Synthesis of Eco-Friendly Silver Nanoparticles from *Securidaca Longepedunculata* and *Artocarpus Heterophyllus* Powders and Evaluation of their Antibacterial Properties

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Abstract

Aqueous extracts of *Securidacalongepedunculata* and *Artocarpusheterophyllus* were screened for photochemicals and both were found to contain saponins, tannins, flavonoid, cardiac glycosides, anthraquinones and alkaloids. Biosynthesis of silver nanoparticles was carried out from these extracts. The formation and stability of the reduced nanoparticles in the colloidal solutions were monitored using UV-visible spectroscopy. The Fourier transform infra-red spectroscopy (FTIR), indicated the presence of bio-molecules responsible for reduction and stabilization of the nanoparticles. The morphology of the nanoparticles formed from *Securidacalongepedunculata* was flake like and that from *Artocarpusheterophyllus* was plate-like as shown by scanning electron microscopy (SEM). The nanoparticles from both plants showed potent anti-bacterial activities towards Staphylococcus aureus, a gram positive bacterium.

Keywords: *S. longepedunculata*, *A. heterophyllus*, Eco-friendly, antibacterial activity

Introduction

The synthesis of nano-materials and their characterization has become an emerging field of nanotechnology from the past two decades, due to their application in the field of physics, chemistry, biology and medicine (Albrecht *et al,* 2006). Nanoparticles attract greater attention due to their various applications in different fields especially in nano-medcine. Metal nanoparticles are of use in various catalytic applications, electronics, biology and biomedical applications, material science etc (Raveendran *et al,* 2006). Most techniques of synthesizing nanoparticles, such as chemical reduction of ions in aqueous solutions with or without stabilizing agents (Liz-Marzan *et al,* 1996), thermal decomposition in organic solvents (Esumi *et al*, 1990), chemical reduction and photo-reduction in reverse micelles (Pileni*et al*, 2000; Sun *et al*, 2001) and radiation chemical reduction (Henglein, 1993; Henglein, 1998) etc. Most of these methods are extremely expensive and also involve the use of toxic, hazardous chemicals which may pose potential environmental and biological risks.

The use of plants for the synthesis ofnanoparticles is a rapid, low cost, eco-friendly and a single step method for biosynthesis process (Huang *et al*, 2007). The usage of plants can also be suitably scaled up for large-scale synthesis of nanoparticles in a controlled manner according to their size, shape and dispersity. As the synthesis of nanoparticles using plant extracts is extracellular, it is more beneficial than other processes. Among the nanoparticles, silver nanoparticles are attractive especially for antimicrobial sterilization. Silver is the metal of choice as they hold the promise to kill microbes effectively (Ip *et al*, 2006).

Nanoparticles have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and their ability to permeate cells. Nanotechnology has projected the effectiveness of silver particles as antimicrobial agents (Popescu *et al*, 2010).

In this work, silver nanoparticles were synthesized via eco-friendly methods using plant extracts from *Securidaca longe pedunculata* and *Artocarpus heterophyllus*. The anti-microbial activities of these nanoparticles were also evaluated to establish their potency against microorganisms.

Materials and Methods

Collection and Preparation of Plant materials

The leaves of *Securidaca longe pedunculata* and *Artocarpusheterophyllus* were obtained from Buruku Local Government of Benue state. The plants were identified in the Botany Laboratory of the Biological Science Department of Benue state University Makurdi. The plants were washed, sundried and powdered. The powdered samples were stored in clean cellophane bags in a dry place for further analysis.

Plant Extraction

The aqueous extract of each plant powder was prepared by soaking 20 g of the material in 200 mL of distilled water at room temperature. After 24 hrs, the macerates were filtered on Whatmann filter paper no. 1 and the aqueous extracts were evaporated to dryness on a water bath at 40 ^{o}C. The dried materials were maintained in a cool place in the dark (Verastegui *et al*, 1996).

Phytochemical Screening

Test for alkaloids

About 0.5 g of the sample of extract was dissolved in 5 mL of 1% HCl and kept in a boiling water bath; 1 mL of the filtrate was treated with few drops of Meyer's reagents. Turbidity of the precipitate observed was an indication that alkaloids were present (Mathur *et al*, 2011).

Test for tannins

About 0.5 g of the sample of each extract was mixed with 10 mL boiling water and filtered. A few mL of 6% $FeCl_3$ were added to the filterate and the appearance of a deep colour confirmed the presence of tannins (Mathur *et al*, 2011).

Test for flavonoids

About 0.2 mL of the sample of each extract was dissolved in CH_3OH and heated; a chip of Mg metal was added to the mixture followed by a few drops of HCl. The appearance

of a reddish orange colour indicated the presence of flavanoids (Mathur *et al*, 2011).

Test for steroids

About 0.5 mL of the sample of each extract was dissolved in 3 mL of $CHCl_3$ and filtered. To the filtrate concentrated H_2SO_4, was added which formed a lower layer. A reddish brown colour was an indication of a steroid ring (Mathur *et al*, 2011).

Test for Saponins

About 0.5 mL of the sample of each extract was shaken well with 2 mL of distilled water. The persistent foam shows the presence of saponins (Mathur *et al*, 2011).

Test for cardiac glycosides

About 0.5 mL of the sample of each extract was treated with 2 mL of glacial acetic acid and a drop of H_2SO_4 gives a brown ring indicating the presence of cardiac glycosides (Mathur*et al*, 2011).

Test for Anthraquinones

About 0.5 mL of the sample of each extract was shaken with chloroform and a few drops of ammonium solution was added, shaken slightly and kept to stand. The presence of a pink cherry red colour in the ammonia layer indicates the presence of free anthraquinones (Mathur *et al*, 2011).

Biosynthesis of silver nanoparticles

Silver nanoparticles were prepared from *S.longepedunculata* and *A. heterophyllus*. 2 g of the powdered leaves were boiled in 100 mL of distilled water for 10 minutes and left for 24 hours. The macerates were filtered and dried. 5 mL of 10% ammonium solution was added to 10 mL of 1M $AgNO_3$ solution followed by the addition of 20 mL of the plant extract and the final volume was adjusted to 50 mL using distilled water. The Erlenmeyer flasks were incubated at 37^{o}C under agitation for 24-48 hours. A colour change from yellowish to bright yellow and to dark brown was observed (Nester *et al*, 2008).

Characterization of silver nanoparticles

The optical property of the silver nanoparticles was determined by UV-vis spectrophotometer (UV-2500 PC series), the spectra were taken at different times interval between 200 to 400 nm. The FTIR analysis of the nanoparticles was taken in the range of 4000-500 cm-1 using FTIR spectrometer (Shimadzu 8400s FTIR). The morphological features of the silver nanoparticles were studied by Scanning Electron Microscope. The samples were characterized in the SEM at an accelerating voltage of 15 KV.

Antimicrobial Activity

Sensitivity nutrient agar was prepared. A sterile cotton swap was dipped into the inoculums; the excess fluid of the inoculums was squeezed against the side of the tube and then streaked all over the plate to obtain a uniform spreading in all direction on the sterile sensitivity agar plate. The appropriate impregnated disc was placed on the surface of the agar plate at a regular interval of 20 mm from each other. Pre-diffusion of the active agent in the disc was allowed for 30 minutes at room temperature, the plate was then incubated at 37^{o}C for 24 hours. The clear zones were then observed.

Result and Discussion

The leaf extracts of *Securidaca longe pedunculata* and *Artocarpus heterophyllus* were subjected to phytochemical screening using conventional methods to test for the presence of alkaloids, tannins, flavonoids, steroids, saponins, cardiac glycosides and anthraquinones. The results of the phytochemical screening is presented in Table 1

Table 1: Phytochemical Screening of *S. longepedunculata* and *A. heterophyllu*

Parameter	*S. longepedunculata*	*A. heterophyllus*
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Steriods	+	+
Saponins	+	+
Cardiac glycosides	+	+
Anthraquinones	+	+

These secondary metabolites; alkaloids, tannins, flavonoids, steroids, saponin, cardiac glycosides, and anthraquinones were present in both *S. longepedunculata* and A. *heterophyllus* extracts. The phytochemicals act as reducing and stabilizing agents for the bio-reduction reaction during the synthesis of nanoparticles (Dubey *et al*, 2009).

UV-visible spectroscopy was used to establish the preliminary formation of silver nanoparticles. The colour was noticed to change from pale yellow to dark brown. The development of the dark brown colour was due to plasma resonance property of the silver particles (Noginov *et al*, 2006). The reduction of pure silver ions was monitored by measuring the absorption maxima corresponding to the plasma resonance of silver. The maxima were obtained at 354-399.20 nm for *Securidaca longe pedunculata* as silver nanoparticles and at 362-399.20 nm for *Artocarpus heterophyllus* as silver nanoparticles respectively. The UV-visible spectra of *S. longepedunculata* and *A. heterophyllus* are shown in Figure 1 and 2 respectively.

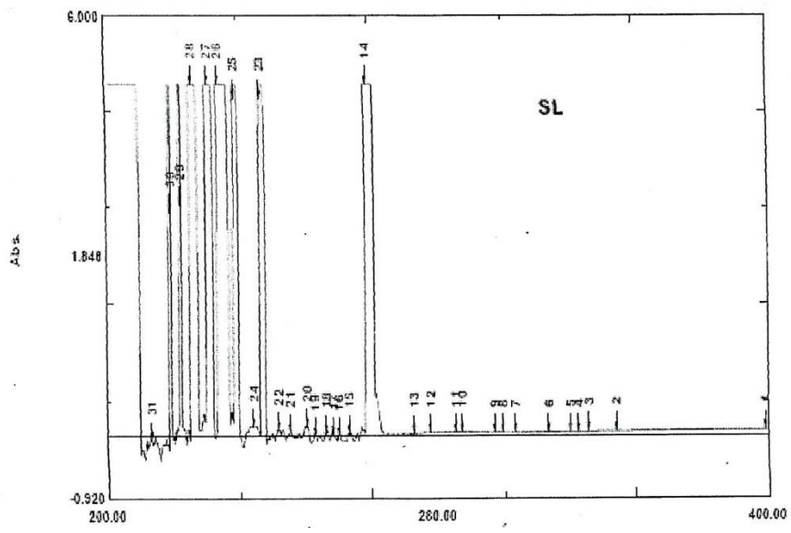

Figure 1: UV-Visible Spectrum of Silver Nanoparticles from *S. longedunculata* Extracts

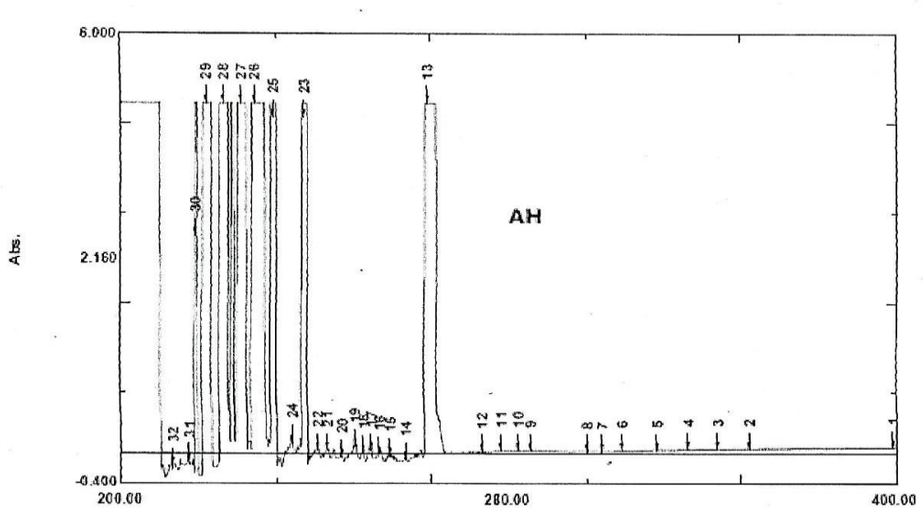

Figure 2: UV-Visible Spectrum of Silver Nanoparticles from *A. heterophyllus* Extracts

The FTIR spectra for *Securidaca longe pedunculata*, Fig. 3 and that of *Artocarpus heterophyllus*, Fig. 4 shows characteristic peak at 3350 and 3370 cm^{-1} corresponding to O-H stretching vibration, characteristic of alcohol for *S.longepedunculata* and *A.heterophyllus* respectively. Peaks at 1650 cm^{-1} for *S.longepedunculata* and 1750 cm^{-1} for *A.heterophyllus* are due to carbonyl stretch in the amide linkages of the protein. The FTIR spectra thus indicated that the secondary structures of the proteins were not affected as a consequence of the reaction with the Ag+ ions or binding with the silver nanoparticles. This result suggests that the biological molecules could possibly perform a function for the formation and stabilization of the silver nanoparticles in an aqueous medium.

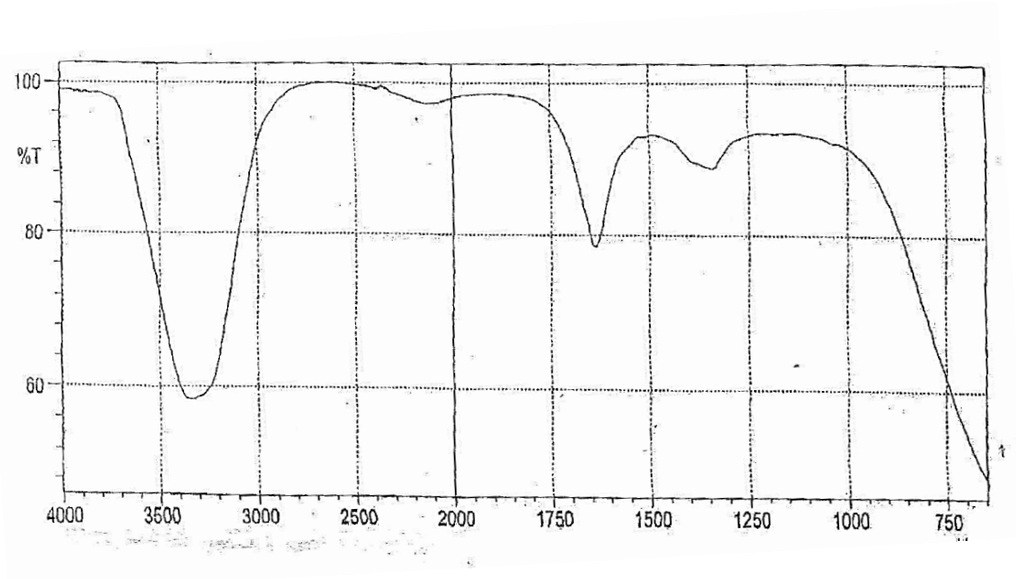

Figure 3: FTIR spectrum of *Securidaca longepeduncula*

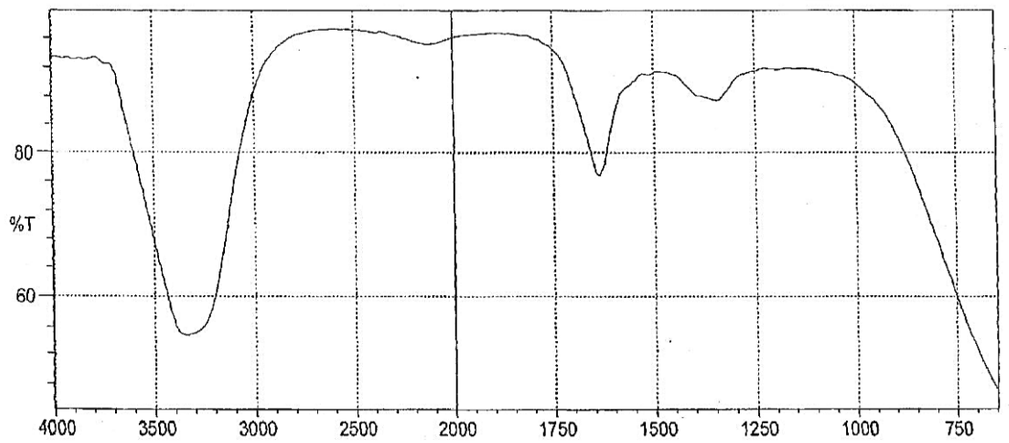

Figure 4: FTIR spectrum of *Artocarpusheterophyllus*

The morphology of the silver nanoparticles was determined by the scanning electron microscopy. The formation of silver nanoparticles as well as their morphological dimensions in the SEM study demonstrated that the average size was from 35–55 nm with inter-particle distance. The SEM spectra show a flake-like morphology for *S.longepedunculata* and a plate-like morphology for *A. heterophyllus* as silver nanoparticles, as shown in Figure 5 and 6 respectively

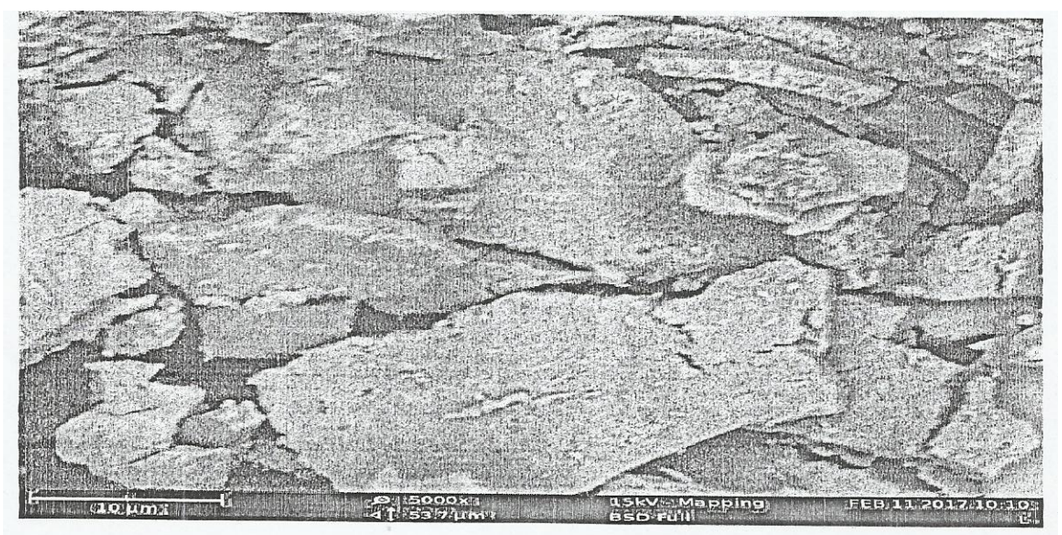

Figure 5: SEM Image of Silver Nanoparticles from *S. longepedunculata*

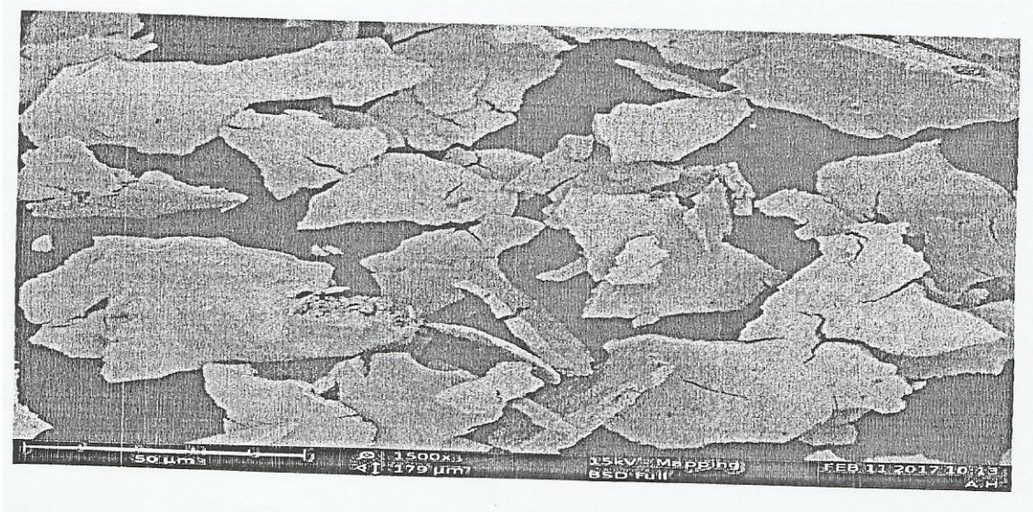

Figure 6: SEM Image of Silver Nanoparticles from *A. heterophyllus*

The results of the anti-microbial activities of the synthesized nanoparticles from *Securidaca longe pedunculata* and *Artocarpus heterophyllus* is presented in Table 2.

Table 2: Anti-Microbial Studies of Silver Nanoparticles from *S. longepedunculata* and *A. heterophyllus*

Sample No.	Inhibition (mm)	Sample No	Inhibition (mm)
SL_1	13	AH_1	13
SL_2	13	AH_2	13
SL_3	13	AH_3	13
SL_4	11	AH_4	11
SL_5	11	AH_5	11
SL_6	9	AH_6	11
SL_7	9	AH_7	9
SL_8	9	AH_8	9
SL_9	7	AH_9	7
SL_{10}	7	AH_{10}	7

SL = *Securidaca longe pedunculata*, AH = *Artocarpus heterophyllus*

From the impregnated disc of serial dilution of the inoculums of decreasing concentration from 0.5 to 0.001 labeled 1-10 for both *S. longepedunculata* and *A. heterophyllus* silver nanoparticles, it was observed that the highest zone of inhibitions against the gram positive bacteria, Staphylococcus aureus for both nano particles were at 13 mm. the inhibition decreases with decreasing concentration of the inoculums.

Conclusion

The phytochemicals present in the leaf extracts of *Securidaca longe pedunculata* and *Artocarpus heterophyllus* were evaluated. The extracts provide simple and efficient way to the synthesis of silver nanoparticles which are eco-friendly, fast and of low cost. The characterization of these nanoparticles was done using UV-visible which confirms the reduction of silver ions to silver nanoparticles. The SEM images suggest that nanoparticles were flake-like and plate-like for *S. longepedunculata* and *A.heterophyllus* respectively. The FTIR studies suggest that the protein might play an important role in the stabilization of silver nanoparticles. The anti-microbial studies show efficient anti-microbial function for both *S. longepedunculata* and *A. heterophyllus* nanoparticles.

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