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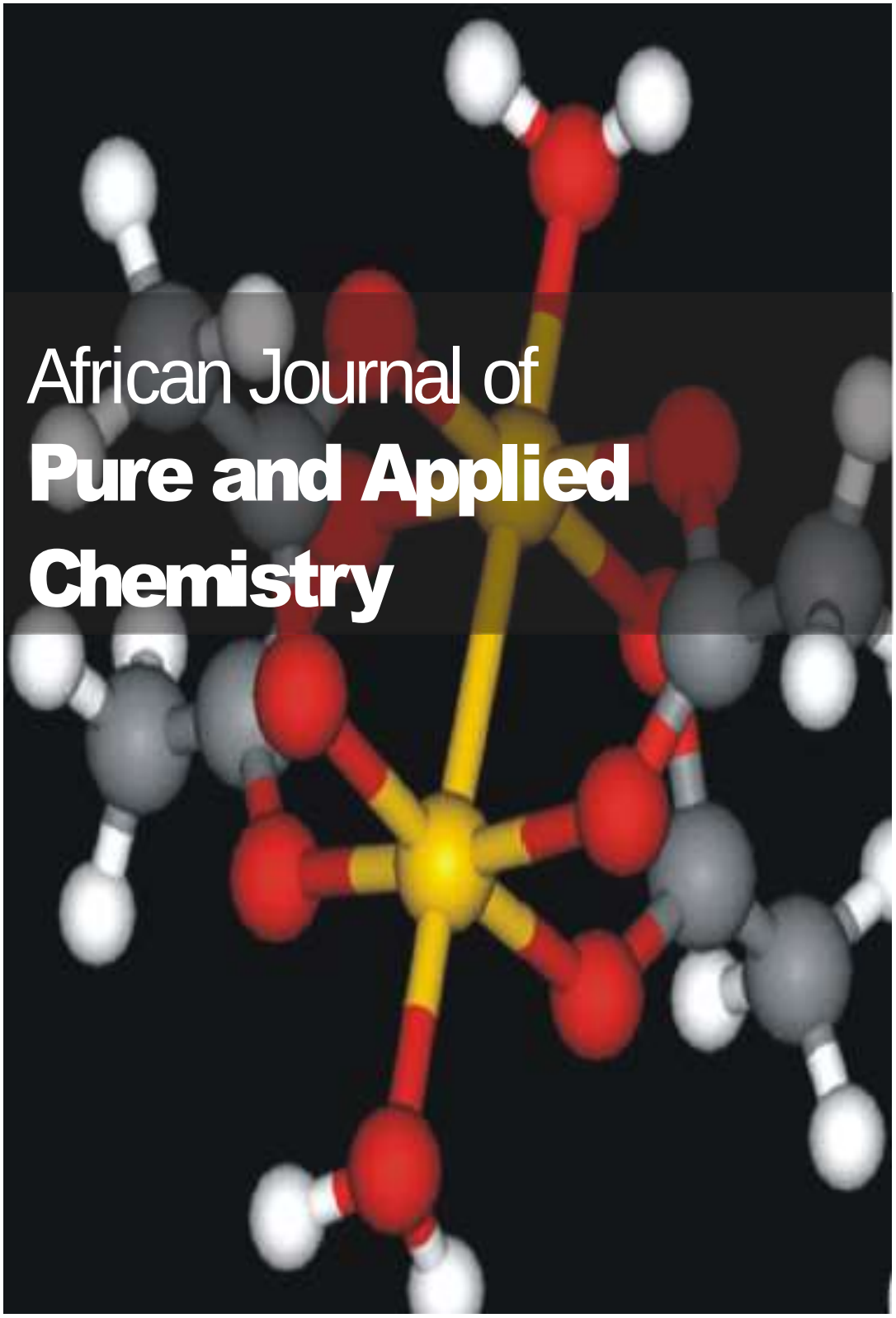

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Full Length Research Paper

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 transmition 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 transmition 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 (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 terpernoids 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 stabizing 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

i-0 min ii-60 min

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 (Woehrle 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 (Gebru 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

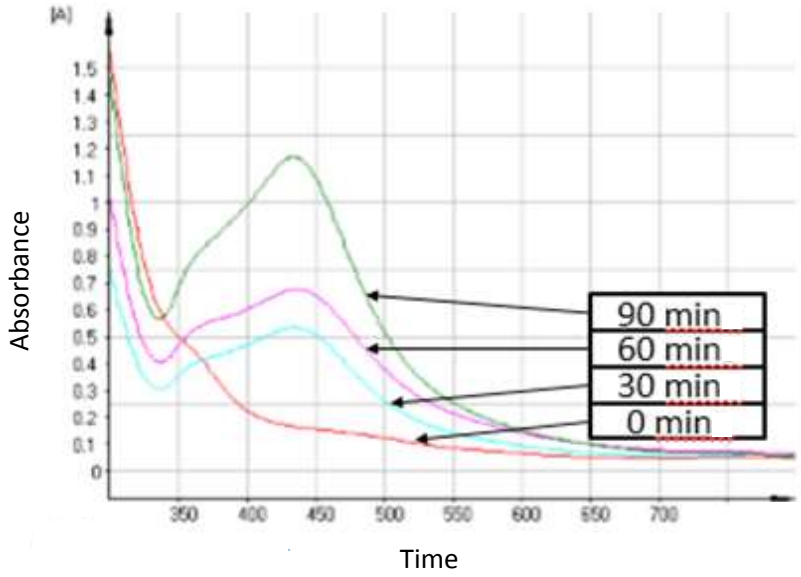

Figure 3. UV-Vis spectra on formation of AgNPs using *B. micrantha* bark extract.

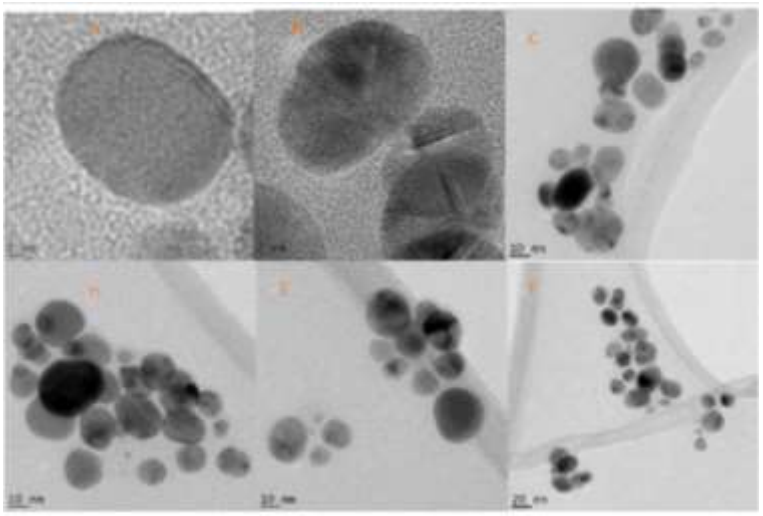

Figure 4. Transmission electron microscope micrographs of AgNPs at different magnifications.

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

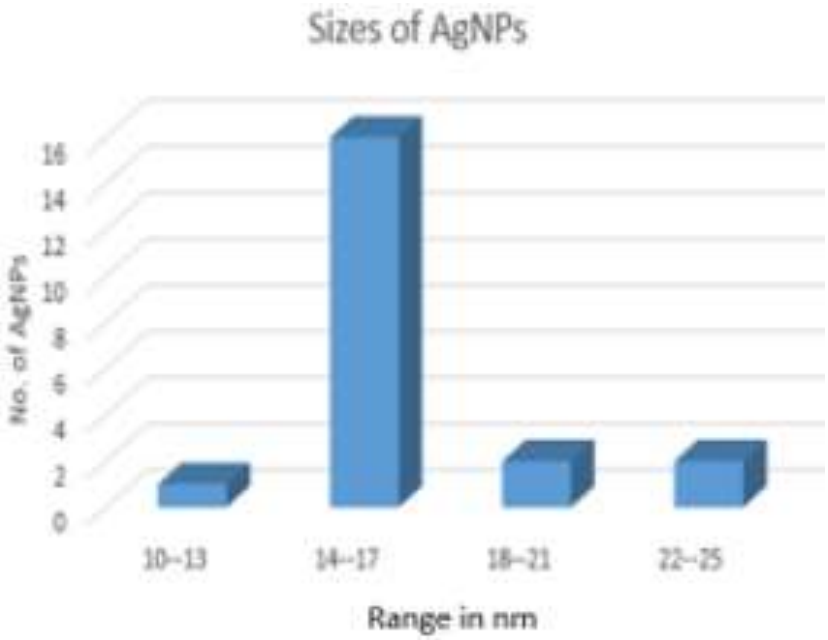

Figure 5. Size distribution of AgNPs.

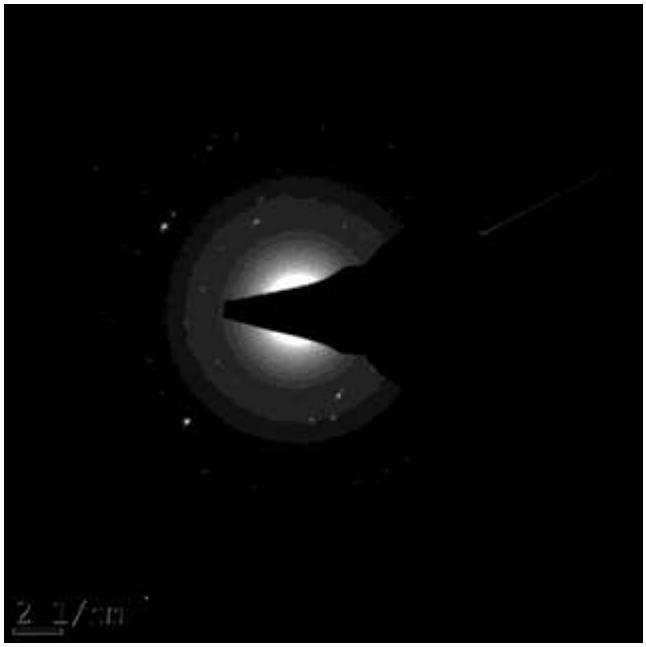

Figure 6. SAED micrograph of AgNPs.

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 shinny rings confirming crystalline nature of the AgNPs (Figure 6). SAED images with shiny sports in circular rings are due to crystalline nature of metals (Rajeshkumar and Veena, 2018).

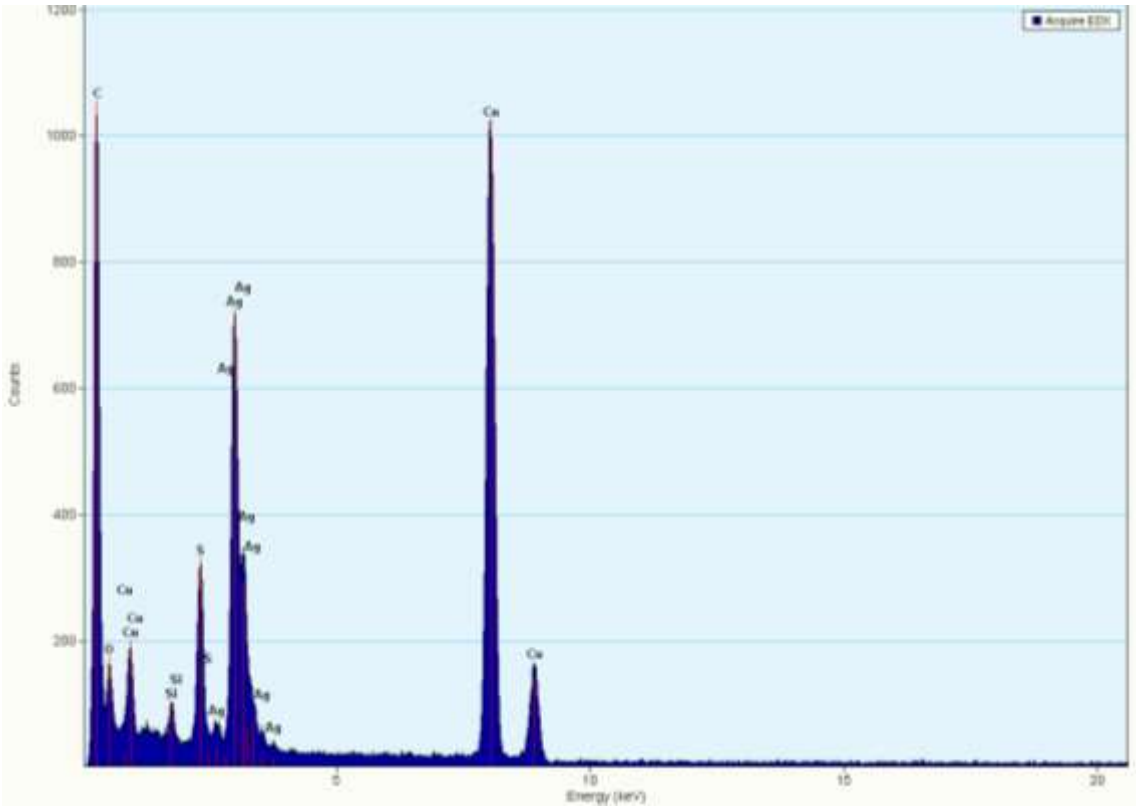

Figure 7. EDX spectrum of AgNPs.

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 biomolecueles 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 prescence 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, Ciprofloxaxin 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

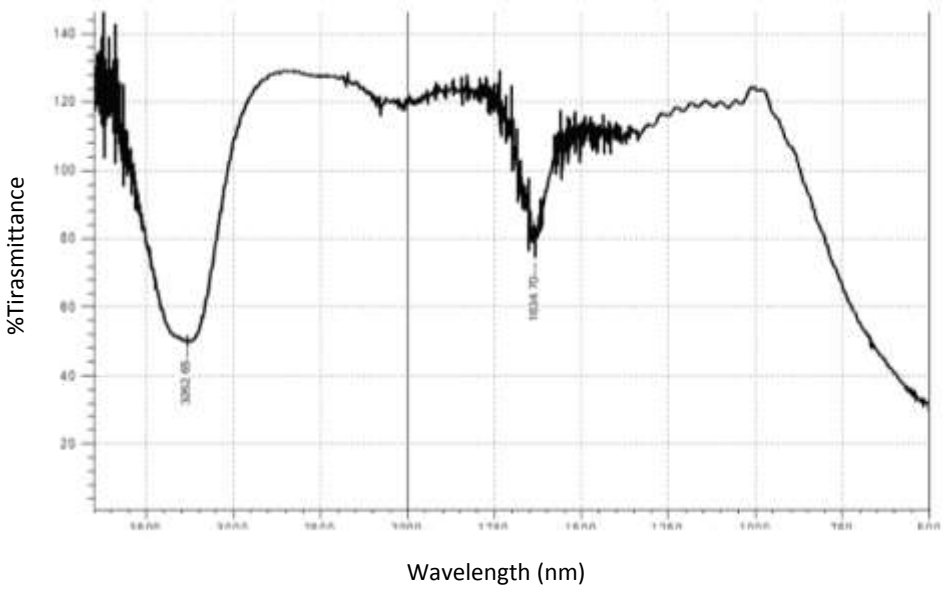

Figure 8. FTIR Spectrum of AgNPs.

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

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

N/A: Not applicable.

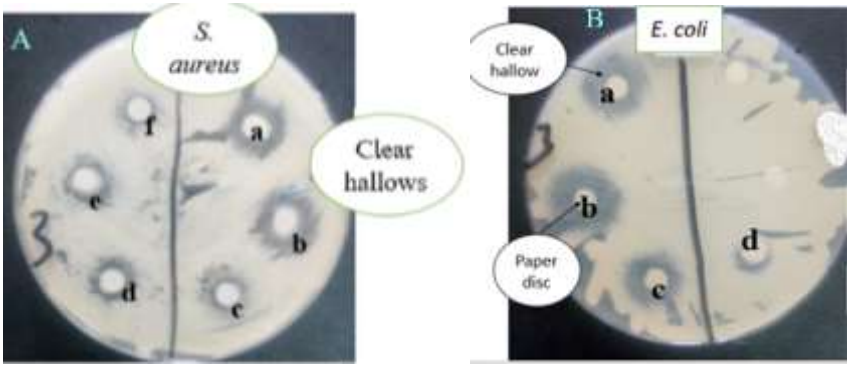

Figure 9. Effect of AgNPs on the bacteria. (A) Effect of AgNPs on *S. aureus*. (B) Effect of AgNPs on *E. coli*.

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 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, 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 nanoparticleas 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 declard any conflict of interests.

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Antioxidant activity, total phenolic and flavonoid content of selected Kenyan medicinal plants, sea algae and medicinal wild mushrooms

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Medicinal plants are good sources of bioactive compounds that guards the body against cancer and chronic ailments. A lot of studies have been done on medicinal values of higher plants but less on marine sources and wild non-edible mushrooms. The study aimed to assess the antioxidant activity, total flavonoid and total phenolic content from selected Kenyan medicinal plants, sea algae and mushrooms. The plants were selected based on their availability and folklore medicinal value. The antioxidant activity of the extracts was assessed using 2, 2-diphenylpicryl-1-hydrazyl (DPPH) free radical scavenging method. The total phenolic analysis was achieved using the Folin-Ciocalteu technique while the flavonoid content was determined by the use of aluminium chloride calorimetric method. The total phenolic content was expressed as gallic acid equivalent (GAE) and flavonoid content as quercetin equivalent (QE). *Ganoderma applanatum* had the highest scavenging ability (95.56%), while *Urtica dioica* leaves had the lowest (11.99%) at 0.3 mg/ml of extract. *G. applanatum* also had the lowest IC_{50} (<0.025 mg/ml), an indication that it had the highest antioxidant potential. *Ganoderma lucidum* showed the highest total phenolic content (GAE/g) of 156 ± 3.45 mg and *U. dioica* showed the lowest. *G. lucidum* also showed the highest total flavonoid content (QE/g) of 31.16 ± 0.04 mg. The study reveals that the Kenyan plant species can be potential sources of new natural antioxidants.

Key words: Antioxidant activity, DPPH, Folin-Ciocalteu, IC_{50}, flavonoids, phenolic content.

INTRODUCTION

Medicinal plants are vital in the survival of living organisms. Phytochemicals from the medicinal plants can be used as an organism defense mechanism and source of vital medicines. Medicinal plants are used for management of human diseases due to the presence of phytochemicals (Wadood, 2013). Approximately 80% of the population in developing countries depends on medicinal plants for their primary health care needs (Evans, 1997; Farnsworth and Soejarto, 1991). The use of medicinal plants has been motivated by several factors,

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including the belief that they have few side effects, easy accessibility and are cheaper as compared to modern synthetic drugs as well as the effectiveness of some plant remedies (Zheng and Wang, 2017). Phenolic compounds are important constituents present in plants and act as natural antioxidants (Ngueyen et al., 2009).They are composed of a large number of metabolites including flavonoids, polyphenols, tocopherols, tannins and lignins. Secondary metabolites of phenolic nature can act as part of plant's defense against insects, animals or plant pathogens and can protect the body from excess free radicals and retard the progress of many chronic diseases (Lai et al., 2001; Pengelly, 2004).

In humans, they act as antibacterial, anti-inflammatory, anthelmintic and cytotoxicity (Dore et al., 2014). An important function of phenolics is the antioxidant activity. Antioxidants have been reported to act as cardiovascular protectants, anti-aging agents and possible anti-cancer activity (Liu, 2003). The antioxidant properties of phenolic compounds such as flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelating of metal ions and inhibition of enzymes responsible for the generation of free radicals (Acker et al., 1996). Plants, sea algae and mushrooms have varying levels of total phenolic content and antioxidant activity (Kajal et al., 2017; Krishnendu A. 2010). These plants have potential to be good sources of antioxidants and arrest free radical damage. Oxidation is a very useful process in the metabolism of living organisms. During the process, there is production of free radicals. These radicals abstract electrons from other molecules to attain stability hence the damages. Antioxidants reduce free radicals in living organism's cells, hence are useful in the treatment of many human diseases such as cancer (Soni and Sosa, 2013). Antioxidants can neutralize free radicals by two major mechanisms, hydrogen atom transfer (HAT) and single electron transfer (SET) (Aqil et al., 2006). The end result is the same, regardless of the mechanism (Ismail and Tan, 2002; Wright et al., 2001).

The aim of this study was to determine antioxidant activity, total phenolic and flavonoid content of selected Kenyan medicinal plants, mushrooms and seaweeds. The plants were selected based on the availability of plant materials and due to several studies on medicinal potency, such as antimicrobial, anti- inflammatory, anti-diabetic, malaria, fever and anti-cancer (Iwu, 2004; Kokwaro, 2009). The plants will include the following: *Eucheuma denticulatum, Ganoderma applanatum, Ganoderma lucidum, Trametes elegans, Prunus africana Bridelia micrantha* and *Urtica dioica. P. africana* commonly known as African cherry has a wide distribution in Africa growing in mountainous regions. The stem bark is of commercial value (Gulcin et al., 2004). It has several bio components such as polyphenols fatty acids, esters and alkanols (Kadu et al., 2012). *B. micrantha* is a medium-tall tree, growing up to 20 m and belonging to the family of Phyllanthaceae. (Mburu et al., 2016: Munayi RR 2016) *U. dioica* is a herbaceous perennial flowering plant in the family of *Urticaceae* commonly known as stinging nettle or common nettle (Ilhami et al., 2004).

Both edible and non-edible mushrooms are regarded as good sources of bioactive compounds in human diets for the antioxidant and anti-inflammatory purpose. They store secondary metabolites, which includes compounds like flavonoids, polyphenols, polyketides, terpenes and steroids with pharmacological and nutritional value (Abugri and Mcelhenney, 2013; Nguyen et al., 2016).

Studies on non-edible *G. applanatum* and *G. lucidum* mushrooms have secondary metabolites responsible for antioxidants and anti-inflammatory activity (Rašeta et al., 2016, Jeon et al., 2008). Anti-oxidant and anti-inflammatory activities in marine algae are attributed by high content of hydrophilic polyphenols and soluble polysaccharides (Delgado et al., 2013; Balakrishnan et al., 2014).

In this study, a method based on the scavenging of the stable radical, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used to measure the antioxidant activity potential of plant extracts from Kenya (Brand et al., 1995; Kulisic et al., 2004; Ansari et al., 2013). Folin-Ciocalteu reagent, a commonly used complexing coloring reagent was used for measuring the total phenolic content while the flavonoid content was determined by the use of aluminium chloride calorimetric method.

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, and quercetin were purchased from Aldrich Co. Germany. Methanol, sodium carbonate, aluminum chloride, sodium nitrate, and ascorbic acid were of analytical grade.

Sample collection

Roots, stem bark, leaves and whole plant were collected from their natural habitats in Kenya between January and September 2018. Mushrooms: *G. applanatum* and *T. elegans* were collected in February while *G. lucidum* was collected in September from Kitale, Trans-Nzoia County. The samples were collected in the afternoons from dead acacia tree along the river. The mushrooms were transported on the same night in polythene bags. *B. micrantha* bark*, U. dioica* leaves and roots and *P. africana* bark were collected in July from Manyatta constituency, Embu County. The plants and mushrooms were identified and voucher specimens deposited in the herbarium at the National Museums of Kenya, Nairobi. Sea algae: *Eucheuma denticulatum* was collected in August from the Kenyan South Coast, Shimoni, Kwale County and the specimen was identified at Kenya Marine and Fisheries Research Institute (KEMFRI), Mombasa, Kenya.

Sample preparation

All plants, mushrooms and seaweeds were washed thoroughly

three times with tap water, shade dried and ground to fine powder. The fine powder of different samples was stored in air-tight polyethylene bags before extraction.

Extraction

100 g of each ground plant sample was weighed, 500 ml methanol was added and soaked overnight and then sonicated at 60°C over ultrasonic bath for two hours to enhance extraction. The extract was filtered using Whatman filter paper No.1. The clear filtrate obtained was concentrated at reduced pressure by rotary evaporations to produce a crude extract.

DPPH free radical scavenging activity assay

Antioxidant activity was performed according to the method described by Brand et al., 1995 and Jaita et al., 2010 with slight modifications. Series of dilutions of the methanolic extract (0.025-0.3 mg/ml) was prepared. A measure of 2 ml of the extract was mixed with 3 ml of 0.3 mg/ml of DPPH radical. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance of the mixture was read at 517 nm using UV-VIS Spectrophotometer (Analytik Jena model). The absorbance of the resulting solution was converted into a percentage of antioxidant activity (% inhibition) by the use of the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Ao} - \text{A1})}{\text{Ao}} \times 100$$

Where Ao = Absorbance of the control solution containing only DPPH solution; A1 = Absorbance in the presence of extract in DPPH solution; and Ascorbic acid was used as a standard.

Determination of total phenolic content

The number of total phenolics was determined using the Folin-Ciocalteu method (Jaita et al., 2010) with some modification. 0.1 g of the extract was dissolved in 5 ml methanol. 200 µl of the extract solution and 1 ml Follin-Ciocalteu reagent were mixed and 1 ml of 7.5% of sodium carbonate solution was added after 3 min. The mixture was shaken and allowed to stand for 2 h in the dark. The absorbance of the solution was read in triplicate using UV-VIS Spectrophotometer (analytic Jena model) at a wavelength of 515 nm. A blank solution was prepared and read similarly. A calibration curve of gallic acid was obtained from serial dilutions of various concentrations of gallic acid prepared from its stock solution. The results were expressed as GAE (gallic acid equivalents/g) of the extract.

Determination of total flavonoid content

The total flavonoid content was determined spectrophotometrically (Quettier-Deleu et al., 2000). 4 ml of distilled water was added to 1 ml of the extract in a 10-ml volumetric flask, followed by 1 ml of 5% sodium nitrate. 1 ml of 10% aluminum chloride was added after 5 min. This was left to settle for 5 min where 2 ml of sodium hydroxide was then added and topped up to the mark with distilled water. The absorbance readings were taken at 510 nm against a blank (water). The flavonoid content was determined using a standard curve with quercetin (10-180 mg/ml). The mean of three readings was used and expressed as milligrams of quercetin equivalents (QE/g of extract).

RESULTS AND DISCUSSION

Antioxidant activity

The effect of antioxidant on DPPH radical is thought to be due to their hydrogen donating ability. The extracts of plant samples are allowed to react with stable DPPH radical in methanol solution. The reduction capability of DPPH radicals is determined by the decrease in its absorbance at 515 nm, induced by antioxidant. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50% is termed as inhibition concentration (IC_{50} or EC_{50}). This parameter is widely used to measure the antioxidant activity (Sanchez-Moreno et al., 1998). A lower IC_{50} value corresponds with a higher antioxidant ability. The plant extracts showed significant DPPH scavenging activity (59.01 – 95.56%) as compared with values obtained for standard ascorbic acid (94.32%) at 0.3 mg/ml. The antioxidant activity increased with the concentration of extracts as shown in Figure 1.

G. applanatum showed the highest scavenging activity of 95.56% (IC_{50} < 0.025 mg/ml), higher than that of ascorbic acid 94.32% (IC_{50} = 0.03 mg/ml) followed by *P. africana* bark 93.6% (IC_{50} = 0.033) at 0.3 mg/ml. This was in agreement with the scavenging ability of 84% reported for methanol extract of *P. africana* bark by Edwin et al. (2018). *U. dioica* leaves showed the lowest scavenging activity of 11.99% (IC_{50} > 0.3 mg/ml) as shown in Figure 1. The results of antioxidant activity of other plant extracts were as follows: *B. micrantha* 92.67% (IC_{50} = 0.038 mg/mL), *G. lucidum* 91.24% (IC_{50} = 0.04 mg/ml), *E. denticulatum* 90.21% (IC_{50} = 0.042 mg/ml) *U. dioica* roots 84.32% (IC_{50} = 0.045 mg/ml) and *T. elegans* 59.01% (IC_{50} = 0.22 mg/ml). In this study, mushroom showed a good scavenging activity. This could be due to the absorption of nutrients from the components they grow from. The scavenging ability of *G. applanatum* was higher than the one reported of 71% (Nagaraj, 2014). *T. elegans* had a scavenging activity value of 59.01% which was lower than the one reported of 65% (Awala, 2015). Antioxidant activity of *B. micrantha* (92.6%) was lower than the one reported of 97.70% (Nwaheujo 2014). This variation may be due to the variation in geographical area and the bioactive compounds present. Different parts of the plants showed variation in antioxidant activity due to the presence of different types of phytochemicals which have different antioxidant activity.

Total flavonoid content

The highest total flavonoid content was observed in *G. lucidum* (31.16 ± 0.04 mg QE/g), which was higher than the reported value of 10.82 mg QE/mg (Raseta, 2016). *B. micrantha* bark had a value of 30.47 ± 0.03 mg QE/g and *T. elegans* had a value of 103.19 ± 1.23 mgQE/g, higher than the reported value of 0.97 mg QE/g (Awala, 2015). It was observed that *U. dioica* leaves had the lowest total flavonoid content (3.97 ± 0.06 mg QE/g) as shown in

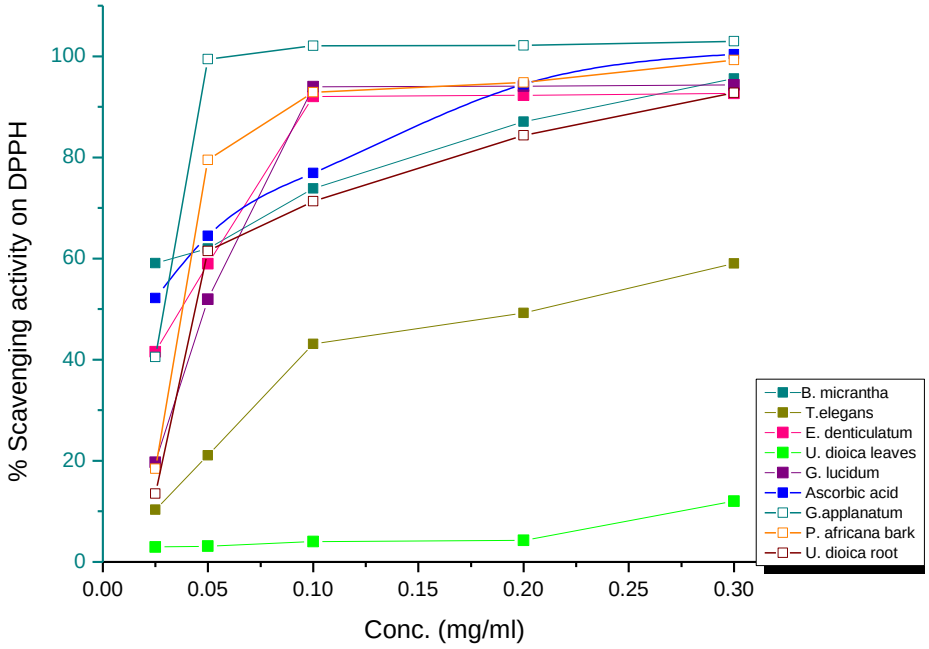

Figure 1. % radical Scavenging activity of extracts on DPPH. Determination of antioxidant activity with concentration.

Table 1. Total phenolic and flavonoid content and antioxidant activity of extracts.

Extract	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	Antioxidant activity on DPPH at 0.3 mg/ml of extract %	IC_{50} (mg/ml)
Ascorbic acid			94.32	0.03
G. applanatum	127.23 ± 0.64	14.53 ± 0.28	95.56	< 0.025
P. africana bark	148.55 ± 4.05	9.29 ± 0.06	93.60	0.033
B Micrantha bark	128.79 ± 1.54	30.47 ± 0.03	92.67	0.038
G. lucidum	156.07 ± 3.45	31.16 ± 0.04	91.24	0.04
E. denticulatum	146.15 ± 1.11	9.36 ± 0.12	90.21	0.042
U. dioica roots	144.04 ± 3.89	28.54 ± 0.67	84.32	0.045
T. elegans	103.19 ± 1.23	9.97 ± 0.32	59.01	0.22
U. dioica leaves	43.19 ± 1.15	3.97 ± 0.06	11.99	> 0.3

Table 1. The extracts with high antioxidant activity also had high total flavonoid content. According to literature, high flavonoid content may provide protection against oxidative stress along with other oxidative defenses such as vitamins and enzymes (Tripathy et al., 2014).

Total phenolic content

All the extracts under the study contained noticeable phenolic content with significant variations (P-value = 0.0004). The highest phenolic content was observed in *G. lucidum* (156.07 ± 3.45 mg GAE/g) > *P. africana* bark (148.55 ± 4.04) > *E. denticulatum* (146.15 ± 1.11) > *B. micrantha* bark (12879. ± 1.54) > *G. applanatum* (127.23 ± 0.64 mg GAE/g) as shown in Table 1. The highest phenolic content in *G. lucidum* may be due to different polyphenolic content. The results obtained for *G. applanatum* were lower compared to 191.76 mg GAE/g reported by Raseta (2016). The value for *G. lucidum* was higher than the reported value of 60.41 mg GAE/g (Raseta, 2016). *T. elegans* had a higher value (9.97 ± 0.32 mg GAE/g) than reported (4.79 mg GAE/g) (Awala, 2015). The total phenolic content was lowest in *U. dioica* leaves (3.97 ± 0.06 mg GAE/g). The results showed a correlation between antioxidant activity and phenolic content (r = 0.9467) as observed from the results. The higher the phenolic content the higher the antioxidant

activity. Natural extracts with proven antioxidant activity usually contain compounds with a phenolic moiety such as flavonoids, tocopherols, carotenoids, tannins (Dapkevicius et al., 1998). This implies that phenolic components are among the compounds responsible for reducing the DPPH radical.

Conclusion

Results of this study indicate that methanolic plant extracts have a significant effect on the scavenging of free radicals. The antioxidant activity increases with concentration. Most of the extracts had high total phenolic and flavonoid content. *G. lucidum* extracts had the highest total flavonoid and phenolic content, with *G. applanatum* showing the highest antioxidant activity. *B. micrantha* and *P. africana* showed high antioxidant activity of 92.67 and 93.60 respectively. Their total phenolic and flavonoid content were relatively high. The study revealed that these Kenyan plant species can be potential sources of new natural antioxidants.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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