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Article

Ultrasonic Synthesis of Silver Nanoparticles Mediated by *Prunus africana* Plant Extracts and Their Antibacterial Activity

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Abstract: Metal nanoparticles possess unique properties influenced by their size, distribution and morphology. There is increasing interest and demand in metal nanoparticles due to their wide applications in various fields such as medicine, electronics, catalysis, cosmetics and energy. Chemical and physical methods used in synthesis of nanoparticles are costly and unfriendly to the environment due to toxic chemicals involved. Green synthesis is rapid; cost friendly and involve non-toxic chemicals. Plants provide unique platform for green synthesis of metal nanoparticles over other green synthetic methods as they stabilize and cap the nanoparticles with biomolecules. In this study, *Prunus africana* stem bark extract was used to synthesize silver nanoparticles (AgNPs) on ultrasonic bath. The formation of AgNPs was monitored visually through colour change and by UV-Vis spectroscopy. The Energy Dispersive X-ray (EDX) analysis showed that the AgNPs were pure silver. HRTEM analysis indicated evenly distributed, monodispersed and spherical AgNPs with the average size of 23±3.06nm. FTIR analysis showed the presence of hydroxyl and -C=C- groups an indication of presence of phenolic compounds. The antibacterial activity of the synthesized AgNPs was tested against *E. coli* and *S. aureus* and were found to be toxic against these pathogens with maximum zones of inhibition of 14.21±0.208mm and 16.03±0.204mm respectively. MIC for both *E. coli* and *S. aureus* was 0.25mM. No previous reports on synthesis of silver nanoparticles using *P. africana.* The

study contributes towards application of *P. africana* extracts in generation of novel silver nanoparticles for development of drugs useful in fight against bacterial infections.

Keywords: AgNPs, *P. africana*, HRTEM, EDX, antibacterial activity.

1. Introduction

Nanotechnology is an important field of modern research dealing with synthesis, strategy and manipulation of particles of size ranging from approximately 1 to 100nm (Ahmed *et al.*, 2016). It has the potential to create new materials and certainly provide answers to world's problems related to agriculture, nutrition, health, water and energy (Hassan *et al.*, 2016). Metal nanoparticles with controlled shape and size form basis for advanced functional materials for electronic, sensor and optical devices (Gurunathan *et al.*, 2009). Generally, Metal nanoparticles are prepared by physical and chemical methods which are costly and potentially harmful to the environment (Prabhu and Poulose, 2012). Chemical methods involve use of toxic chemicals like Sodium borohydride ($NaBH_4$), Sodium Citrate and hydrazine as reducing agents (Arya, 2010). $NaBH_4$ can be a source of caustic salts and flammable gases, which contribute to various biological risks (Patnaik, 2007). Green synthesis of nanoparticles is an alternate, feasible method being developed by researchers which embrace environmentally friendly techniques. The process is rapid, economical, nonpathogenic and ecofriendly (Mukherjee *et al.*, 2008). Equally, biosynthesis of silver nanoparticles is of great interest because of their diverse range of application in bioengineering, food packaging, medical industry, cosmetics, catalysis and electrochemistry.

The green synthesis technique for assembly of metal nanoparticles involves use of plant extracts (Song and Kim, 2009), fungi (Vigneshwaran *et al.*, 2007), bacteria (Tsibakhashvil *et al.*, 2010), molds (Elgorban *et al.*, 2016) biodegradable polymers and ultrasonic probes and baths. Plant extracts are novel, greener, cost-effective and can be used as reducing agent as well as capping and stabilizing agents in the synthesis of metal nanoparticles. Biomolecules like proteins, alkaloids, flavonoids-polyphenolic compounds, vitamins, polysaccharides and terpernoids in plants are responsible for reducing, capping and stabilization of the silver nanoparticles (Gebru *et al.*, 2013). Plants synthesized nanoparticles are more stable and they can be produced faster than those synthesized by microorganisms (Firdhouse and Lalitha, 2015).

Sonication has been used for rapid green synthesis of silver nanoparticles. Aqueous extract of *Portulaca oleracea* leaves was used to synthesize AgNPs under sonication. X-ray diffraction (XRD)

and Scanning Electron Microscope (SEM) showed the size of the AgNPs was less than 60nm (Firdhouse and Lalitha, 2012).

Camellia sinensis fortified with lemon and honey was used to synthesize AgNPs by use of ultrasonic probes. The extract acted both as reducing agent as well as capping agent. The reaction rate was found to be increased through ultrasonic irradiation (Kothai and Jayanthi, 2014).

Ocimum sanctum leaf extract was used to reduce silver ions to crystalline AgNPs with particle sizes ranging from 4 to 30nm (Pavani *et al.*, 2013). The AgNPs exhibited antibacterial activity against *E. coli* and *S. aureus* (Singhal *et al*., 2011).

AgNPs synthesized using an aqueous solution of *Pulicaria glutinosa* plant extract as a bio reductant were pure and well crystalline with face-centered cubic structure. The results showed the size of the AgNPs depended on the concentration of the plant extract (Khan *et al.*, 2013)

The properties of AgNPs depend on their size, shape, morphology and distribution. The size of the AgNPs synthesized by biological methods varies between 1 and 600 nm (Rudramurthy *et al.*, 2016). Due to the unique properties of the AgNPs, they have found various medical applications in bone implants, catheters and cardiovascular treatment as they lower risk of pathogenic formation of biofilm (Cao, 2004). Additionally, AgNPs exhibit potential antibacterial effects against invasion and hinder growth of *Bacillus subtillis, S. aureus, Pseudomonas aeruginosa, E. coli* and *Treponema pallidum* (Rudramurthy *et al.*, 2016).

Studies have shown that, different plant extracts produce silver nanoparticles of different sizes and shapes, and in certain cases they produce mixed shapes (Ikram, 2015). This makes it difficult to predict how a metal would respond to a particular plant extract. Also, different AgNPs sizes have different antibacterial activities.

P. africana commonly known as African cherry, has a wide distribution in Africa growing in mountainous regions. Cameroon is one of the countries in Africa that commercially *P. africana* and exports its stem bark mostly to Europe for medicinal purpose (Cunningham, 1993). In Kenya it's found in central Kenya. It has several bio components: polyphenols (Verschaeve *et al.*, 2004) fatty acids, esters and alkanols (Kadu *et al.*, 2012) . *P. africana* has been reported to treat many disorders including fevers, malaria, stomach pain, kidney disease, gonorrhea and it's being investigated for cancer treatment (Iwu, 2014; Kokwaro, 2009).

The aim of the study was to synthesize silver nanoparticles using *P. africana* stem bark extract over ultrasonic bath. There has been no previous reports on synthesis of silver nanoparticles using *P. africana.* Formation of the AgNPs was monitored using UV-Vis and characterized by FTIR, EDX and HRTEM. The antibacterial activities of the synthesized AgNPs were tested against *E. coli* and *S. aureus* bacteria.

2. Materials and Methods

2.1. Sample Collection

Stem bark of *P. africana* plant (Figure 1) were collected from Manyatta constituency, Embu County Upper Eastern Kenya. The plant specimen was identified by a taxonomist from the Department of Plant Sciences and a voucher specimen deposited at the herbarium in Kenyatta University.

Figure 1: *P. africana* tree.

(Photo by Wilson Njue)

2.2. Plant Extraction

The stem bark was cleaned using distilled water to remove dust particles and any other impurities, then chopped and air dried for two weeks at room temperature. The dry bark was then pulverized. The plant extract (10mg/ml) was prepared by mixing 10 grams of the powder with 100ml of distilled water in a conical flask then immersed in water bath at 60^{o}C for 4 hours. The extract was filtered by vacuum filtration using Whatman filter paper No. 1. The extract was centrifuged at 4000 rpm to remove the fine plant particles. The supernatant clear solution was then stored at -4^{o}C for further use.

2.3. Ultrasonic Synthesis of Silver Nanoparticles (AgNPs)

Ultrasonic synthesis was done by the method described by Mason (1997). Ultrasonic bath (WUC-A03H) was used to facilitate reaction process. Plant extract (10mg/ml) was mixed with 0.001M $AgNO_3$ solution in a conical flask in the ratio of 1:9 and immersed in the ultrasonic bath at 25^{o}C until there was no further colour change. The formation of the AgNPs was monitored by visual observation of colour change and by measuring UV-Visible spectra at regular intervals.

2.4. Characterization and Analysis

UV-Vis spectroscopy was used to monitor the formation of the AgNPs. Scanning was done between 300 to 800 nm since absorption band for AgNPs range from 400nm to 450nm (Rashid *et al.*, 2013). Fourier Transform Infra-Red spectroscopy analysis (FTIR, Shimadzu IRT racer-100) was done to determine the nature of biomolecules responsible for capping and stabilizing the AgNPs. High Resolution Transmission Electron Microscope (HRTEM FEI Technai F20) analysis determined the morphology, shape and size distribution of the silver nanoparticles. Samples for HRTEM analysis were prepared by drop coating synthesized AgNPs solution on to carbon-coated copper HRTEM grids (Woehrle *et al.*, 2006).

2.5. Anti-bacterial Activity

Antibacterial activity was done using paper disc diffusion technique (Valgas *et al.*, 2007). The test bacterial strains were sub cultured for 24 hours. The concentration of the bacteria was determined by comparing its turbidity with McFarland solution. The inoculum (1.5×10^8 colony forming units/ml) was swabbed on the nutrient agar in sterile petri dishes. Paper discs (6mm) impregnated with AgNPs were placed on the same petri dishes then incubated for 24hrs at 37 ºC. Zones of inhibition were then measured. The magnitude of antibacterial effect against, *E. coli* (ATCC No.25922) and *S. aureus* (ATCC No14028) was determined based on the zones of inhibition (Gebru *et al.*, 2013). Vancomycin was used as the positive control for *S. aureus* and Ciprofloxacin for *E. coli*. Distilled water was used as the negative control.

3. Results and Discussion

3.1. Visual Observation and UV-Vis Spectra

The mixture of the *P. africana* stem bark extract and $AgNO_3$ solution in the ultrasonic bath changed from orange to dark brown after 120 minutes. The change in colour suggested formation of AgNPs as observed earlier on the study of biosynthesis of AgNPs using *Fusarium solani* fungi (Ingle *et al.*, 2009). The dark-brown colour was due to excitation of surface Plasmon vibrations of AgNPs (Ahmad *et al.*, 2010). The surface Plasmon resonance was a result of the combination vibrations of electrons of the AgNPs in resonance with the light wave. The specific oscillations depend on the shape and size of the particles (Henglein, 1993).

The UV-Vis spectra (Figure 2) showed peaks at λ_{max} 425nm which is characteristic of surface Plasmon resonance of AgNPs. Similar absorption at λ_{max} 425nm was obtained by Wu *et al*, 2011 on the study of surface Plasmon resonance-induced visible light photocatalytic reduction of graphene oxide: using Ag nanoparticles as a Plasmonic photocatalyst. The absorbance intensity increased steadily up to 150 minutes and after that it remained the same indicating that the reaction was

complete. Increase in the absorbance was due to the increase in concentration of silver nanoparticles. All the peaks appeared at λ_{max}425 nm indicating the silver nanoparticles formed were stable and of same size. Study done by Mafune, 2000 on formation and size control of AgNPs by laser ablation, showed nanoparticles of similar size and shape have absorption peaks at the same wavelength.

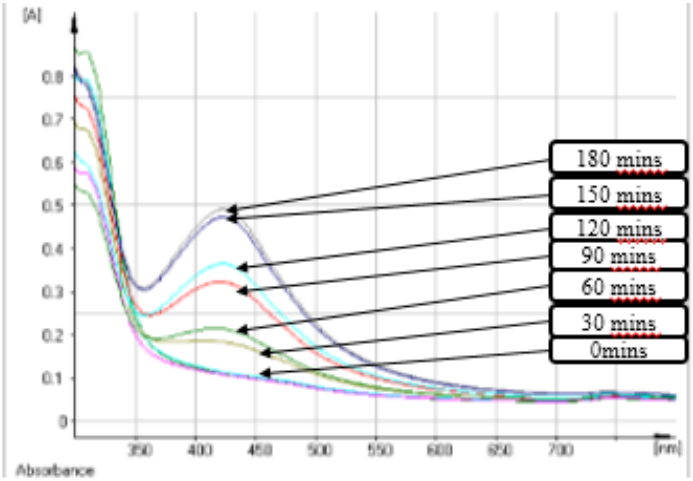

Figure 2: UV-Vis spectra on formation of AgNPs at different time intervals

3.2. High ResolutionTransmission Electron Microscope(HRTEM) Analysis

The images of HRTEM microscopy investigation of the synthesized AgNPs using *P africana* bark extract are presented in Figure 3.The synthesized AgNPs were evenly distributed and spherical. The AgNPs had non uniform surface.

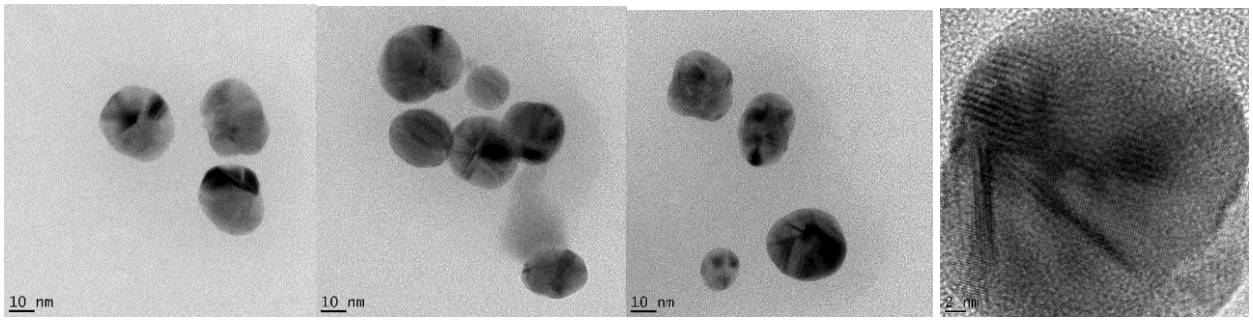

Figure 3: HRTEM micrographs of AgNPs synthesized using *P. africana* stem bark

The sizes of the synthesized nanoparticles ranged from 16 nm to 30 nm with modal class in the range of 21 nm to 25 nm (Figure. 4). This indicated that, the nanoparticles were monodispersed. The average size of the nanoparticles was 23±3.06 nm.

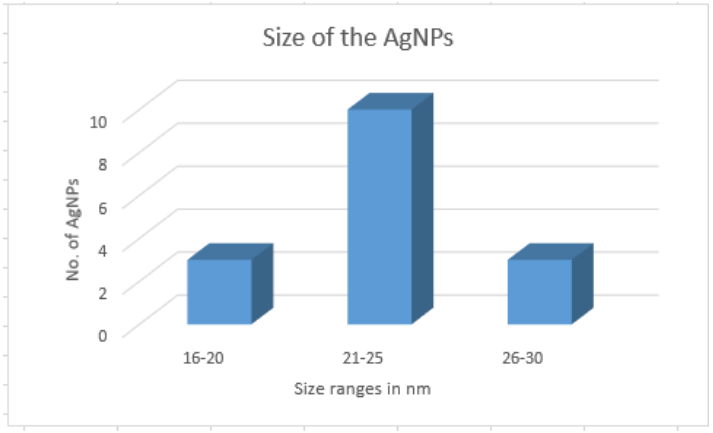

Figure 4: Size distribution of AgNPs synthesized using *P. africana* stem bark

The Selected Area Electron Diffraction (SAED) micrograph analysis (Figure 5) showed discrete shinny sports affirming the synthesized nanoparticles were crystalline.

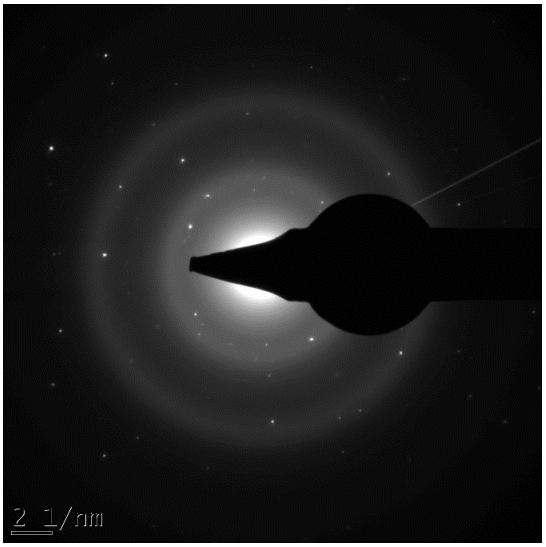

Figure 5: Selected Area Electron Diffraction image of AgNPs

The Energy Dispersive X- ray (EDX) was used to determine whether the synthesized nanoparticles were of silver. The EDX spectrum confirmed the presence of AgNPs with peaks at 3.0 KeV (Figure 6) characteristic of silver (Vijayakumar *et al.*, 2013).

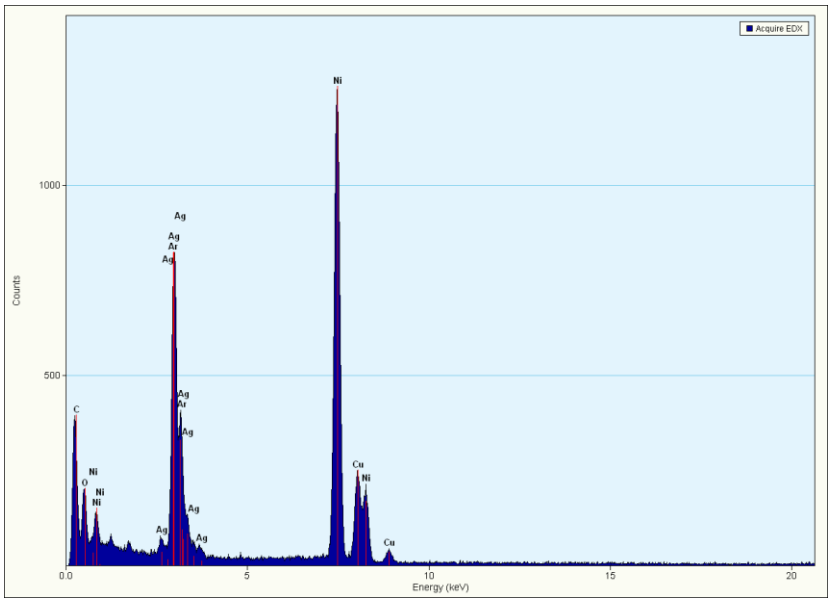

Figure 6: Energy Dispersive X-ray spectrum for AgNPs

3.4. Fourier Transform Infra-Red (FTIR) Analysis

The FTIR spectrometer was used to identify the functional groups of the plant biomolecules responsible for stabilizing and capping of the silver nanoparticles. The FTIR spectrum of AgNPs (Figure 7) showed peaks at 3438 cm^{-1} attributed to O-H stretching, 2925 cm^{-1} of C-H interlayer stretching,-1637 cm^{-1} and 1429 cm^{-1} could be attributed to- C=C- stretching for benzene ring and absorption at 1075 cm^{-1} could be C-O stretch. The above data indicates the possible involvement of phenolic compounds as capping and stabilizing agents.

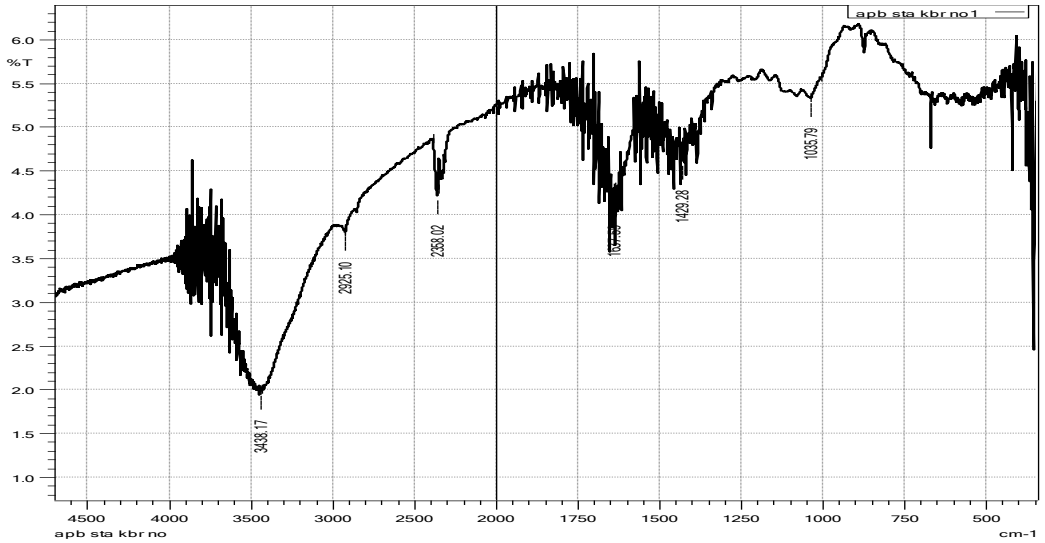

Figure 7: FTIR spectrum of AgNPs

Antibacterial activity of AgNPs from *P. africana* stem bark extract

The antibacterial activity of silver nanoparticles exhibited high inhibition on *E. coli* and *S. aureus* pathogens. Maximum inhibition zones are shown in Table 1.

Table 1: Inhibition zones of AgNPs on *E. coli* and *S. aureus*

Sample	Maximum Zones of inhibition (mm)	
	E. coli	*S. aureus*
P. africana bark AgNPs	14.21±0.208	16.03±0.204
Bark extract	9.03±0.128	7.97±0.124
Vancomycin	24.5±0.18	NA
Ciprofloxacin	NA	34.31±0.14

Note: NA – not applicable

On the gram positive bacteria *S. aureus*, the AgNPs showed inhibition zones of 16.03±0.204 compared to positive control vancomycin with an inhibition zone of 24.5±0.18 mm an indication that *S. aureus* was susceptible to AgNPs. On the gram negative bacteria, AgNPs showed maximum inhibition zone of 14.2±0.208 compared to positive control ciprofloxacin which had an inhibition zone of 34.31±0.14 nm. *E. coli* was intermediate to AgNPs according to Interpretation of zones of inhibition using Bauer-Kirby Antibiotic Susceptibility testing (Bauer *et al.*, 1966). The activity of the AgNPs can be attributed to the large surface area to volume ratio of the AgNPs. The *P. africana* bark extract showed an inhibition of 9.03±0.128 mm on *E. coli* and 7.97±0.124 mm on *S. aureus.* The Minimum Inhibitory Concentration (MIC) for *S. aureus* was 0.25mM as shown in Figure 8.

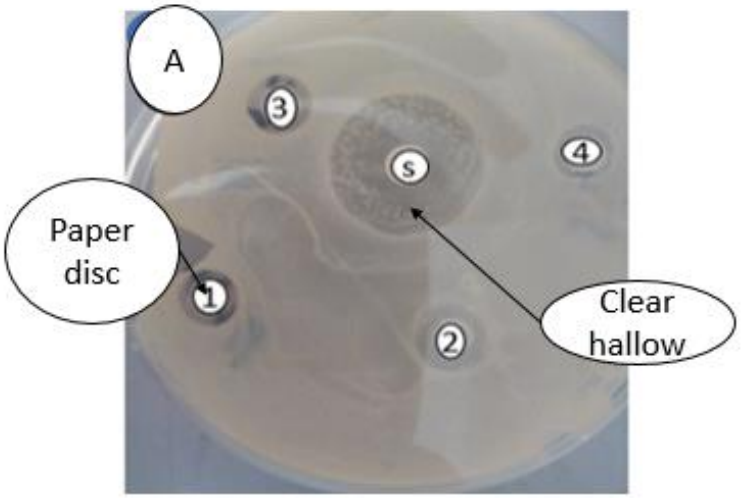

Figure 8: Zones of inhibition of AgNPs against *S. aureus*

KEY:

The clear hollows (translucent regions) show inhibition zones caused by silver nanoparticles of different concentrations; 1:-1mM AgNPs, 2:- 0.75mM AgNPs, 3:- 0.5mM AgNPs, 4:- 0.25mM AgNPs and S- vancomycin (Standard drug).

The Minimum inhibitory concentration for *E. coli* was 0.25mM as shown in Figure 9 disc 4.

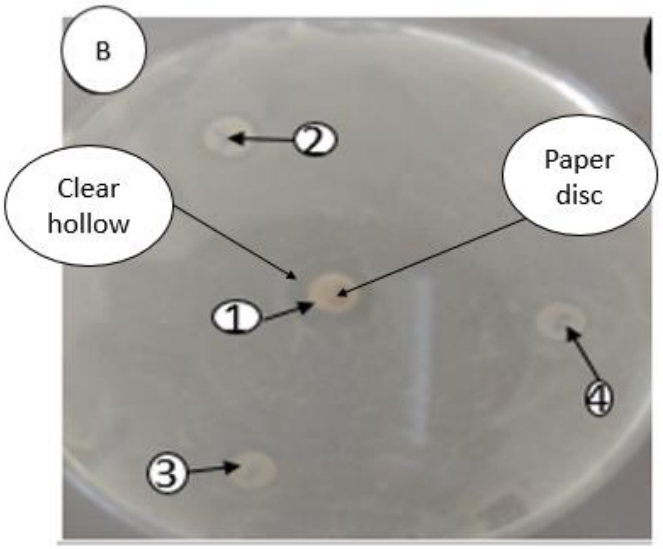

Figure 9: Zones of inhibition of AgNPs against *E. coli*

KEY:

Zones of inhibition of AgNPs synthesized using *P. africana* bark. The clear hollows (translucent regions) show inhibition zones caused by silver nanoparticles of different concentrations; 1:-1mM AgNPs, 2:- 0.75mM AgNPs, 3:- 0.5mM AgNPs and 4:- 0.25mM AgNPs.

The observed results concur with literature that AgNPs penetrate the cell walls of bacteria releasing Ag^+ which can interact with thiol groups of many enzymes and deactivate them. This stops the growth of the bacteria (Matsumura *et al.*, 2003, Li *et al.*, 2006). In this study, biosynthesized AgNPs were found to exhibit good activity on the inhibition of bacterial growth.

4. Conclusions

Biosynthesis of silver nano particles was achieved rapidly and cheaply by use of *P. africana* stem bark extract as bio reductants and stabilizing agents. High Resolution Transmition Electron Microscope (HRTEM) analysis showed the nanoparticles had non uniform surface, were spherical and monodispersed with an average size of 23±3.06 nm. The nanoparticles were pure silver as revealed by EDX. SAED indicated the nanoparticles were crystalline. The antibacterial activity showed that *E. coli* and *S. aureus* bacteria were susceptible to the AgNPs. The study showed that *P. Africana stem bark* extracts can be used to synthesize silver nanoparticles of known sizes which can be used in development of novel drugs to fight opportunistic pathogens.

Potential Conflicts of Interest

The authors declare no conflict of interest.

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References

[1] Ahmed, S., Ahmad, M., Swami, B. L. and Ikram, S. (2016). Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *Journal of Radiation Research and Applied Sciences*, **9** (1): 1-7.

[2] Ahmad, N., Sharma, S., Alam, M. K., Singh, V., Shamsi, S., Mehta, B. and Fatma, A. (2010). Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. *Colloids and Surfaces B: Biointerfaces*, **81** (1): 81-86.

[3] Arya, V. (2010). Living systems: eco-friendly nanofactories. *Digest Journal of Nanomaterials and Biostructures*, **5** (1): 9-21.

[4] Bauer, A., Kirby, W., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, **45** (4): 493.

[5] Cao, G. (2004). *Nanostructures and nanomaterials: synthesis, properties and applications*: World Scientific.

[6] Cunningham, A. B., & Mbenkum, F. T. (1993). Sustainability of harvesting Prunus africana bark in Cameroon. *People and Plants working paper*, **2**: 28.

[7] Elgorban, A., Aref, S., Seham, S., Elhindi, K., Bahkali, A., Sayed, S. and Manal, M. (2016). Extracellular synthesis of silver nanoparticles using Aspergillus versicolor and evaluation of their activity on plant pathogenic fungi. *Mycosphere*, 7(6): 844-852

[8] Firdhouse, M. J. and Lalitha, P. (2012). Green synthesis of silver nanoparticles using the aqueous extract of Portulaca oleracea (L.). *Asian Journal of Pharmaceutical and Clinical Research*, **6** (1): 92-94.

[9] Firdhouse, M. J. and Lalitha, P. (2015). Biosynthesis of silver nanoparticles and its applications. *Journal of Nanotechnology*, **2015**.

[10] Gebru, H., Taddesse, A., Kaushal, J. and Yadav, O. (2013). Green synthesis of silver nanoparticles and their antibacterial activity. *Journal of Surface Science and Technology*, **29** (1-2): 47-66.

[11] Gurunatha n, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D., Pandian, S. R. K., Muniyandi, J., Hariharan, N. and Eom, S. H. (2009). Biosynthesis, purification and characterization of silver nanoparticles using Escherichia coli. *Colloids and Surfaces B: Biointerfaces,* **74** (1): 328-335.

[12] Hassan, L. A., Elijah, A. T., Ojiefoh, O. C., Joseph, O., Sunday, B. O., Olugbenga, D. E. and Anuoluwapo, A. A. (2016). Biosynthesis of silver nanoparticles using Garcinia kola and its antibacterial potential. *African Journal of Pure and Applied Chemistry,* **10** (1): 1-7.

[13] Ikram, S. (2015). Synthesis of Gold Nanoparticles using Plant Extract: An Overview. *ARCHIVOS DE MEDICINA,* **1** (1): 5.

[14] Ingle, A., Rai, M., Gade, A. and Bawaskar, M. (2009). Fusarium solani: a novel biological agent for the extracellular synthesis of silver nanoparticles. *Journal of Nanoparticle Research,* **11** (8): 2079.

[15] Iwu, M. M. (2014). *Handbook of African medicinal plants*: CRC press.

[16] Li, Y., Leung, P., Yao, L., Song, Q. and Newton, E. (2006). Antimicrobial effect of surgical masks coated with nanoparticles. *Journal of Hospital Infection,* **62** (1): 58-63.

[17] Kadu, C. A., Parich, A., Schueler, S., Konrad, H., Muluvi, G. M., Eyog-Matig, O., Muchugi, A., Williams, V. L., Ramamonjisoa, L. and Kapinga, C. (2012). Bioactive constituents in Prunus africana: Geographical variation throughout Africa and associations with environmental and genetic parameters. *Phytochemistry,* **83:** 70-78.

[18] Khan, M., Khan, M., Adil, S. F., Tahir, M. N., Tremel, W., Alkhathlan, H. Z., Al-Warthan, A. and Siddiqui, M. (2013). Green synthesis of silver nanoparticles mediated by Pulicaria glutinosa extract. *Int J Nanomedicine,* **8** (1): 1507-1516.

[19] Kokwaro, J. O. (2009). *Medicinal plants of east Africa*: University of Nairobi press.

[20] Kothai, S. and Jayanthi, B. (2014). Ultrasound Intensified green synthesis of silver nanoparticles using Camellia sinensis extract fortified with lemon and honey. *Int. J. Chem. Tech. Res,* **6**: 248-253.

[21] Mason, T. J. (1997). Ultrasound in synthetic organic chemistry. *Chemical Society Reviews,* **26** (6): 443-451.

[22] Mafuné, F., Kohno, J.-y., Takeda, Y., Kondow, T. and Sawabe, H. (2000). Formation and size control of silver nanoparticles by laser ablation in aqueous solution. *The Journal of Physical Chemistry B,* **104** (39): 9111-9117.

[23] Matsumura, Y., Yoshikata, K., Kunisaki, S.-i. and Tsuchido, T. (2003). Mode of bactericidal action of silver zeolite and its comparison with that of silver nitrate. *Applied and environmental microbiology,* **69** (7): 4278-4281.

[24] Mukherjee, P., Roy, M., Mandal, B., Dey, G., Mukherjee, P., Ghatak, J., Tyagi, A. and Kale, S. (2008). Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum. *Nanotechnology,* **19** (7): 075103.

[25] Patnaik, P. (2007). *A comprehensive guide to the hazardous properties of chemical substances*. John Wiley & Sons.

[26] Pavani, K., Gayathramma, K., Banerjee, A. and Suresh, S. (2013). Phyto-synthesis of silver nanoparticles using extracts of Ipomoea indica flowers. *American Journal of Nanomaterials,* **1** (1): 5-8.

[27] Prabhu, S. and Poulose, E. K. (2012). Silver nanoparticles: mechanism of antibacterial action, synthesis, medical applications, and toxicity effects. *International Nano Letters,* **2** (1): 32.

[28] Rashid, M. U., Bhuiyan, M. K. H. and Quayum, M. E. (2013). Synthesis of silver nano particles (Ag-NPs) and their uses for quantitative analysis of vitamin C tablets. *Dhaka University Journal of Pharmaceutical Sciences,* **12** (1): 29-33.

[29] Rudramurthy, G. R., Swamy, M. K., Sinniah, U. R. and Ghasemzadeh, A. (2016). Nanoparticles: alternatives against drug-resistant pathogenic microbes. *Molecules,* **21** (7): 836.

[30] Singhal, G., Bhavesh, R., Kasariya, K., Sharma, A. R. and Singh, R. P. (2011). Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. *Journal of Nanoparticle Research,* **13** (7): 2981-2988.

[31] Song, J. Y. and Kim, B. S. (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess and biosysHRTEMs engineering,* **32** (1): 79-84.

[32] Tsibakhashvil, N., Kalabegishvili, T., Gabunia, V., Gintury, E., Kuchava, N., Bagdavadze, N., Pataraya, D., Gurielidzse, M., Gvarjaladze, D. and Lomidze, L. (2010). Synthesis of silver nanoparticles using bacteria. *Nano Stud,* **2:** 179-182.

[33] Valgas, C., Souza, S. M. d., Smânia, E. F. and Smânia Jr, A. (2007). Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology,* **38** (2): 369-380.

[34] Vigneshwaran, N., Ashtaputre, N., Varadarajan, P., Nachane, R., Paralikar, K. and Balasubramanya, R. (2007). Biological synthesis of silver nanoparticles using the fungus Aspergillus flavus. *Materials Letters,* **61** (6): 1413-1418.

[35] Vijayakumar, M., Priya, K., Nancy, F. T., Noorlidah, A., & Ahmed, A. B. A. (2013). Biosynthesis, characterisation and anti-bacterial effect of plant-mediated silver nanoparticles using Artemisia nilagirica. *Industrial Crops and Products*, **41**: 235-240.

[36] Verschaeve, L., Kestens, V., Taylor, J., Elgorashi, E., Maes, A., Van Puyvelde, L., De Kimpe, N. and Van Staden, J. (2004). Investigation of the antimutagenic effects of selected South African medicinal plant extracts. *Toxicology in vitro,* **18** (1): 29-35.

[37] Vidhu, V. and Philip, D. (2014). Catalytic degradation of organic dyes using biosynthesized silver nanoparticles. *Micron,* **56:** 54-62.

[38] Woehrle, G. H., Hutchison, J. E., Özkar, S. and FINKE, R. G. (2006). Analysis of nanoparticle transmission electron microscopy data using a public-domain image-processing program, image. *Turkish Journal of Chemistry,* **30** (1): 1-13.

[39] Wu, T., Liu, S., Luo, Y., Lu, W., Wang, L., & Sun, X. (2011). Surface plasmon resonance-induced visible light photocatalytic reduction of graphene oxide: using Ag nanoparticles as a plasmonic photocatalyst. *Nanoscale,* **3**(5): 2142-2144.