Okaraonye & Ikewuchi, 2009)**. Use of plant extracts in the synthesis and antimicrobial activities of AgNPs have been extensively studied (Azizi *et al.*, 2013) however, studies on synthesis of AgNPs from *Pennisetum purpureum* extract and antimicrobial activities of such AgNPs are limited. Therefore, this study focussed on the synthesis of AgNPs from *Pennisetum purpureum plant* extract and the antimicrobial activity of the Nanoparticles against bacteria.

MATERIALS AND METHODS

Collection of Elephant grass and preparation of extract

Fresh Elephant grass (*Pennisetum purpureum*) was collected from Joseph Ayo Babalola University campus, Ikeji-Arakeji, Osun State. The Elephant grass samples was washed several times with distilled water to remove dust particles and subsequently dried at room temperature. The elephant grass extract used in this study was obtained by adding 10 g of the dried elephant grass into an Erlenmeyer flask containing 150 ml of distilled water and boiled for 10 minutes at 60^{o}C before decanting it. The extract was allowed to cool and filtered using Whatman No.1 filter paper and was then stored in the refrigerator at 4^{o}C and used for further experiments.

Synthesis of silver nanoparticles

Silver nanoparticle was prepared by the adding 5 ml of 1 mM silver nitrate solution to 5ml of the elephant grass leaf extract and incubated at 60^{o}C in a thermostatted water temperature. The bio reduction of silver ion in solution was monitored at different time intervals by sampling aliquots of the reaction mixture for measurements of UV-Visible spectra of the solution. Reduction of the silver ions was observed by the change in colour of the reaction mixture to dark brown. The silver nanoparticle was purified by centrifuging the dark brown solution obtained several times at 2500 rpm for 20 minutes and the residues obtained were re-dispersed in water.

Characterization of silver nanoparticles

The absorption spectrum of the coloured reaction solution was recorded on a UV-Visible spectrophotometer by diluting small aliquots with distilled water in the range 200- 1200 nm. FTIR measurements were recorded on a Thermo Scientific Nicolet IF5 spectrophotometer (resolution 1000 – 4000 cm^{-1}). The analysis was carried out by pipetting a small aliquot of the sample into a potassium bromate plate and allowed to dry.

Screening of antibacterial activity of the synthesized nanoparticles

Antibacterial activities of the synthesized silver nanoparticles were assessed against two Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* bacteria using agar well diffusion method with Muller Hinton Agar. Chloramphenicol (50 µl) was used as control. The experimental setup was replicated thrice. The plates were then incubated at 37°C for 24 hours and then examined for zones of inhibition.

RESULTS AND DISCUSSION

The UV–Visible spectrum of the silver nanoparticles synthesized from leave extract of *Pennisetum Purpureum* is as shown in Figure 1. When the extract was added to the boiling $AgNO_3$solution, the initial yellow colour of the solution turned dark brown colour, indicating formation of silver nanoparticles. The colour change was due to the reduction of silver ions. Metallic nanoparticles scatter and absorb light at certain wavelengths due to the resonant collective excitations of charge density at the interface between a conductor and an insulator known as surface plasmon resonance.

Metal nanoparticles have electrons which give surface Plasmon resonance absorption band due to the combined vibration of free electrons on its surface in resonance with light wave. In this study, the absorption band of silver nanoparticles was observed at 430 nm (Fig. 1). The colour of the mixture of extract and silver ions changed from yellow to dark brown (Figure 2) on completion of reaction. This characteristic colour change is due to the excitation of the surface plasmon resonance of the silver nanoparticles silver nanoparticles (Mallikarjuna *et al.*, 2011) which gives rise to the observed colour change from yellow to brown in this study.

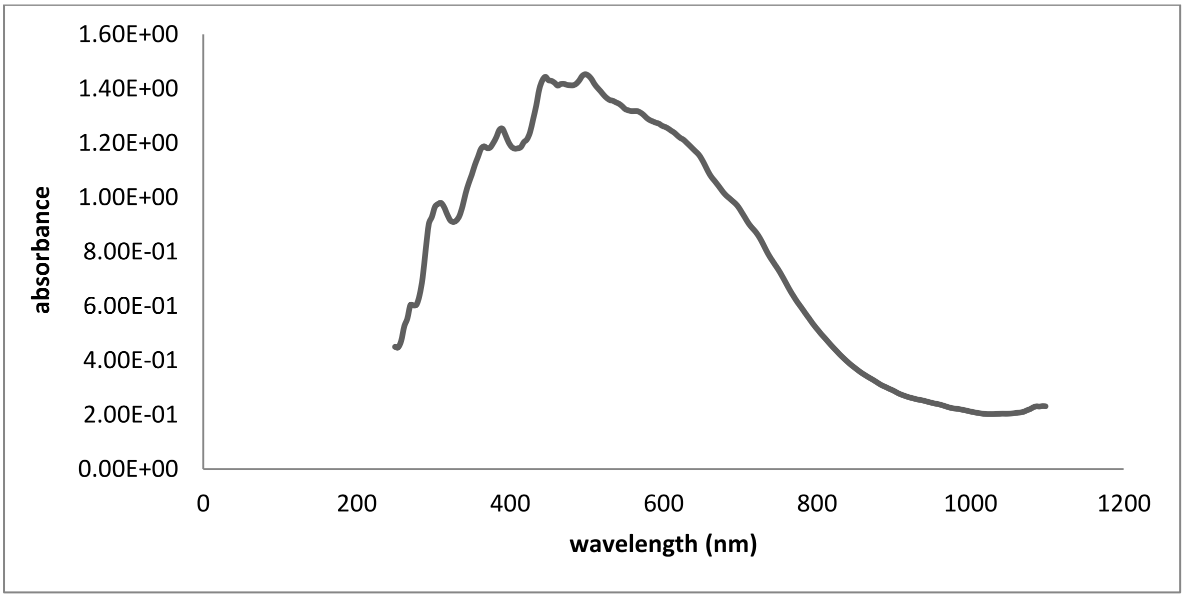

Figure 1: UV-Vis absorption spectrum of silver nanoparticles (SNP) synthesized from Pennisetum purpureum leaf extract

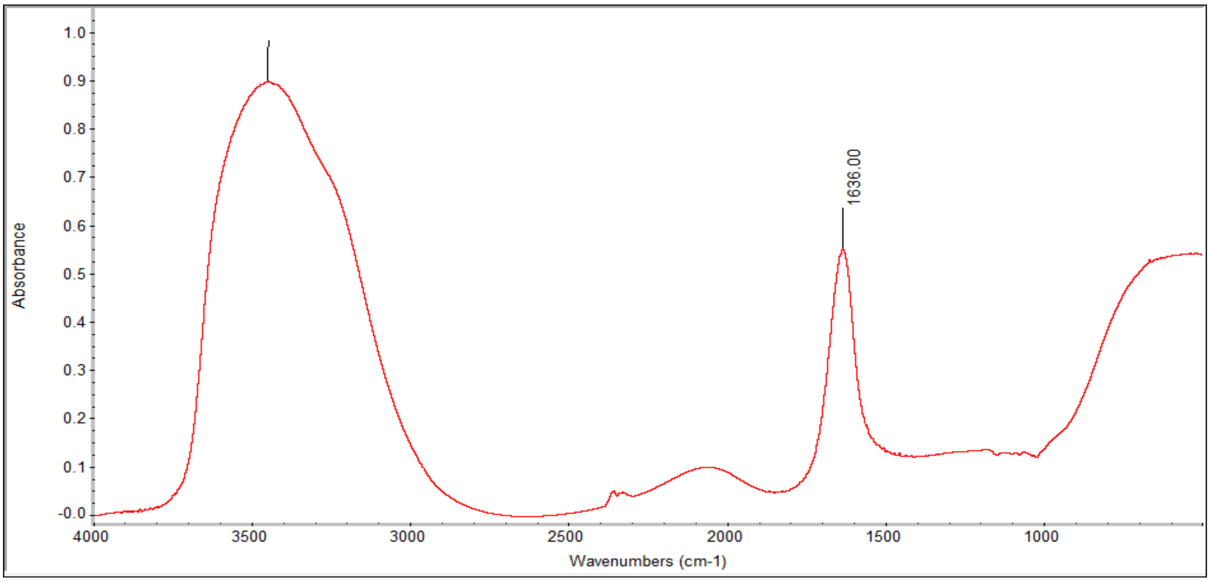

Figure 2: FTIR spectra of silver nanoparticles synthesized from *Pennisetum purpureum* extract.

FTIR measurements were carried out to identify the biomolecules responsible for capping and stabilization of nanoparticles synthesized from elephant grass leaf extract. The FTIR spectrum (Fig. 3) shows absorption bands at 3470 and 2050 cm^{-1} representing O-H and C-H stretching vibration of polyols. The absorption peaks located at 1634 and 650 cm^{-1} represented C=O and N-H vibration stretching of carboxylic acid and amines respectively. These peaks indicate that polyols (phenols and flavonoids), terpenoids and protein compounds were responsible for the reduction of silver ions to nanosilver and were further incorporated onto the silver colloids as capping agent preventing the agglomeration of the colloids thus stabilizing it (Kouvaris *et al.*, 2012).

The mean zones of inhibition exhibited by the elephant grass leaf extract synthesized silver nanoparticles; elephant grass leaf extract and bare silver nitrate solution against *S. aureus* and *B. subtilis* are shown in Table 1. The highest antibacterial activity/inhibition of 8.10±0.15 was recorded against *B*. subtilis while 4.2 ±0.21 was recorded against *S. aureus*

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Table 1: Zones of inhibition (mm) of AgNPs against test bacteria strains

Bacteria strains	AgNPs	Extract	$AgNO_{3\ (aq)}$	Control
S. aureus	4.2±0.45	negative	3.10± 0.80	9.70±0.20
B. subtilis	8.5± 0.20	negative	5.40±0.15	10.30±0.43

Control- Chloramphenicol

In comparison with the antibacterial activity of plant extract alone, the synthesized nanoparticle is clearly an effective antibacterial agent. This is due to the fact that the leaf extract showed no antibacterial activity against any of the test strains. However, it is rich in phytochemicals that was sufficient for the bio-reduction of Ag^+ to Ag^o (Okaraonye & Ikewuchi, 2009). Though the antibacterial activities of the synthesised silver nanoparticles were lower than that of the control (chloramphenicol) in this study, the percentage inhibition of the silver nanoparticles against *S. aureus* and *B. subtilis* was still above average; 64 and 82.5 % respectively.

CONCLUSION

In this study, we have described a simple and green method for the synthesis of silver nanoparticles using the leaf extract of elephant grass (*Pennisetum purpureum*). The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy. The FTIR spectrum showed that the phytochemicals found in the extracts were incorporated onto the silver nanoparticles as capping and stabilizing agents. The synthesized nanoparticles showed considerable bacterial inhibition against two gram positive bacterial *S. aureus* and *B. subtilis* in comparison to chloramphenicol, a potent antimicrobial drug.

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