

Full Length Research Paper

Green sonochemical synthesis of silver nanoparticles using *Bridelia micrantha* extract and evaluation of their antibacterial activity

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The emergence of drug resistance and multiple drug resistance has necessitated the discovery of novel strategies for development of new generation of antimicrobial agents from natural substances for control of microbial infections. Metal nanoparticles have proven to have antimicrobial properties, but the chemical methods used in their production use hazardous chemicals and nanoparticles produced are unstable. In this study, an alternative feasible environmentally friendly method was used for the synthesis of silver nanoparticles using *Bridelia micrantha*, a Kenyan medicinal plant as a reducing, stabilizing and capping agent. The reaction was done over ultrasonic bath. Formation of the nanoparticles was monitored by visual observation and also by use of UV-VIS spectrophotometer. The synthesized silver nanoparticles had an absorption peak at λ_{\max} 431 nm due to plasmon resonance. Energy dispersive X-ray (EDX) analysis showed the synthesized nanoparticles were pure silver. High resolution transmission electron microscope (HRTEM) analysis showed the nanoparticles had non uniform surface and were spherical with an average size of 16.07 ± 3.192 nm. Scanning area electron diffraction (SAED) showed distinct shiny spots, confirming the crystallinity of the nanoparticles. Fourier transform infrared (FTIR) analysis indicated the presence of biomolecules capping the nanoparticles. The silver nanoparticles inhibited growth of *Escherichia coli* and *Staphylococcus aureus*. The data from this study will significantly contribute in designing novel methods geared towards development of drugs to combat pathogens by use of silver nanoparticles synthesized in an environmentally and ecofriendly way.

Key words: Antibacterial activity, *Bridelia micrantha*, energy dispersive X-ray (EDX), scanning area electron diffraction (SAED), high resolution transmission electron microscope (HRTEM), silver nanoparticles (AgNPs).

INTRODUCTION

The rise in emerging infectious diseases and their impact in increased incidences of drug resistance is well

documented (Weisblum, 1998). Thus, there is a pressing demand to discover novel strategies and identify new

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Figure 1. *Bridelia micrantha*.
Source: Photo by Wilson Njue.

antimicrobial agents from natural and inorganic substances to develop the next generation of drugs or agents to control microbial infections (Taylor, 2013).

Nanoparticles have shown great antimicrobial properties. The unique properties of nanoparticles in the size range from 1 to 100 nm (European, 2011) have made the field of nanotechnology to be one of the most active areas of research in modern material science. The size, shape and morphology of nanoparticles are the vital parameters for the properties and applications of the nanoparticles (Sithara et al., 2017). Different methods have been used in the synthesis of nanoparticles, which can be categorized into chemical, physical and green methods (Ahmed et al., 2016). Chemical methods used are not environmentally friendly as they involve the use of toxic chemicals like sodium borohydride and hydrazine as reducing and capping agents (Arya, 2010). Nanoparticles synthesized via chemical methods have some toxic chemicals adsorbed on the surface that may have adverse effects in medical applications (Geethalakshmi and Sarada, 2010).

Plant extracts (Song and Kim, 2009), fungi (Vigneshwaran et al., 2007), bacteria (Tsubakhashvil et al., 2010), molds (Elgorban et al., 2016), biodegradable polymers and sonicators (Perelshtein et al., 2008) have been used in green synthesis of metal nanoparticles. The green methods of synthesis of metal nanoparticles are rapid, economical, ecofriendly, compatible for pharmaceuticals and can be easily scaled up for large scale synthesis (Mukherjee et al., 2008). Plants contain biomolecules like proteins, alkaloids, flavonoids,

polyphenolic compounds, vitamins, polysaccharides and terpenoids which can reduce, cap and stabilize nanoparticles (Gebru et al., 2013). Plant biosynthesized nanoparticles are more stable and are produced faster than those synthesized by microorganisms (Firdhouse and Lalitha, 2015). Metal nanoparticles have been synthesized via sonochemical method by applying ultrasound radiation (20 KHz to 10 MHz) or use of ultrasonic bath (sonicator) where metal nanoparticles of different sizes are formed (Esmaeili-Zare et al., 2012). The physical phenomenon in sonochemistry involves cavitation and nebulization. In sonication, cavitation involves formation, growth and implosive of a bubble in liquid which creates conditions suitable for synthesis of a wide variety of nanostructures (Bang and Suslick, 2010). Silver nanoparticles of varied sizes, 4 to 56 nm, have been synthesized via sonochemical method using k-carageenan as reducing and stabilizing agent at different concentrations (Elsupikhe et al., 2015). Also, ultrasonication has been used to synthesize silver nanoparticles of size 100 nm using citrate as the reducing and capping agent (Skiba et al., 2018).

Synthesis of metal nanoparticles using different plant extracts and their probable application has been reported (Ashishie et al., 2018; Thakkar et al., 2010). The greatest challenge in the biosynthesis is that, different plants produce metal nanoparticles of different sizes and shapes (Ikram, 2015). Metal nanoparticles of different sizes tend to have different antimicrobial activities. Silver nanoparticles have been reported to exhibit strong antiseptic, antibacterial, antifungal and antiviral properties thus making them to be of great interest in the medical field (Franci et al., 2015). Hence, there was need to evaluate the use of *Bridelia micrantha* in synthesis of silver nanoparticles and test their antibacterial activity. *B. micrantha* belong to the family Euphorbiaceae and is known by Kenyan local names as *Mukoigo* (Kikuyu) and *Odugu-Kulo* (Luo). *B. micrantha* is traditionally used in Asia and Africa for treatment of various ailments like bronchitis, anaemia and sexually transmitted diseases (Kokwaro, 2009; Munayi, 2016). A study by Munayi (2016) indicated that *B. micrantha* can be used in treatment of diabetes mellitus, syphilis, tape worm, abdominal pain, headache, pneumonia, sore eyes and coughs. The analysis of *B. micrantha* phytochemicals showed that it contains various secondary metabolites like flavonoids, saponins, lignans and triterpenes (Ngueyem et al., 2009). It was thus identified for this research because of the variety of medicinal properties reported including antioxidants and is readily available. There has been no previous reports of *B. micrantha* mediated biosynthesis of silver nanoparticles (AgNPs).

MATERIALS AND METHODS

Stem bark of *B. micrantha* (Figure 1) was obtained from Manyatta constituency, Embu County, Kenya. The plant specimen was



Figure 2. *B. micrantha* bark extract and AgNO_3 solution on sonication (i) 0 min (ii) 60 min of sonication.

identified by taxonomist from Department of Plant Sciences and voucher specimen deposited at the herbarium in Kenyatta University.

Sample preparation

The *B. micrantha* 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. Retsch grinder (Retsch 200 made in Germany), was used to pulverize the bark into fine powder. The plant extract was prepared by mixing 10 g of the dried powder with 100 mL of distilled water then heated for 3 h at 60°C . The extract was filtered using What man filter paper No. 1 to obtain a clear filtrate. The filtrate was then centrifuged for 10 min to remove the fine plant particles. The extract was stored at -4°C for further use.

Ultrasonic synthesis of AgNPs

The synthesis was done by the method described by (Mason, 1997) with some modifications. Sonicator bath (WUC-A03H) of frequency 20 KHz was used to facilitate the synthesis. The plant extract was mixed with 0.001 M AgNO_3 solution in the ratio of 1:9 in a conical flask then immersed in the ultrasonic bath until there was no further colour change in the solution.

UV-Vis spectroscopy

UV-Vis spectroscopy (Specord 200 Analytik jena) was used to monitor the formation of AgNPs. Scanning was done at regular intervals to check the intensity of the optical density of the absorption band in the range from 400 to 450 nm (Rashid et al., 2013). Water was used as blank.

Fourier transform infra-red spectroscopy (FTIR) analysis

Measurements were done using FTIR (Shidmanzu IRt racer-200) to determine the functional groups of biomolecules capping and stabilizing the silver nanoparticles (AgNPs). The sample was

centrifuged at 5,000 rpm for 20 min to obtain a solid at the bottom of the centrifuge tube. The solid was ground with KBr. The solid material was pressed to obtain a pellet, which was used for FTIR analysis.

High resolution transmission electron microscope (HRTEM) analysis

The size, shape and morphology of the AgNPs were determined by HRTEM (FEI Tecnai F20). The samples for HRTEM analysis were prepared by drop coating the AgNPs solution onto carbon-coated copper TEM grids (Woehrl et al., 2006).

Anti-bacterial activity

The antibacterial activity was done using paper disc diffusion technique as per method by Piddock (1990). The test bacterial strains were sub cultured for 24 h. 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 (6 mm) impregnated with AgNPs were placed on the same petri dishes then incubated for 24 h at 37°C . Zones of inhibition were then measured. The magnitude of antimicrobial effect against, *Escherichia coli* (ATCC No.25922) and *Staphylococcus aureus* (ATCC No14028) was determined based on the inhibition zone measured (Gebu et al., 2013). The AgNPs exhibiting activity had the minimum inhibitory concentration (MIC) determined. Vancomycin was used as the positive control for *S. aureus* and Ciprofloxacin for *E. coli*. Distilled water was used as the negative control.

RESULTS AND DISCUSSION

The reaction mixture of *B. micrantha* stem bark extract and silver nitrate solution changed from red brown to dark brown in 60 min an indication of formation of AgNPs (Figure 2).

The colour change to dark brown was due to excitation

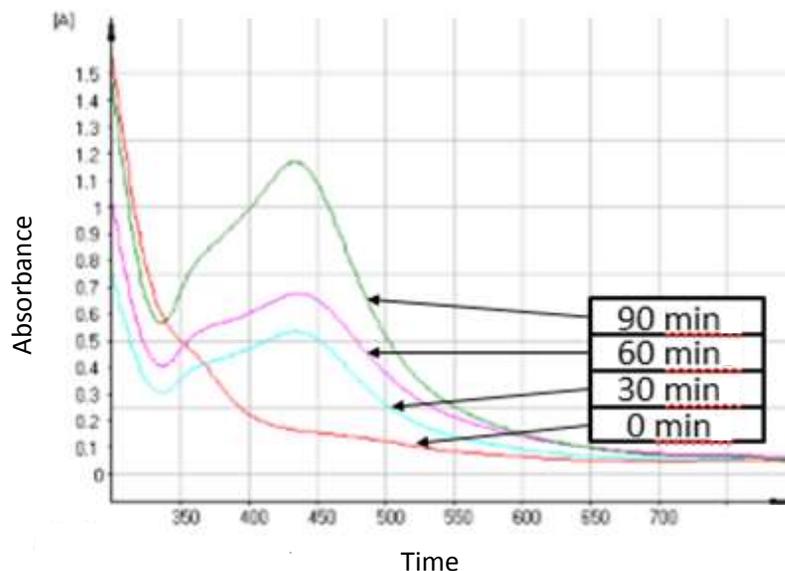


Figure 3. UV-Vis spectra on formation of AgNPs using *B. micrantha* bark extract.

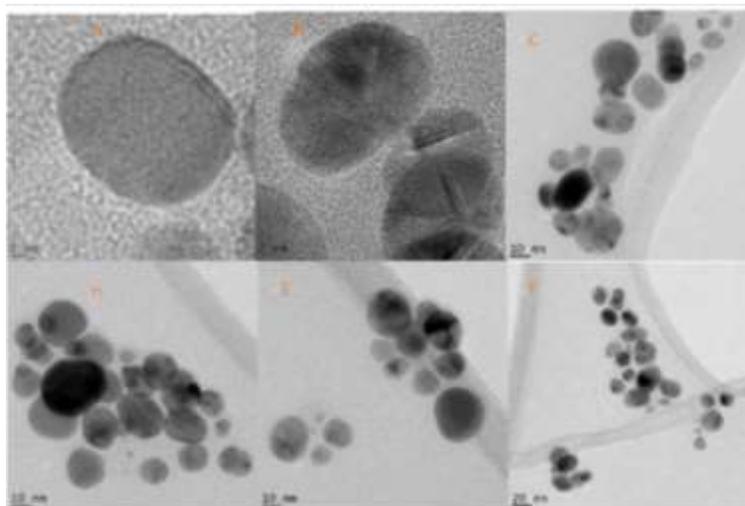


Figure 4. Transmission electron microscope micrographs of AgNPs at different magnifications.

of surface Plasmon vibrations. This is the combination vibrations of electrons of the AgNPs in resonance with the light wave (Sathiya and Akilandeswari, 2014).

The UV-Vis absorption spectra of the synthesized nanoparticles at different time intervals is as shown in Figure 3. There was a peak at λ_{max} 431 nm.

The steady peak at the same wavelength (λ_{max} 431 nm) indicated that, the nanoparticles were mono dispersed in the solution without aggregation. Similar observation was made on the study of photo-chemically grown AgNPs with wavelength-controlled size and shape (Callegari et al., 2003). The optical density increased with

time up to 90 min. Increase in optical density of the solution suggested increase in concentration of AgNPs (Maillard et al., 2003).

High resolution transmission electron microscope (HRTEM) analysis

The high resolution transmission electron microscope of the images of the synthesized silver nanoparticles are as shown in Figure 4. The AgNPs had non-uniform surface and were quasi spherical as indicated in Figure 4. The

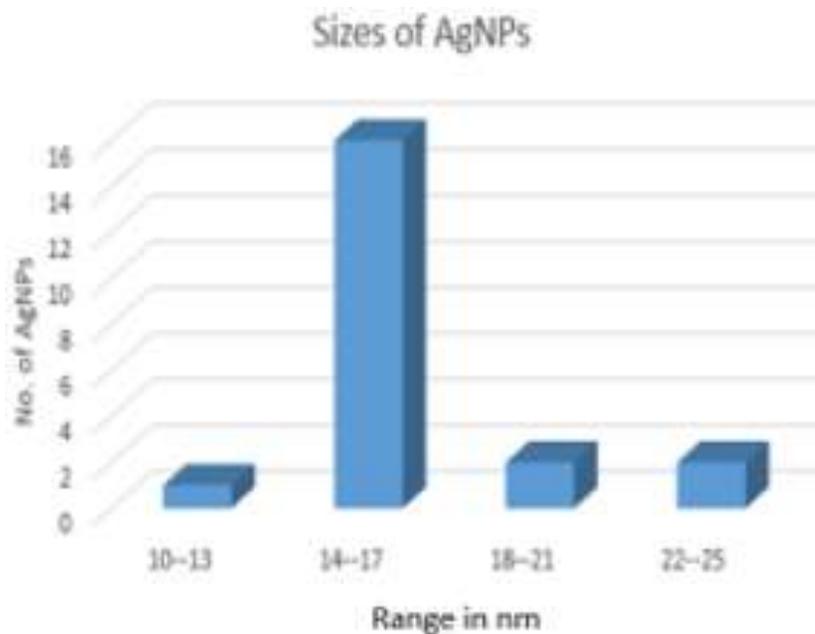


Figure 5. Size distribution of AgNPs.

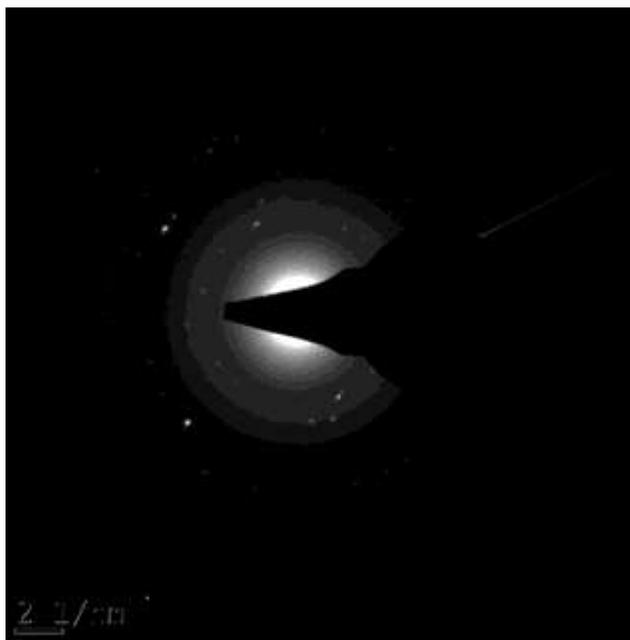


Figure 6. SAED micrograph of AgNPs.

particles were evenly distributed.

The size distribution of the AgNPs determined from HRTEM analysis are shown in Figure 5. The sizes ranged from 10 to 25 nm with mode range being 14 to 17 nm. The average diameter of the nanoparticles was 16.07 ± 3.192 nm. The narrow range of the size indicated the nanoparticles was monodispersed.

Scanning area electron diffraction (SAED) analysis

The SAED image showed discrete shiny rings confirming crystalline nature of the AgNPs (Figure 6). SAED images with shiny spots in circular rings are due to crystalline nature of metals (Rajeshkumar and Veena, 2018).

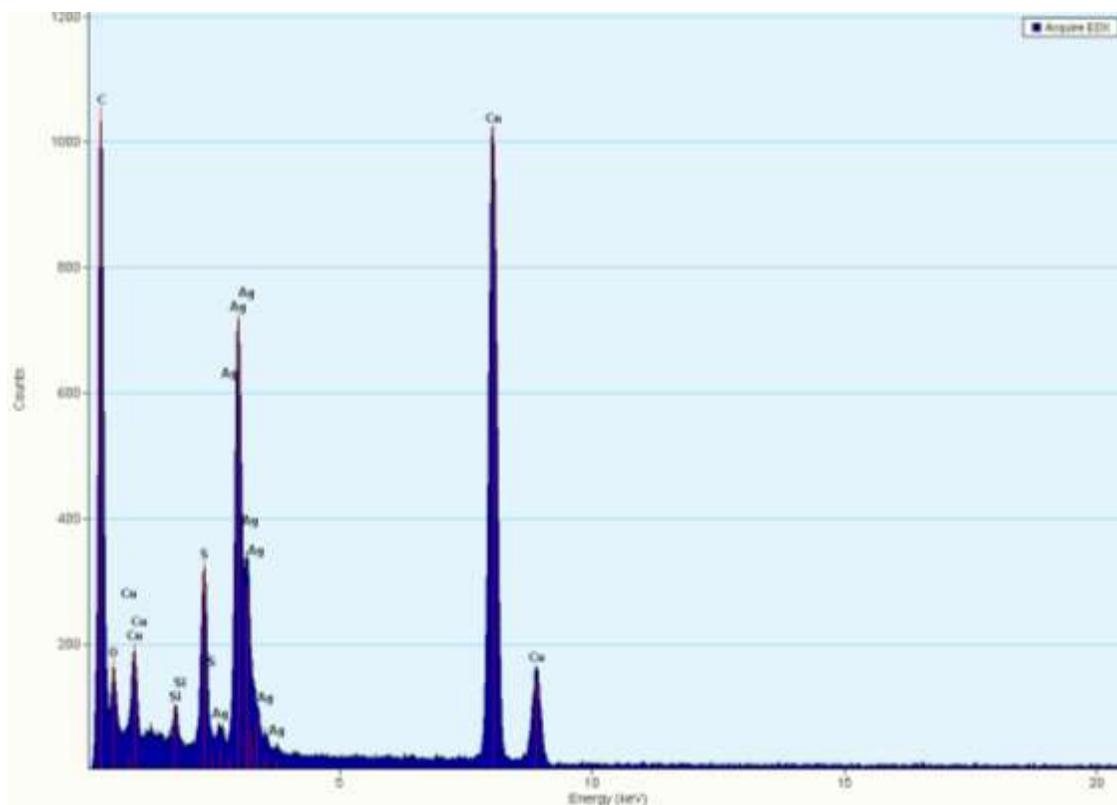


Figure 7. EDX spectrum of AgNPs.

Energy dispersive X-ray (EDX) analysis

The EDX spectrum as shown in Figure 7 which had overlaying peaks at 3.0 Kev confirming the synthesized nanoparticles was of silver. Similar peaks in EDX spectrum were observed in the study of synthesis of AgNPs using *Kigelia africana* (Ashishie et al., 2018).

Fourier transform infra-red spectroscopy (FTIR) analysis

FTIR analysis of AgNPs showed bands at 3433 cm^{-1} for O-H bond stretching and at 1634 cm^{-1} corresponding to -C=C- stretching (Figure 8) an indication of involvement of biomolecules in capping of silver nanoparticles. The functional groups -OH and -C=C- stretching frequencies were probably due to phenolic compounds especially flavonoids. Phytochemical studies on extracts from *B. micrantha* showed the presence of phenols (Wilkins and Bohm, 1976).

Antibacterial activity of synthesized AgNPs

AgNPs from *B. micrantha* plant extract showed

antibacterial activity on both Gram negative and Gram positive bacteria. The zones of inhibition measured after 24 h of inoculation were recorded in Table 1.

AgNPs had inhibition zones of 19.05 ± 0.0481 mm on *S. aureus*. *S. aureus* was susceptible to the AgNPs as compared to the zone of inhibition (22.1 ± 0.12 mm) of the standard, Vancomycin. The minimum inhibitory concentration of AgNPs on *S. aureus* was 0.125 mM. On Gram-negative bacteria (*E. coli*), AgNPs formed had inhibition zones of 16.13 ± 0.098 mm while the standard, Ciprofloxacin had inhibition zone of 33.4 ± 0.542 mm. The MIC for *E. coli* was 0.25 mM. The *B. micrantha* extract showed inhibition zones of 11.0 ± 0.002 mm on *S. aureus* and 10.5 ± 0.012 mm on *E. coli*. Thus increase in the inhibition zones was due to the AgNPs. Studies have shown that, AgNPs can penetrate through the bacteria cell membrane thus making the bacteria to lose viability and eventually leading to death (Matsumura et al., 2003).

AgNPs had higher inhibition (19.05 ± 0.0481 mm) on the Gram-positive bacteria compared to Gram-negative bacteria (16.13 ± 0.098 mm). On the contrary, AgNPs have been shown to have higher inhibition on Gram-negative than on Gram-positive. In this study, the higher inhibition on Gram-positive than Gram-negative can be attributed to the synergistic effect of the plant extract and the AgNPs. Study done on synthesis of AgNPs using *K. africana* fruit extract and evaluation of their antimicrobial

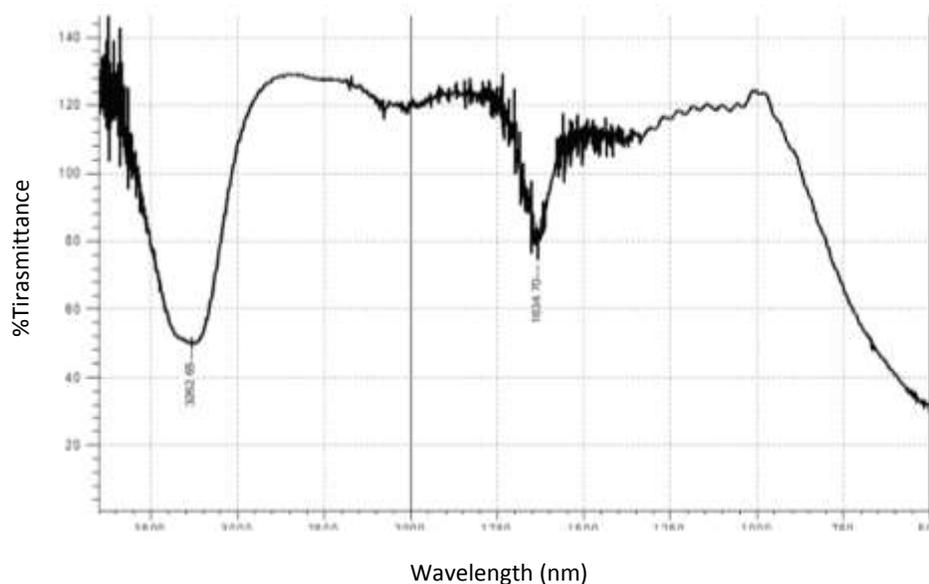


Figure 8. FTIR Spectrum of AgNPs.

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

Sample	Zones of inhibition (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
<i>B. micrantha</i> bark AgNPs	16.13±0.098	19.05±0.048
<i>B. micrantha</i> stem bark extract	10.5±0.012	11.0±0.002
Vancomycin	N/A	22.1±0.12
Ciprofloxacin	33.4±0.542	N/A
Distilled water	6	6

N/A: Not applicable.

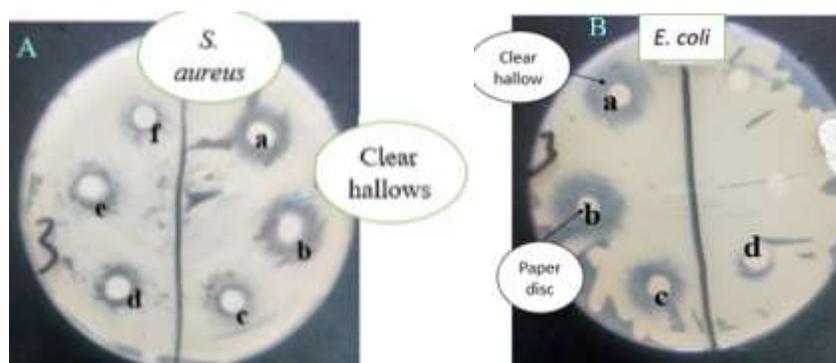


Figure 9. Effect of AgNPs on the bacteria. (A) Effect of AgNPs on *S. aureus*. (B) Effect of AgNPs on *E. coli*.

activities also showed high inhibition effect of AgNPs on Gram-positive than Gram-negative (Ashishie et al., 2018).

Zones of inhibition of AgNPs against Gram-positive bacteria *S. aureus* are as shown in Figure 9A. The clear hallow (translucent regions) show inhibition zones

caused by silver nanoparticles of different concentrations; a: 1 mM AgNPs, b: 0.75 mM AgNPs, c: 0.5 mM AgNPs, d: 0.25 mM AgNPs, e: plant extract, f: 0.125 mM AgNPs. The minimum inhibitory concentration was 0.125 mM as shown in Figure 9A disc f.

Zone of inhibition of AgNPs against Gram-negative bacteria *E. coli* is displayed in Figure 9B. The clear hollows (translucent regions) show inhibition zones caused by silver nanoparticles of different concentrations; a: 1 mM AgNPs, b: 0.75 mM AgNPs, c: 0.5 mM AgNPs, d: 0.25 mM AgNPs. The minimum inhibitory concentration for *E. coli* was 0.25 mM as shown in Figure 9B disc d.

Conclusion

AgNPs were successfully synthesized using *B. micrantha* extract as reducing as well as capping agent. The method was cheap, ecofriendly and rapid. The UV-Vis spectrometer confirmed the formation of silver nanoparticles with an absorption peak at λ_{\max} 431 nm. The functional groups -OH and -C=C- stretching frequencies in FTIR were probably due to phenolic compounds found in *B. micrantha*. HRTEM analysis showed that the synthesized silver nanoparticles were spherical, monodispersed with average size of 16.07 ± 3.192 nm. The synthesized nanoparticles showed ability to inhibit the growth of *S. aureus* and *E. coli*. They had inhibition zones of 19.05 ± 0.0481 mm for Gram-positive *S. aureus* and 16.13 ± 0.098 mm for Gram-negative *E. coli* hence the novel silver nanoparticles synthesized can be used in development of new drugs to fight bacterial pathogens. This is the first report on green chemistry route in biosynthesis of AgNPs using *B. micrantha* plant extract.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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