

SONOCHEMICAL BIOSYNTHESIS OF SILVER NANOPARTICLES USING *BRIDELIA MICRANTHA* AND EVALUATION OF THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Metal nanoparticles have proven to have antimicrobial properties, but the chemical methods used in their production involve use hazardous chemicals. In this study, ecofriendly method was used for the synthesis of silver nanoparticles using B. micrantha. The reaction was done over ultrasonic bath. Formation of the nanoparticles was monitored by use of UV-VIS spectrophotometer and absorption peak at λ_{max} 431 nm was obtained. EDX analysis showed the nanoparticles were pure silver. HRTEM analysis showed the nanoparticles had non uniform surface and were spherical with an average size of 16.07 ± 3.192 nm. SAED showed distinct shiny spots, confirming crystallinity of the nanoparticles. FTIR analysis indicated the presence of biomolecules capping the nanoparticles. The silver nanoparticles inhibited growth of E.coli and S. aureus. The study contributes in designing novel methods geared towards development of drugs to combat pathogens by use of silver nanoparticles.

KEY WORDS: Antibacterial activity, *Bridelia micrantha*, HRTEM, EDX, SAED, Silver nanoparticles.

1. 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 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 of 1 to 100 nm (EUROPEAN, C. 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 (Tsibakhashvil *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 (Gebbru *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 (20KHz –10MHz) 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).

Synthesis of metal nanoparticles using different plant extracts and their probable application has been reported (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).

2. MATERIALS AND METHODS

2.1 Materials

Stem bark of *Bridelia micrantha* (Figure 1) was obtained from Manyatta constituency, Embu County, Kenya.

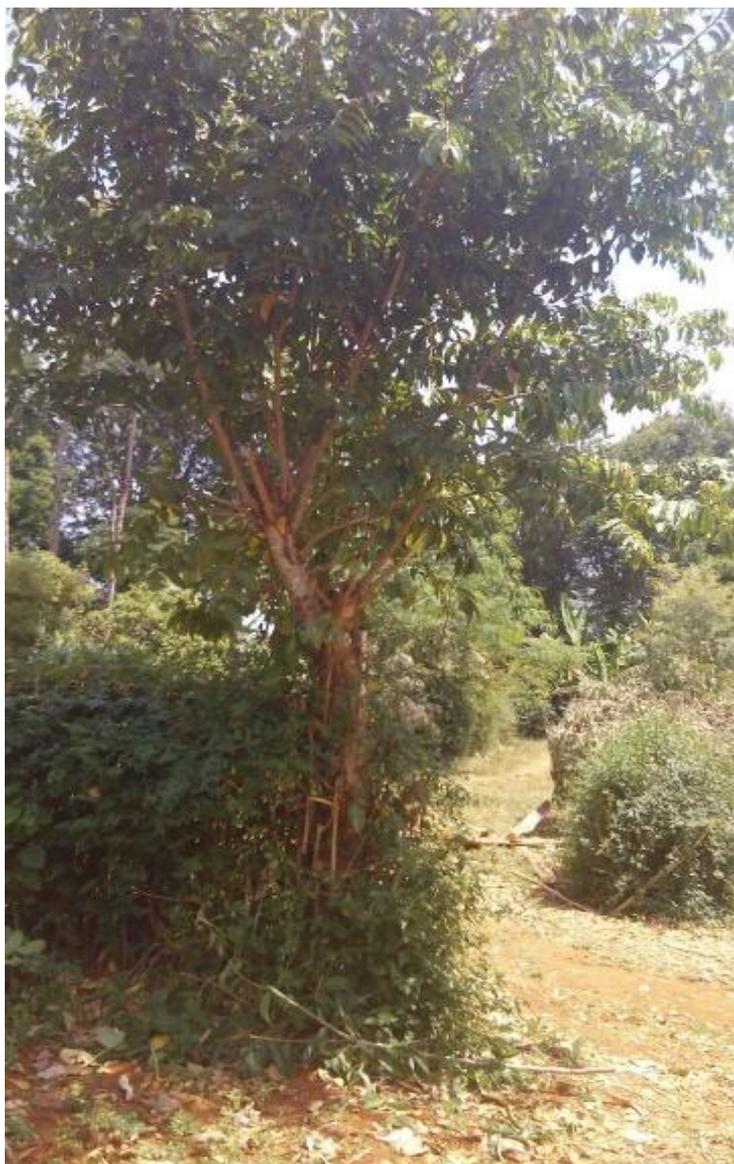


Figure 1: *Bridelia micrantha* Photo by Wilson Njue

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

2.4 Sample preparation

The *Bridelia 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 10g of the dried powder with 100mL of distilled water then heated for 3 hours 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 minutes to remove the fine plant particles. The extract was stored at -4 °C for further use.

2.6 Ultrasonic biosynthesis of AgNPs

The synthesis was done by the method described by (Mason, 1997) with some modifications. Sonicator bath (WUC-A03H) was used to facilitate the synthesis. The plant extract was mixed with 0.001M AgNO₃ 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.

2.7 UV-Vis spectroscopy

UV-Vis spectroscopy 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 400nm to 450nm (Rashid *et al.*, 2013). Water was used as blank.

2.8 Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis

FTIR measurements were done to determine the functional groups of biomolecules capping and stabilizing the silver nanoparticles (AgNPs). The sample was centrifuged at 5,000 rpm for 20 minutes 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.

2.9 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 on to carbon-coated copper TEM grids (Woehrle *et al.*, 2006).

3.0 Anti-bacterial activity

The antibacterial activity was done using paper disc diffusion technique as per method by LJV Piddock, 1990. 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°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 (Gebbru *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.

3. RESULTS AND DISCUSSION

3.1 Colour development and UV-Vis spectra

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

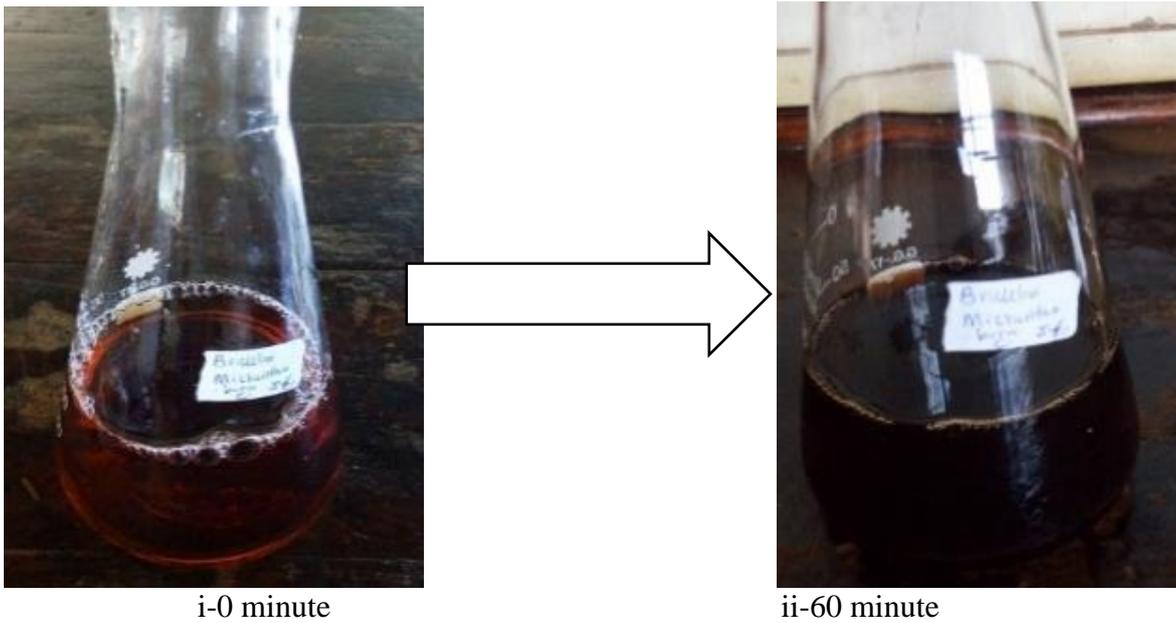


Figure 2: *B. micrantha* bark extract and AgNO₃ solution on sonication (i) 0 minute (ii) 60 minutes of sonication.

The colour change to dark brown was due to excitation 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 shown in Figure 3. There was a peak at λ_{max} 431 nm.

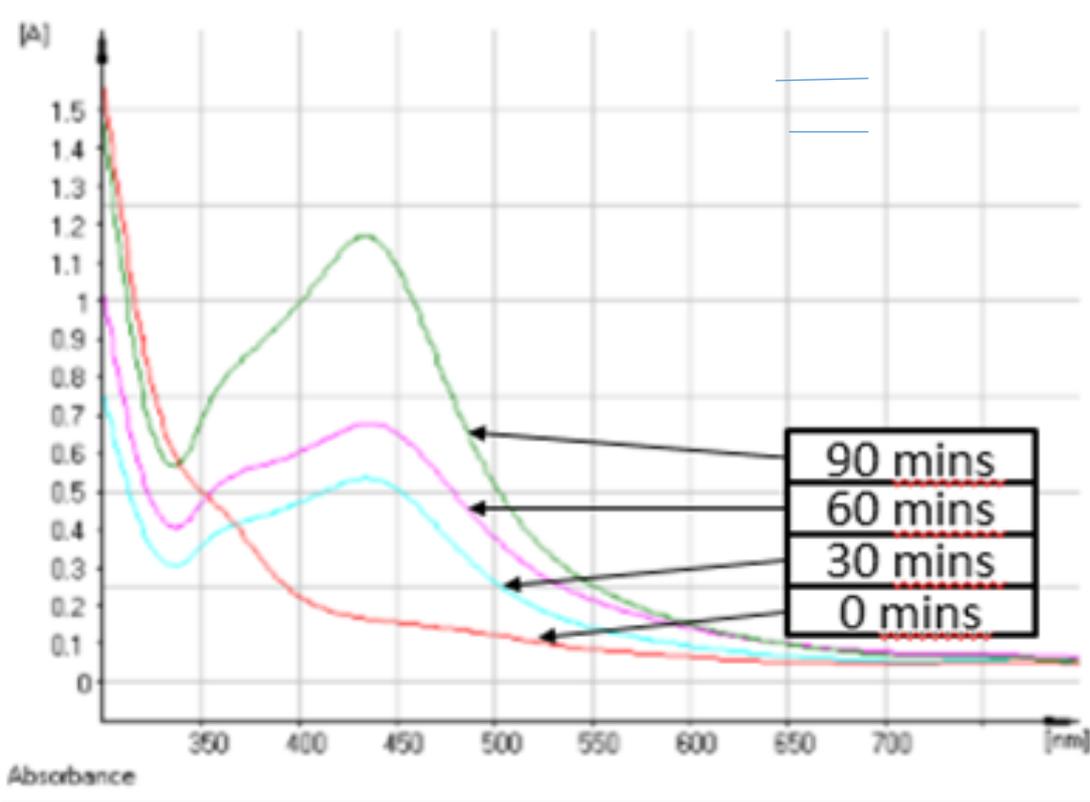


Figure 3: UV-Vis spectra on formation of AgNPs using *B. micrantha* bark extract

The steady peak at the same wavelength (λ_{\max} 431 nm) indicated that, the nanoparticles were monodispersed in the solution without aggregation. Similar observation was made on the study of photochemically grown AgNPs with wavelength-controlled size and shape (Callegari *et al.*, 2003). The optical density increased with time up to 90 minutes. Increase in optical density of the solution suggested increase in concentration of AgNPs (Maillard *et al.*, 2003).

3.2 High resolution Transmission Electron Microscope (HRTEM) analysis

The High Resolution Transmission Electron Microscope images of the synthesized silver nanoparticles are shown in Figure 4. The AgNPs had non-uniform surface and were quasi-spherical. The particles were evenly distributed.

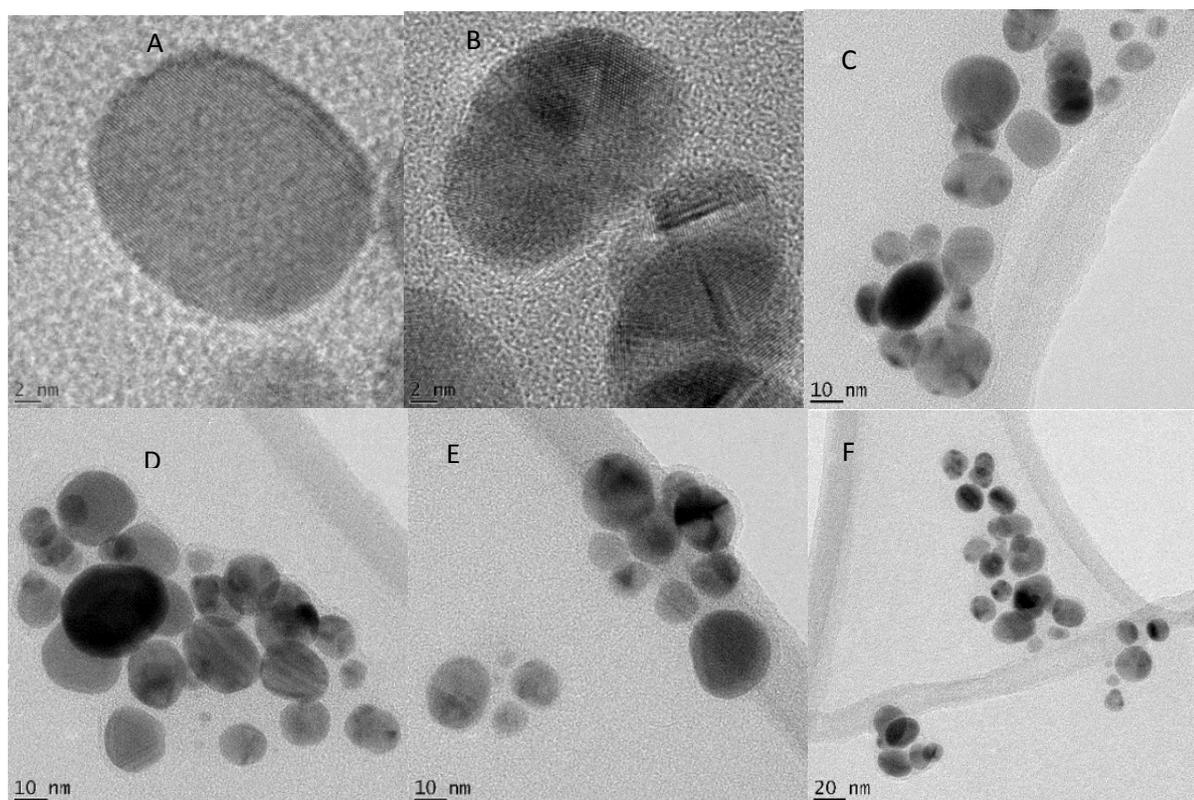


Figure 4: Transmission Electron Microscope micrographs of AgNPs at different magnifications

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 were monodispersed.

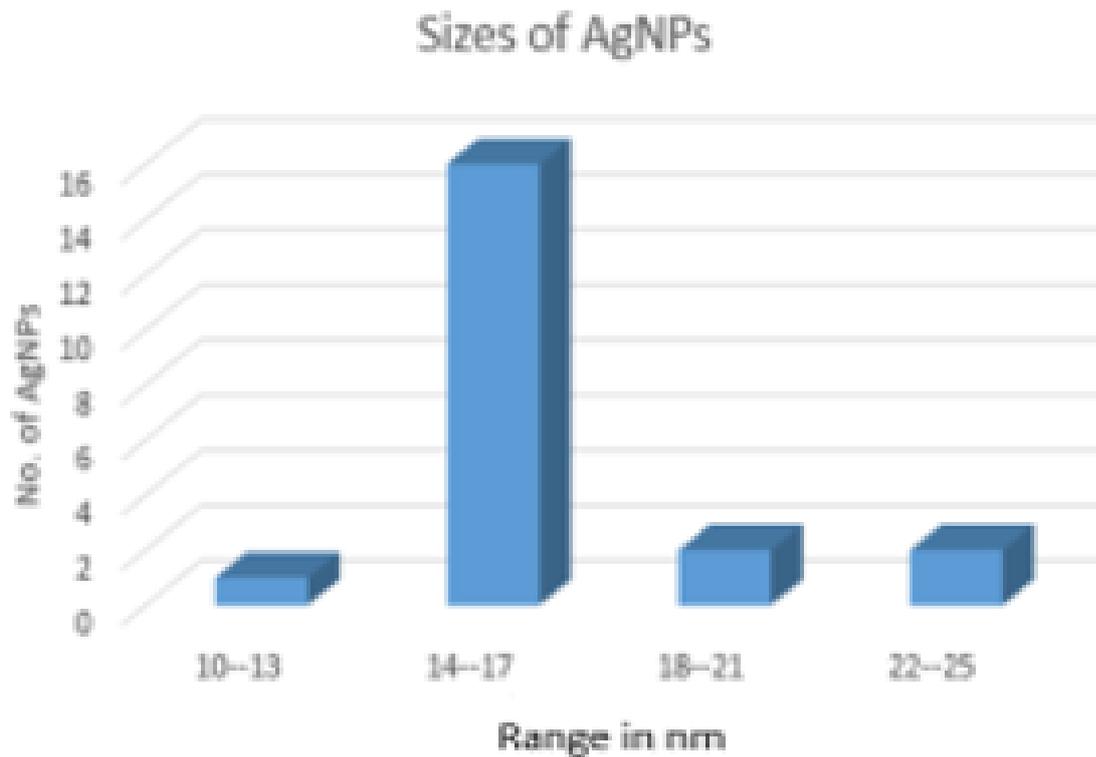


Figure 5: Size distribution of AgNPs.

3.3 Scanning Area Electron Diffraction (SAED) Analysis

The SAED image showed discrete shiny rings confirming crystalline nature of the AgNPs (Figure 6).

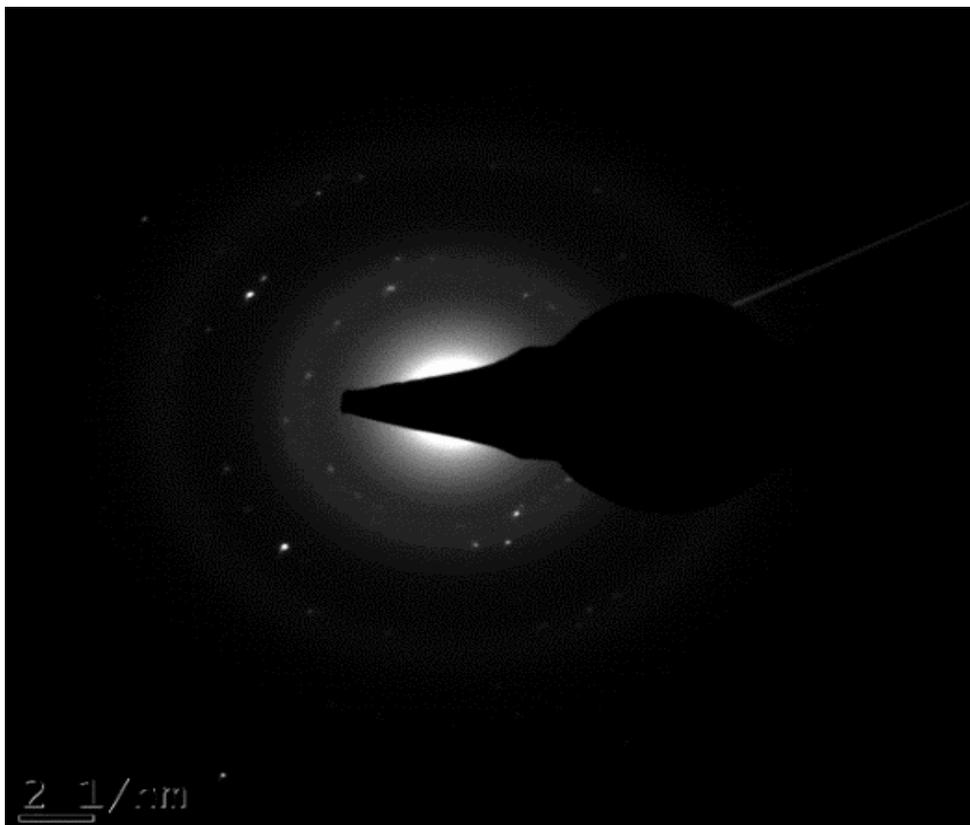


Figure 6: SAED micrograph of AgNPs

3.4 Energy Dispersive X-Ray (EDX) Analysis

The EDX spectrum shown in Figure 7 had overlaying peaks at 3.0 Kev confirming the synthesized nanoparticles were of silver.

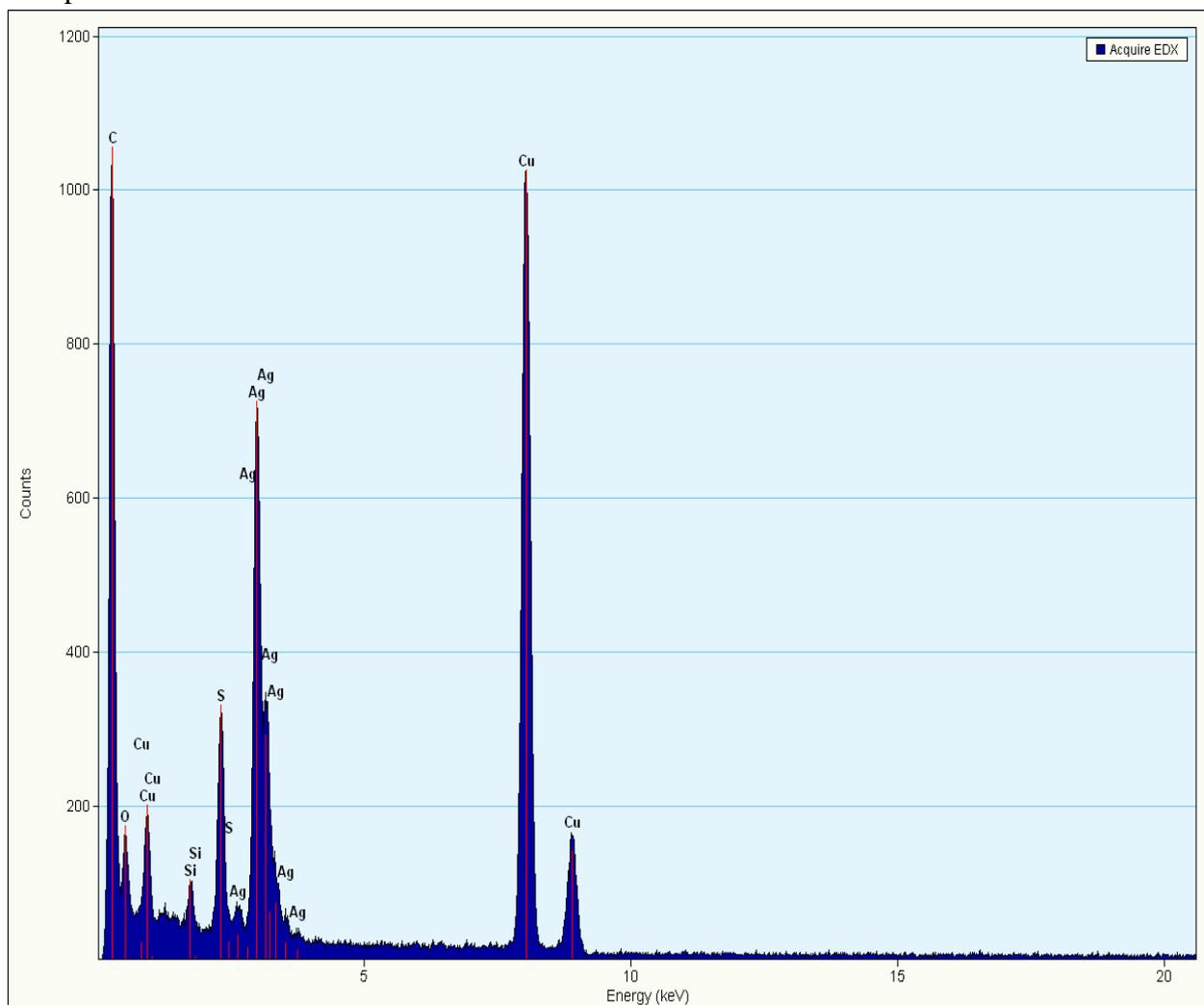


Figure 7: EDX spectrum of AgNPs

3.5 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.

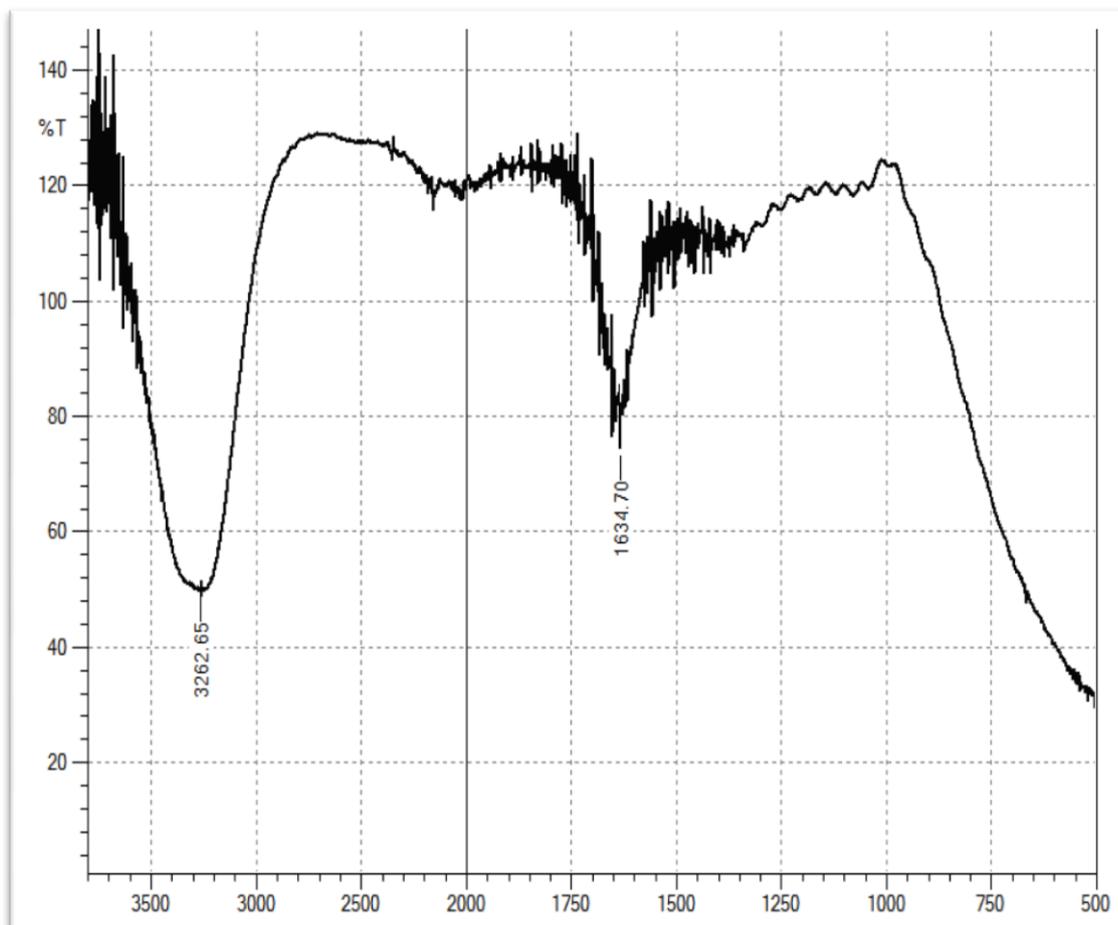


Figure 8: FTIR Spectrum of AgNPs.

3.6 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 hours of inoculation were recorded in table 1.

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

NB: N/A- not applicable

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 0.125mM. 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 0.25mM. 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).

Zones of inhibition of AgNPs against gram positive bacteria *S. aureus* are shown in Figure 9 A. The clear hollows (translucent regions) show inhibition zones caused by silver nanoparticles of different concentrations; a:-1mM AgNPs, b:- 0.75mM AgNPs, c:- 0.5mM AgNPs d:- 0.25mM AgNPs E - plant extract, f - 0.125 mM AgNPs. The minimum inhibitory concentration was 0.125mM as shown in the Figure 9A disc f.

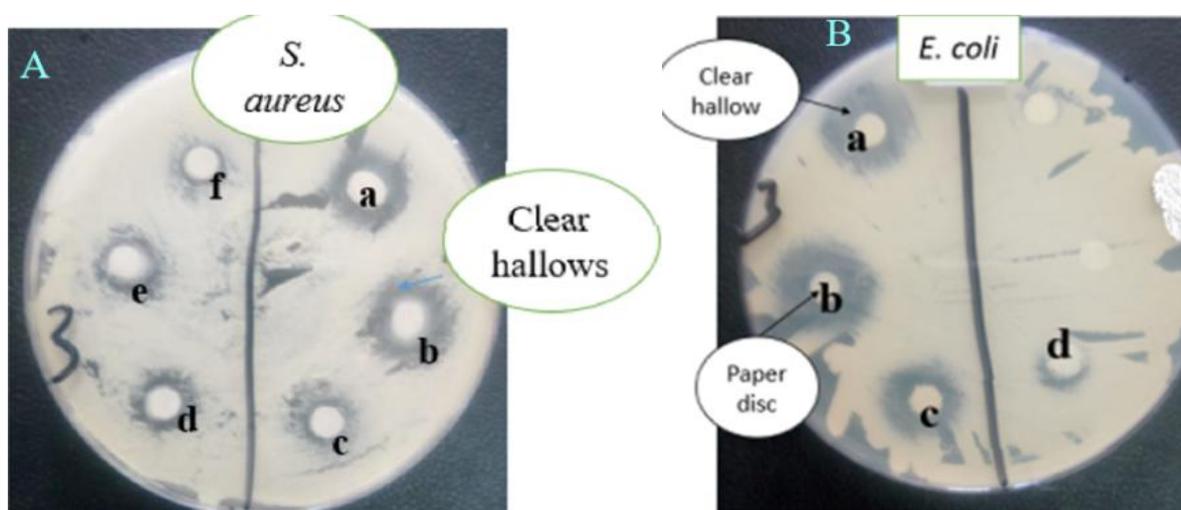


Figure 9: Effect of AgNPs on the bacteria

A) Effect of AgNPs on *S. aureus*

B) Effect of AgNPs on *E. coli*

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

4. 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 nm 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.

Potential Conflicts of Interest

The authors declare no conflict of interest.

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