

GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM LEAF EXTRACTS OF *PARQUETINA NIGRESCENS* AND *SYNEDRELLA NODIFLORA* AND THEIR ANTI-MICROBIAL ACTIVITY.

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ABSTRACT

In this work, metallic silver nanoparticles were synthesized from leaf extracts of *Parquetina nigrescens* and *Synedrella nodiflora*. Silver ion was reduced to metallic silver on treatment of AgNO₃ solution with aqueous extracts of the two plants within 30 minutes. The effects of time and the volume of extract to silver salt solution were investigated on the formation of the nanoparticles. The nanoparticles were characterized using the ultraviolet visible spectroscopy, X-ray diffraction, Scanning electron microscope, and Fourier transformed infra-red spectroscopy. Antimicrobial activities of the nanoparticles were investigated against ten human pathogens using agar well diffusion method. XRD analysis showed that the synthesized silver nanoparticles were crystalline in nature. The SEM result confirmed that nanoparticles from *S. nodiflora* appeared spherical in nature while those of *P. nigrescens* were characterized with different shapes and sizes. The antimicrobial results showed that the nanoparticles have activity against all the tested bacteria but are less active against some isolated fungi.

INTRODUCTION

The emergence of particles in the “nano” dimension has brought tremendous advancement in the field of material science and research. Nanoparticles consist of aggregates of atoms in the range of 1-100 nm. Particles in the “nano” sense greatly exhibit remarkable differences in properties, reactivities and hence, applicabilities when compared to the normal size materials. Nanoparticles possess catalytic, optical, electronic, antibacterial and magnetic properties and have been found useful in various industries, medical and environmental remediation (Gajjar *et al.*, 2009; Feynman, 1991; Parak *et al.*, 2003; Gutierrez *et al.*, 2010). Metallic nanoparticles are considered most promising in terms of their excellent anti-microbial property which is attributed to their large surface area to volume ratio (Cho *et al.*, 2005; Mittal *et al.*, 2013).

Synthetically, nanoparticles can be prepared using either the ‘top down’ or ‘bottom up’ approach (Ahmed *et al.*, 2015). The ‘top down’ approach involved the gradual breakdown or reduction of bulk material into the nano sized particles. Methods in this series include mechanical milling or ball milling, chemical etching, thermal or laser ablation, explosion processes and sputtering. Most of these methods have low rate of production, besides being expensive and energy

intensive. Moreover, the nanoparticles produced are characterized by imperfection or heterogeneity of surface structure. In the “bottom up” approach, particles are assembled or built into their molecular structure from their smaller entities. This can be achieved via chemical process or electrochemical precipitation, vapour deposition, atomic or molecular condensation, sol-gel processes, spray or laser pyrolysis and aerosol processes (Mittal *et al.*, 2013). The particles are generally uniform and are more heterogeneous in nature than in the ‘top down’ approach. However, the dry methods of irradiation are not environmentally benign and most of the wet chemical processes required the use of reactants in a liquid medium together with reducing and stabilizing agents which are toxic and generally hazardous; thus damaging the environment and limiting their use in the field of medicine and drug delivery (Mittal *et al.*, 2013).

The biological process remains the most preferable and more attractive method of preparing nanoparticles because of its environmental friendliness, sustainability and relatively low cost of production (Kharissova *et al.*, 2013). In the biogenic synthesis, plant extracts, bacteria, fungi etc are used to reduce metallic salts such as silver, gold, zinc, iron and other metals. Of the metallic nanoparticles, silver nanoparticles are of utmost importance because of its wide range

of antibacterial activities over other metals. Silver nanoparticles have been produced from several plants in the literature. These include: *Acalypha indica* (Krishnaraj *et al.*, 2010), *Chenopodium album* leaf (Dwivedi and Gopal, 2010), *Vitexnegundo* L. (Zargaret *et al.*, 2011), *Portulacaoleracea* (L) (Firdhouse and Lalitha, 2012), *Centellaasiatica* L., (Rout *et al.*, 2013), pine apple (Elimeke *et al.*, 2014), *Boerhaaviadiffusa* (Nakkala *et al.*, 2014), *Meliadubia* leaf (Kathiravan *et al.*, 2014), *Pistaciaatlantica* (Sadeghi *et al.*, 2015), banana peel (Ibrahim, 2015).

P. nigrescens is a herbaceous plant, commonly found in most African countries especially Nigeria. The plant has been reported to exhibit antioxidant analgesic, anti-inflammatory, antipyretic, antidiabetic, haematinic, sympathomimetic properties (Ayoola *et al.*, 2011; Owoyele *et al.*, 2009; Saba *et al.*, 2010; Aderibigbe *et al.*, (2011). The methanolic extract of the plant has protective property against ethanol induced ulceration. Aderibigbe *et al.*, (2011) reported that different part of the plants can be used to treat diseases like rickets, diarrhea, skin lesions, menstrual disorders, gonorrhoea, and sickle cell anaemia. *Synedrella nodiflora* (L.) is an annual weed reported to be native to America, but found in the plains of India. Martin and Gopalkrishnan (2005) reported the leaves to be active as pollutice for sore rheumatism, leaf juice for earache and aerial part as Insecticidal agents. It has antibacterial and antioxidant activities. A hydro-ethanolic extract of the plant has been reported to ameliorate hyperalgesia and allodynia in vincristine-induced neuropathic pain (Amoateng *et al.*, 2015)

The biosynthesis of silver nanoparticles from *P. nigrescens* has not been reported in the literature. However, Shyamal *et al.* (2014) reported recently the green synthesis of nanoparticles from aqueous extracts of *Synedrella nodiflora* using sunlight as source of irradiation and the antibacterial activities of the nanoparticles against five pathogenic bacterial strain were examined. In the present study, silver nanoparticles were synthesized from aqueous extracts of Nigerian grown *Parquetina nigrescens* and *Synedrella nodiflora* using a hot plate. Ultraviolet visible spectroscopy, X-ray diffraction spectroscopy, Scanning electron microscope, and Fourier transformed infra-red spectroscopy were used to characterize the

nanoparticles. The antimicrobial activity of the nanoparticles was investigated against ten human pathogens using agar well diffusion method.

MATERIALS AND METHODS

Analytical grade AgNO₃ (Sigma Aldrich) was purchase from a chemical store in Nigeria. *Escherichia coli* (E. coli) and other pathogenic organisms were collected from the Department of Microbiology, University of Ibadan. The strains were grown and preserved in the culture media following standard procedures.

The Plant Samples

Leaves of *Synedrella nodiflora* and *Parquetina nigrescens*, were freshly obtained from the University of Ibadan campus. The leaves were thoroughly washed with running tap water and then with distilled water three times to remove dust particles and sand. The plant materials were air-dried at room temperature, after which it was finely cut into small pieces. 25g of the finely cut leaves was weighed into a 400ml beaker containing 100ml of distilled water and boiled for 10mins on the hot plate. The resulting extract was filtered into a 250ml Erlenmeyer flask and stored in the refrigerator for further use.

Synthesis of Silver Nanoparticles (AgNPs)

Equal volume of the prepared plant extracts were added to 1 mM solution of AgNO₃. The mixture was heated on the hot plate at 70°C with a magnetic stirrer for 30 minutes. The bio-reduction of silver nitrate to silver ions was confirmed by a color change. The formation of silver nanoparticles was also monitored by UV – Vis spectrophotometric at the wave length range of 300 to 900 nm. The supernatant liquid was discarded and the pellet obtained was washed three times with distilled water.

Effect of Volume Ratio

The effects of volume was monitored for maximum yield of silver nanoparticles by keeping the volume of plant extract constant and varying the volume of silver nitrate to give a volume ratio of 1:1, 1:2 and 1:3 respectively. The resulting solution was heated at 70°C for about 30 minutes and the absorbance was measured at a wavelength range of 300 to 900 nm.

Effect of Time

The bioreduction of the AgNO_3 by the plant extracts was also investigated with respect to time. The extent of the formation of AgNPs was monitored by sampling an aliquot sample of the mixture at different time intervals of 0, 5, 10, 15, 30 and 60 minutes of the reaction.

Characterization of Silver Nanoparticles

The bio-reduced silver nanoparticles were subjected to various characterization techniques. The surface plasmon resonance (SPR) of the silver nanoparticles was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lambda 25, Germany). Equal volume of the plant extract and 1mM AgNO_3 was heated at 70°C. Aliquot sample of the solution was taken at different time intervals up to 30 minutes and the rate of the reduction of Ag ions was monitored by measuring the absorbance or appearance of plasmon bands with the UV-Vis spectrophotometer at 300 nm to 900 nm. The colloidal solution remaining was cooled, centrifuge, decanted and then dried. The functional groups present in the dried residue of the synthesized silver nanoparticles were taken using FTIR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer). The morphology of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy (Rigaku D/Max-IIIC) while the particle size and shapes were examined using the scanning electron microscope (JEOLJSM-7600F)

Antimicrobial Activity Screening

Ten pathogenic microorganisms, namely, *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Bacillus subtilis* (*B. subtilis*) (Gram negative), *Staphylococcus aureus* (*S. aureus*) (Gram positive), *Candida albicans* (*C. albicans*) (Yeast), *Aspergillus niger* (*A. niger*), *Penicillium notatum* (*P. notatum*), *Rhizopus stolonifer* (*R. stolonifer*), and *Salmonella typhi* (*S. typhi*) were used to determine the antimicrobial activity of the synthesized silver nanoparticles.

The pure cultures of bacteria were sub cultured on Mueller-Hinton agar (MHA) and Sabouraud Dextrose agar (SDA) for yeast. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. 8 mm diameter wells were made on nutrient agar plates using gel

puncture. 50 μL of 20 $\mu\text{g}/\text{ml}$ of nanoparticle colloidal suspension (i.e. 100 μg of nanoparticles in 5 ml of the DMSO) was poured onto each well on all plates. After incubation at 37 °C for 24 h, the diameter of zone inhibition was measured in millimeter, and was recorded. The conventional pour-plate method was used to determine the antibacterial activity of the synthesized silver nanoparticles. In this study, Nutrient Agar medium was used which supports growth of a wide range of bacteria including *Bacillus subtilis* and *Escherichia coli*. The dehydrated medium was constituted as prescribed. For 100 ml Nutrient Agar medium, 2.8 g Nutrient agar powder was added in 100ml distilled water; for the pour-plate method, 0.5 g of agar powder was added in addition to the medium. The medium kept in cotton-plugged glass container was sterilized in an autoclave at 121°C for 15 minutes. It was distributed inside a laminar hood either in culture tubes or on Petri dishes when hot (about 45°C) and allowed to solidify.

Surface Plate Method (fungi)

A sterile Sabouraud Dextrose Agar (62g/l) was prepared accordingly aseptically and was poured into the sterile plates in duplicates and allowed to set properly. 0.2 ml of the 10^{-2} of the agar using sterile spreader to cover the surface of the agar. The wells were made using a sterile cork borer of 8 mm diameter. In each well, the graded concentrations of the extract were introduced into the wells including the controls. The plates were left on the bench for 2 hours to allow extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated uprightly in the incubator for 48 hours at 26-28°C.

RESULTS AND DISCUSSION

Biosynthesis of Silver Nanoparticles

Biosynthesis of silver nanoparticles using *S. nodiflora*, leaf extracts can be monitored from the observed change in colour of the plant extracts. The UV-Vis spectrum was used as a reliable tool in monitoring the progressive formation of the AgNPs. The colour of the reaction mixture changed from yellowish-green to reddish-brown after the addition of silver nitrate in 30 minutes. This is as a result of excitation of surface plasmon vibrations in the silver nanoparticles. Figure 1 represents the change in

colour of the reaction mixture after 30 minutes of reaction which signifies the formation of silver nanoparticles and the reduction of silver ion to its zero valences. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 480 nm, and 460 nm corresponding to *P. nigrescens*, and *S. nodiflora*, respectively (Figure 2). The broadening of the peak showed that the particles are poly dispersed.

In order to determine the optimum condition which would maximize the yield of silver nanoparticles, the effects of time and the ratio of the volume of the plant extract to the silver salt were determined. The results are presented in Figures 3 and 4. The rate formation of AgNPs was observed to increase with respect to time, i.e.,

from 0 to 60 minutes (Figure 3a) for *P. nigrescens* and 0 to 30 minutes (Figure 3b) for *S. nodiflora*. There was a steady increase in the absorbance as the time was increased, though with slight shifts in the absorbance maxima, which signify a variation in the particle size (Muhammad *et al.*, 2012). In Figure 3b, it was noted that there was no visible increase in the spectra taken at 15 and 30 minutes of reaction, respectively for the AgNPs formed from *S. nodiflora* leaf extract. This implied that there was no evidence of size variation after 15 and 30 minutes of reaction for *S. nodiflora* AgNPs. In comparing the two spectral in Figure 3, the AgNPs was formed within 15 and 60 minutes of reaction for the *S. nodiflora* and *P. nigrescens*, respectively. Thus, *S. nodiflora* showed some time advantage over *P. nigrescens*.

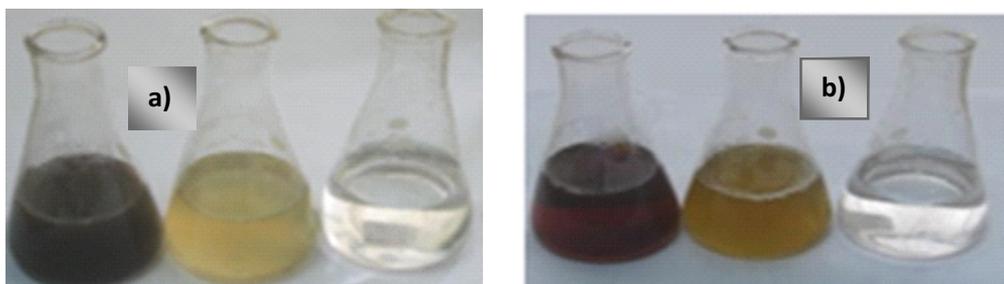


Figure 1: Solutions of silver nanoparticles (first), leaf extract (middle) and silver nitrate (last) from (a) *P. nigrescens* and (b) *S. nodiflora*, respectively

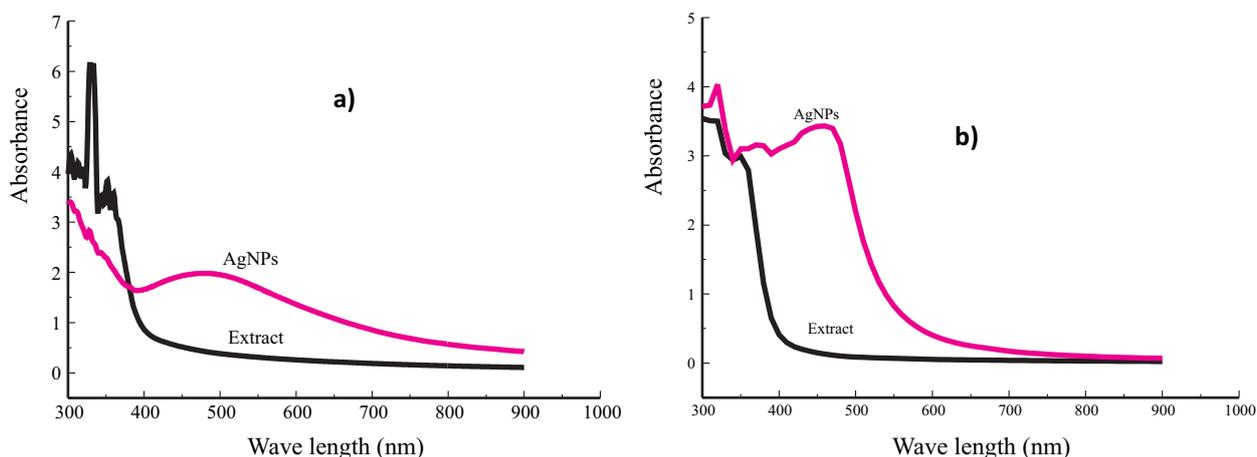


Figure 2: UV-vis spectral of the leaf extract and AgNPs obtained from (a) *P. nigrescens* and (b) *S. nodiflora*

The volume of plant extract to silver nitrate was investigated by keeping the volume of the extract constant and varying that of silver nitrate. It was

found that the optimum ratio for the reaction was 1:3 and 1:1, corresponding to *P. nigrescens* and *S. nodiflora* respectively.

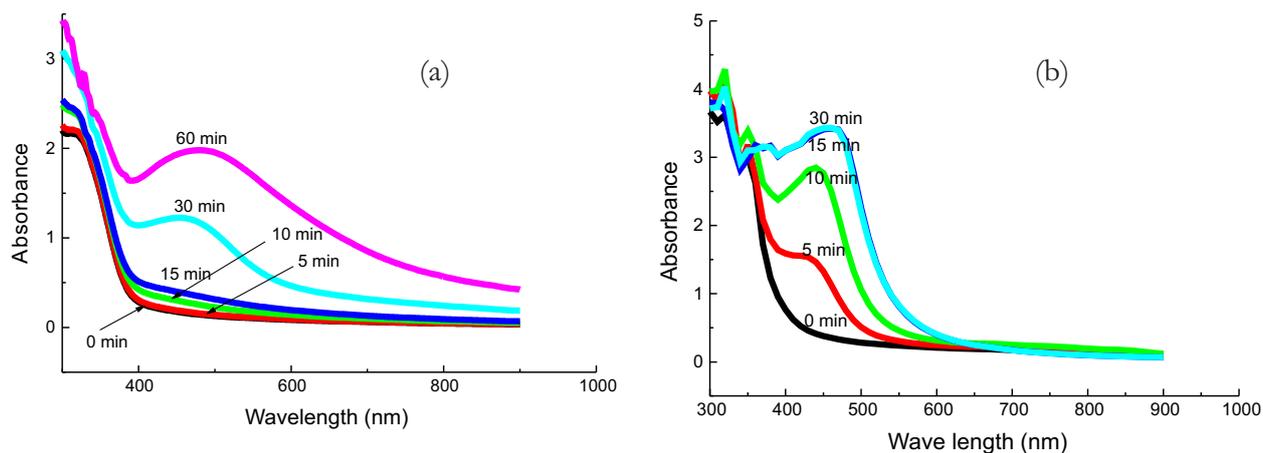


Figure 3: UV-visible spectral of AgNPs from (a) *P. nigrescens* and (b) *S. nodiflora* as a function of time

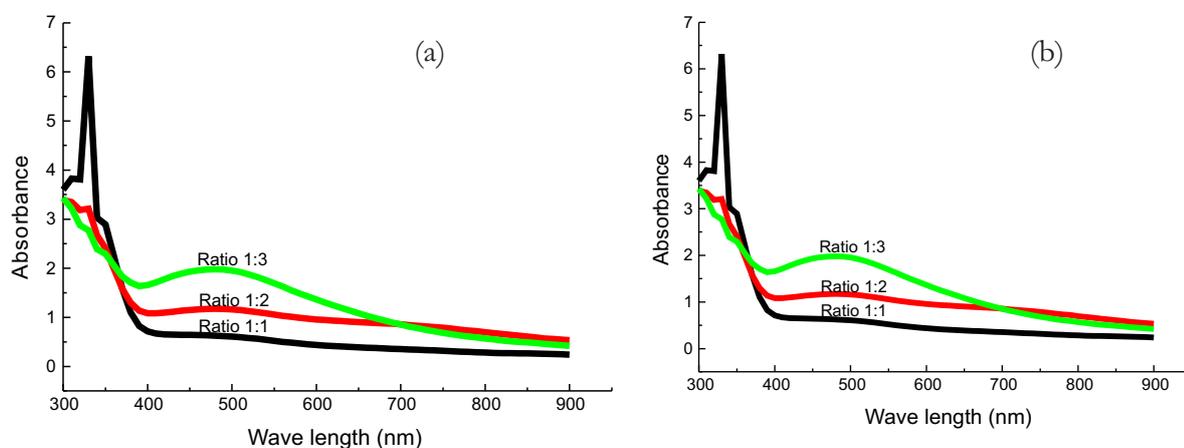


Figure 4: UV-visible spectral of AgNPs from (a) *P. nigrescens* and (b) *S. nodiflora* as a function AgNO_3 / extract ratio

FTIR spectra

The FTIR spectroscopic analysis was taken in order to identify potential biomolecules involved in the bioreduction reaction. The FTIR spectra of the *P. nigrescens* extract and AgNPs is shown in Figure 5a. There was a slight shift in the AgNPs spectrum and the intensity of the peaks observed in the extracts around 3392.17cm^{-1} , 2932cm^{-1} , 1603cm^{-1} , 1384cm^{-1} and 1071cm^{-1} (assigned to O-H, C-H, C-C, C-N and C-O stretching vibrations, respectively) became greatly reduced in the AgNPs spectrum. This suggests that these groups are possibly involved in the bioreduction, capping and stabilization of the AgNPs.

In Figure 5b, the broad band around 3407cm^{-1} is

characteristic of O-H stretching while the peak at 2724cm^{-1} and 1600cm^{-1} is characteristic of aliphatic O-H and C-C vibration frequency of aromatic ring, respectively (Silverstein *et al.* 2005). There is a shift in peaks from 1333cm^{-1} to 1323cm^{-1} (C-N stretch aromatic amines), 1265cm^{-1} - 1245cm^{-1} (C-O stretch of aromatic ethers), showing possible involvement of these group in the bioreduction of the silver salt. The disappearance of the aromatic C=C stretching at 1498cm^{-1} (which has also been ascribed to C-C stretching vibrations of benzenoid) from the AgNPs spectrum showed that it is the main group involved in the capping and stabilization of the synthesized nanoparticles.

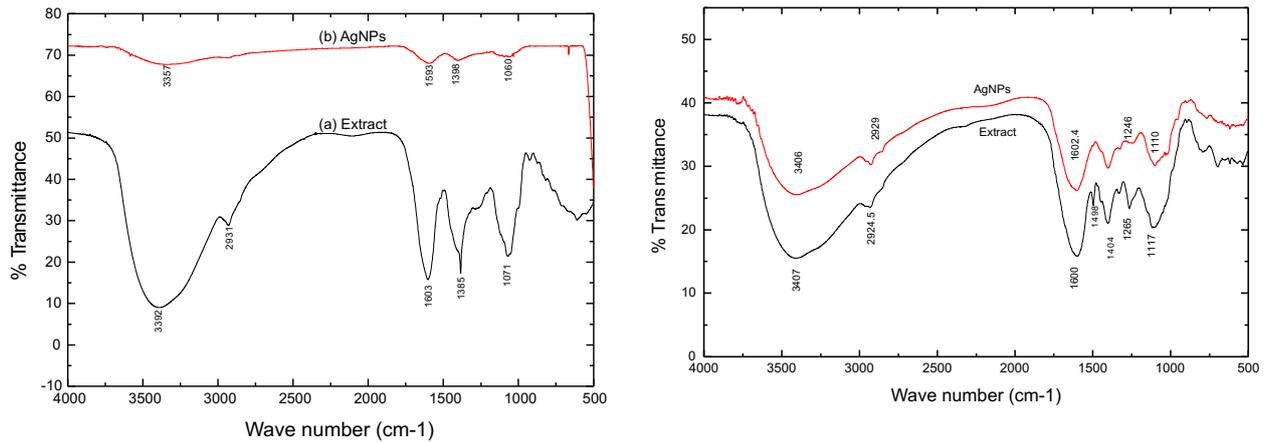


Figure 5: Comparative FTIR spectra of leaf extract and AgNPs obtained from (a) *P. nigrescens* and (b) *S. nodiflora*

Scanning Electron Micrograph

Figure 6 showed the scanning electron micrograph (at different magnifications) of the *S. nodiflora* and *P. nigrescens* extracts treated with 1 mM silver nitrate solution for 30 minutes, respectively. The micrographs of the brown color stable

samples showed the formation of well dispersed silver nanoparticles. The SEM revealed that more uniformly shaped silver nanoparticles were obtained with *S. nodiflora* while those of *P. nigrescens* were irregular.

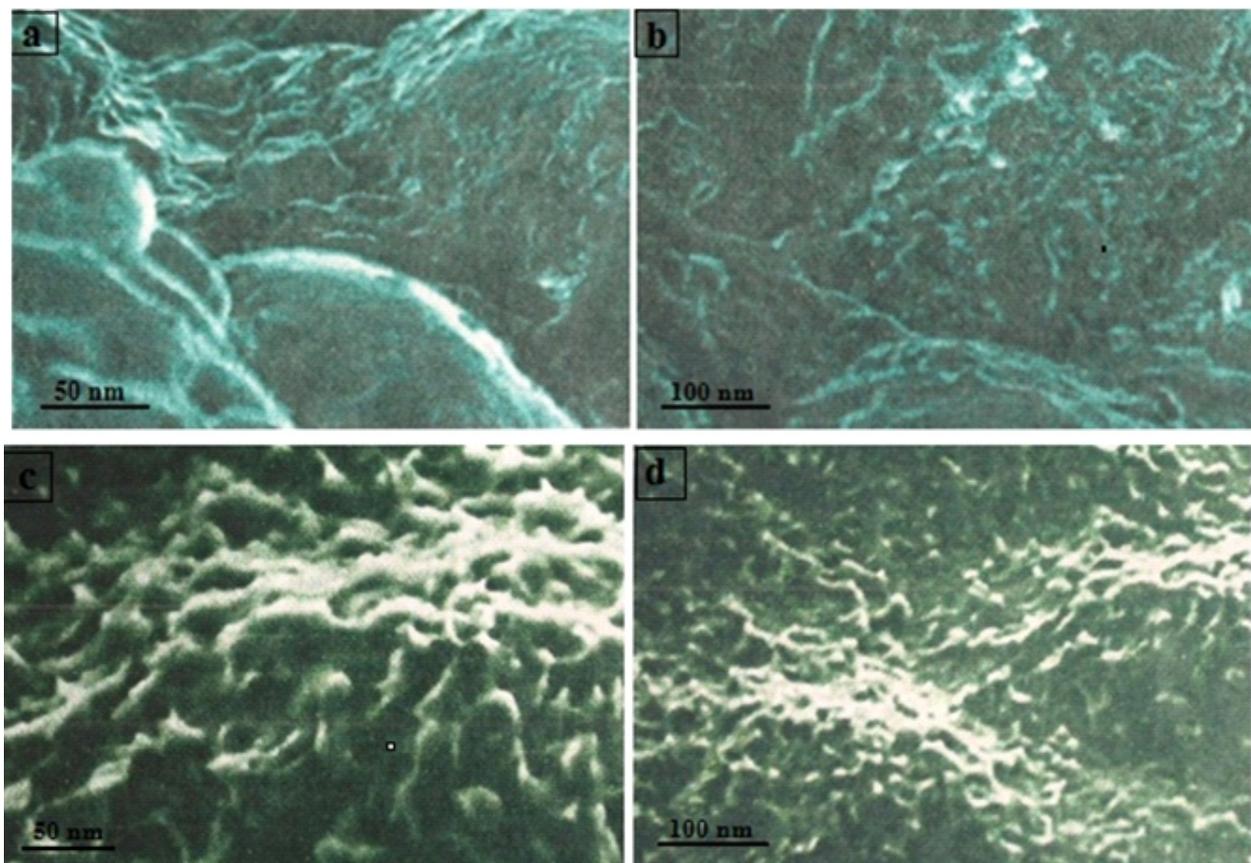


Figure 6: SEM images of AgNPs at different magnifications obtained from the leaf extracts of *P. nigrescens* (a and b) and *S. nodiflora* (c and d)

X-Ray Diffraction Analysis

The XRD was used to confirm the crystalline nature of the AgNPs formed. The AgNPs synthesized from *S. nodiflora*, (figure 7) showed XRD at $2(\theta) = 25^\circ, 33.8^\circ, 55.6^\circ,$ and 65.2° which could be attributed to the 111, 200, 220, and 311 face cubic crystallographic structure of the silver.

The XRD peaks for *P. nigrescens* AgNPs at $2(\theta) = 25.5^\circ, 42.4^\circ,$ and 55.8° can also be indexed to the (200), (220), and (311) Bragg's reflections cubic structure of metallic silver. This is an indication that the synthesized silver nanoparticles from both plant extracts were essentially crystalline in nature.

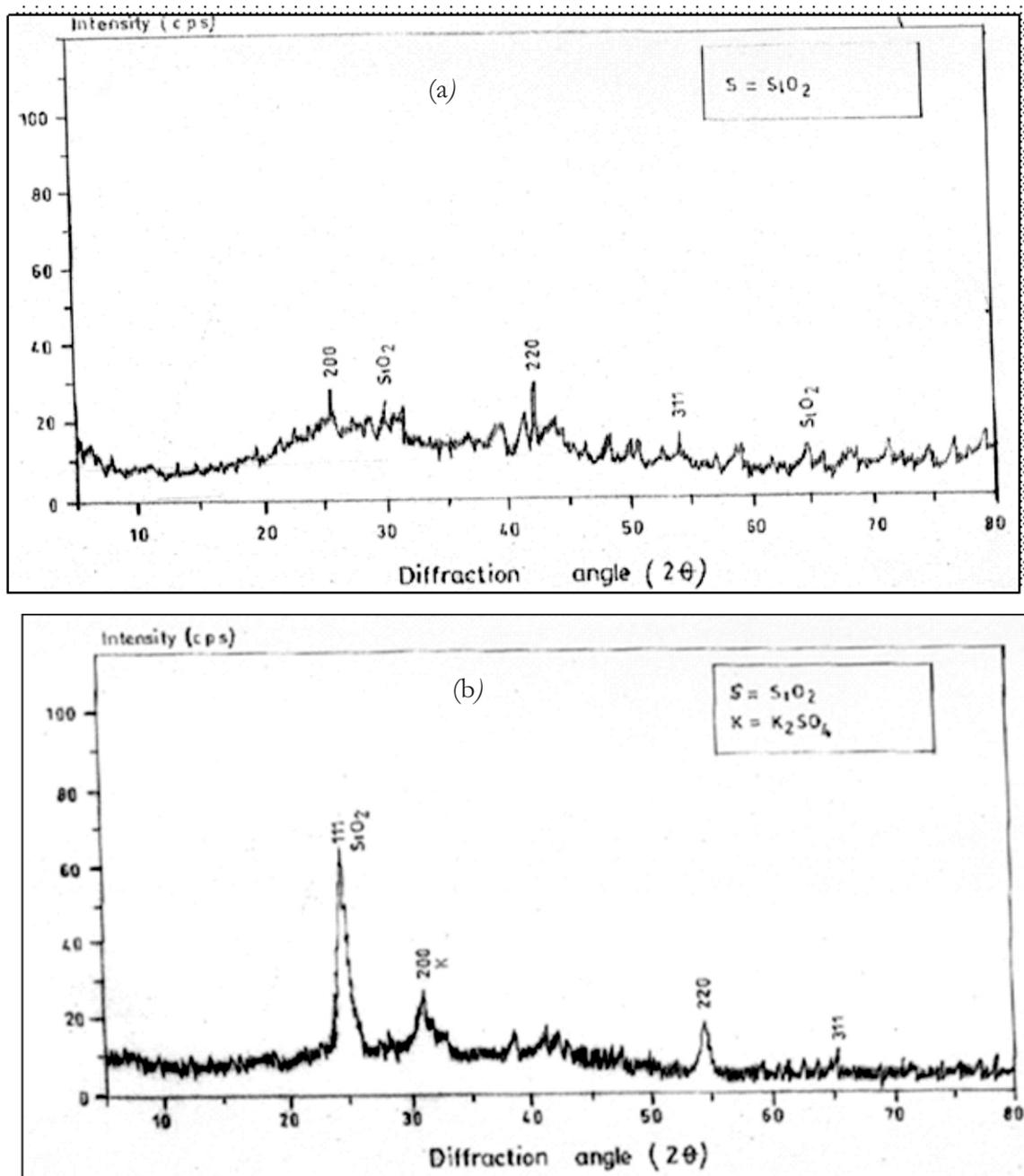


Figure 7: X-ray diffraction patterns of synthesized AgNPs from (a) *P. nigrescens* and (b) *S. nodiflora*

Antimicrobial Assay

The antimicrobial activity of the synthesized silver nanoparticles against various pathogens such as bacteria and fungi was investigated and the zones of inhibition measured in mm is summarized in Tables 1 and 2. The nanoparticles from *P. nigrescens* leaf extract showed activity in eight out of ten tested organisms as shown in Table 1. It was most active on *E. coli*, *S. aureus* and *B. subtilis* even at the lowest concentration of 31.25 mg/ml, but showed no detectable activity on *P. notatum* and *R. Stolonifer*. At 500 mg/ml, the range was from 26 mm to 14 mm while at 250 mg/ml, the range was from 24 mm to 12 mm zone of inhibition. From Table 2,

leaf extract of *S. nodiflora* showed that, at 500 – 31.25 mg/ml, it was most active on *E. coli*, with moderate activity on *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumonia*, *A. niger*, and *C. albicans*. The highest zone of inhibition 24 mm was observed at 500 mg/ml for *E. coli*. Inhibition was however beyond detectable limit for *S. typhi*, *P. notatum*, and *R. stolonifer* indicating selective activity on the ten micro-organisms used in the assay. At the lowest concentration (31.25 mg/ml), no activity was observed except on *E. coli* and *P. aeruginosa* (Table 2). The silver nanoparticles from both plant extracts exhibited antibacterial activity against broad range of Gram positive and Gram negative bacteria and thus would be effective in their treatments.

Table 1: Zones of inhibition (mm) of silver nanoparticles synthesized from leaf extracts of *P. nigrescens**

Conc. (mg/ml)	Zones of inhibition (mm)									
	S.a	E.coli	B. Sub	Ps.a	Klebs.	Sal. T	A.n	C.a	Pen	Rhi
500	26	24	20	20	14	14	18	18	-	-
250	24	20	18	18	12	12	14	14	-	-
125	20	16	14	14	10	10	12	12	-	-
62.5	18	14	12	12	-	-	10	10	-	-
31.25	12	10	10	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	40	40	40	38	36	40	28	26	26	28

Table 2: Zones of inhibition (mm) of silver nanoparticles synthesized from leaf extracts of *S. nodiflora**

Conc. (mg/ml)	Zones of inhibition (mm)									
	S.a	E.coli	B. Sub	Ps.a	Klebs.	Sal. T	A.n	C.a	Pen	Rhi
500	14	24	14	18	18	-	18	14	-	-
250	12	20	12	16	16	-	14	12	-	-
125	10	18	10	14	12	-	12	10	-	-
62.5	-	14	-	12	10	-	10	-	-	-
31.25	-	10	-	10	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	40	40	40	38	36	40	28	26	26	28

*S.a: *Staphylococcus aureus*, E.c: *Escherichia coli*, B.sub: *Bacillus subtilis*, Ps. a: *Pseudomonas aeruginosa*, Klebs: *Klebsiellae pneumonia*, Sal. t: *Salmonella typhi*, A. n: *Aspergillusniger*, C. a: *Candida albicans*, Pen : *Penicillumnotatum*, Rbi: *Rhizopusstolonifer* -ve: Negative control; Dimethylsulfoxide (DMSO); +ve: Positive control; Gentamicin 10 µg/ml for bacteria and Tioconazole 70% for fungi.

CONCLUSION

The result of this study has demonstrated the green synthesis of silver nanoparticles from leaf extracts of *P. nigrescens* and *S. nodiflora*. The nanoparticles were formed within 15 to 30 minutes of reaction and were essentially crystalline in nature. The antimicrobial results showed that the nanoparticles have activity against all the tested bacteria but are less active against some isolated fungi.

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