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Green synthesis of silver monometallic and copper-silver bimetallic nanoparticles using *Kigelia africana* fruit extract and evaluation of their antimicrobial activities

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Aqueous extract of *Kigelia africana* fruits have been utilized in the syntheses of silver nanoparticles (AgNPs) and copper-silver bimetallic nanoparticles (Ag-CuNPs). The synthesized nanoparticles have been characterized using UV-vis, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX), x-ray diffration (XRD) and transmission electron microscopy (TEM). The antimicrobial activities have been evaluated against both Grams-negative and Grams-positive strains of bacteria and fungus. The UV-vis and FTIR techniques revealed the formation of nanoparticles and the active components were adsorbed on the surface of the particles thereby stabilizing the nanoparticles. The SEM reveals uniform microspheres of AgNPs and anisotropic particles for AgCuNPs. TEM shows a particle size of 10 nm. The nanoparticles inhibit the growth of both Grams-negative and Grams-positive bacteria. The present nanoparticles synthesized from aqueous extract of *K. africana* fruits inhibits *Klebsiella pneumoniae* more than any of the antibiotics tested in this study. It competes very well with augmentin against *Pseudomonas aeruginosa* and with meropenem against *Candida albicans* with inhibition zones of 23 and 25 mm, respectively. The bimetallic nanoparticles have demonstrated effectiveness against *Staphylococcus aureus,* with maximum inhibition zone of 27 mm.

Key words: Green synthesis, bioreduction, nanoparticles, bimetallic particles, *Kigelia africana*, antimicrobial activities.

INTRODUCTION

The synthesis of metal and/or bimetallic nanoparticles is a growing field of interest with various inputs from diverse disciplines. This has formed the bedrock for implementation of novel technologies. The intense research interest is due to diverse applications in different fields, for example, biomedical (Chaloupka et al., 2010), drug delivery (Prow et al., 2011), water treatment (Dankovich and Gray, 2011), agriculture (Nair et al., 2010) and sensors (Pandey et al., 2012; Pandey et al., 2013a, b; Tran et al., 2013). Furthermore, the larvicidal, antibacterial, antifungal anticoagulant and thrombolytic activities of silver nanoparticles have been extensively investigated (Lateef et al., 2015, 2016a; Lateef et al., 2016c). Consequently, a number of studies are geared towards development of suitable protocols for the rational synthesis of metal and/or bimetallic nanoparticles in an eco-friendly approach. A number of physio-chemical techniques involving chemical reduction (Park et al., 2008; Ayi et al., 2010, 2015; Khan et al., 2011a), gamma ray radiation (Chen et al., 2007), micro emulsion (Zhang et al., 2006), electrochemical method (Reicha et al., 2012), laser ablation (Abid et al., 2002), autoclave (Yang and Pan, 2012), microwave (Khan et al., 2011b) and photochemical reduction (Alarcon et al., 2012) are commonly employed in the synthesis of metal and/or bimetallic nanoparticles. Although these methods have been quite effective, the risks associated with the use of toxic chemicals as well as high operational cost and energy requirement have limited their use.

Recently, different research groups have reported on the use of green synthesis as an environmentally benign in the preparation of silver nanoparticles. This involves the use of extracts of different parts of plant (Sathishkumar et al., 2009; Tripathi et al., 2009; Prathna et al., 2011; Sivarraman et al., 2009). Mubayi et al. (2012) and Vijaykumar et al. (2014), reported on the use of extract of *Moringa oleifera* and *Boerhaavia diffusa*, respectively, to prepare silver nanoparticles. The biogenic synthesis of silver nanoparticles have been reported recently using a pod extract of *Cola nitida* (Lateef et al., 2015, 2016b), cell-free extract of *Bacillus safensis* (Lateef et al., 2016c) as well as the use of agro-wastes, enzymes and pigments (Adelere and Lateef, 2016). The important plants that have been used to date, include amongst others, the following: *Tinospora cordifolia* (Anuj and Ishnava, 2013), *Aloe vera* (Chandran et al., 2006), *Terminalia chebula* (Edison and Sethuraman, 2012), *Catharanthus roseus* (Mukunthan et al., 2011), *Ocimum tenuiflorum* (Patil et al., 2012), *Azadirachta indica* (Tripathi et al., 2009), *Emblica officinalis* (Ankamwar et al., 2005), *Cocos nucifera* (Roopan et al., 2013) and some common spices like *Piper nigrum* (Shukla et al., 2010) and *Cinnamon zeylicum* (Satishkumar et al., 2009). Plant extracts are known to act as reducing and stabilizing agents in the synthesis of metal nanoparticles. Metabolites, proteins and chlorophyll present in the plant extracts were found to be efficient in controlling the growth and morphology of the metal nanoparticles (Sista et al., 2016; Mondal et al., 2014).

In the present study, we are interested in using the extract of *Kigelia africana* fruits, both as a reducing and stabilizing agents in the synthesis of monometallic silver and copper-silver bimetallic nanoparticles. *K. africana* (syn. *Kigelia pinnata*, *Kigelia aethiopica*) is a tropical plant that belongs to the Bignoniaceae family. It is widely distributed in the South, Central and West Africa with different local names: Ketete (Bette people of Obudu, Nigeria), Ntabinim (Ibibio, Nigeria), Rawuya (Hausa, Nigeria); Uturubein (Igbo, Nigeria); Pandoro, Iyan (Yoruba, Nigeria); Bechi (Nupe, Nigeria); Mwegea (Swahili, Kenya, Tanzania); Umfongothi (Zulu, South Africa) (Mann et al., 2003; Otimenyin and Uzochukwu, 2012). In Hindi (India) it is known as Balmkheera (Saini et al., 2009). The plant is commonly called Cucumber-like or Sausage tree, and different parts (leaves, bark and fruits) have found therapeutic uses traditionally in the treatment of different ailments such as gonorrhea, sphyllis, jaundice, madness, cataract, blood cleanser, high blood pressure, hydrocephalus, measles, hemorrhagia and postpartum bleeding, ulcer etc. (Olatunji and Atolani, 2009; Grace et al., 2002; Abdulkadir et al., 2015, Kamau et al., 2016; Atawodi and Olowoniyi, 2015). Various chemical investigations have been carried out on *K. africana* and many chemical compounds mainly iridoids, naphthaquinones, monoterpenoidnapht-haquinones, isocoumarins, caffeic acid, norviburtinal, lignans, sterols and flavonoids have been identified (Gabriel and Olubunmi, 2009). Chemical analysis of the polar extract of fruit indicated the presence of vermonosides (Picerno et al., 2005), phenylpropanoid derivative identified as 6-p-coumaroyl-sucrose, and flavonoid glycoside (Gouda et al ., 2006). Herein, we report the green synthesis and antimicrobial studies of silver nanoparticles (AgNPs) along with the bimetallic copper-silver nanoparticles mediated by aqueous extract of *K. africana* fruits.

MATERIALS AND METHODS

All the chemicals were of analytical grade and were used as purchased without further purification. *K. africana* fruits were collected from Agasham mountain in Ukwel-Obudu village of Obudu LGA of Cross River State, Nigeria.

Preparation of plant extract

The fruits of *K. africana* weighing 50 g and 0.5 m long (Figure 1a) were washed thoroughly with distilled water, cut into small sizes (with their seeds carefully selected out) and blended together in 100 cm^3 distilled water using a manual blender. The resultant mixture was filtered and the filtrate stored at 4°C.

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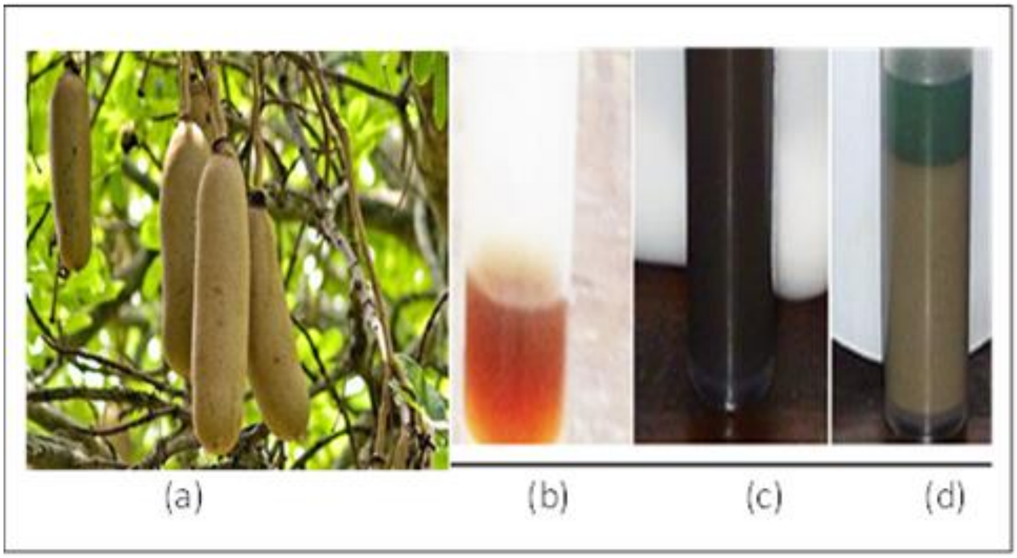

Figure 1. *(a) Kigiela africana* plant showing the fruits used in the present study; (b) Aqueous extract of *Kigiela africana* fruits; (c) AgNPs; (d) AgCuCl bimetallic nanoparticles synthesized by the extract.

Synthesis of the nanoparticles

In this ecofriendly synthetic method, the procedure adopted used silver nitrate ($AgNO_3$), copper chloride ($CuCl_2.2H_2O$) and aqueous extract of *K. africana* fruit. In a typical synthesis of silver nanoparticles (AgNPs), $AgNO_3$ (1.0 g, 5.83 × 10^{-06} mM) was dispersed in 10 cm^3 of aqueous extract of *K. africana* fruit under continuous stirring resulting in a brownish colouration, indicative of Ag^+ reduction. The reaction mixture was then heated at 120°C under reflux. Six portions of 1 cm^3 of the mixture was taken out from the reaction vessel after every 1 h, the reaction was stopped after 6 h. A colour change from coffee brown to dark suspension with a yellow supernatant was observed. The products were centrifuged at 3000 rpm for 15 min, filtered and washed with distilled water, dried at room temperature and stored in airtight container for further analysis. In synthesizing AgCuCl bimetallic nanoparticles, silver and copper salts were mixed together in the ratio of 1: 2. In a typical reaction, $AgNO_3$ (2.0 g, 1.17 ×10^{-05} mM) was dispersed in 6 cm^3 of aqueous extract of *K. africana*, followed by $CuCl_2.2H_2O$ (2.0 g, 2.34 × 10^{-05} mM) under magnetic stirring with the formation of a black homogenous colloidal dispersion. When the reaction mixture was subjected to heat treatment at 120°C under reflux, a colour change from black to green was observed, indicative of oxidation.

Characterization

The bioreductions were monitored using UV-visible spectrophotometer (Evolution 201 spectrophotometer) at regular interval with samples dissolved in ethanol using quartz cuvette operated with a resolution of 1 nm. The active components in the extract responsible for the reduction were analyzed using FTIR Spectrophotometer (Shimadzu IR Affinity-1S) in the spectral range of 4000 to 500 cm^{-1} using KBr pellets. Scanning electron microscopy (SEM) was performed on a Hitachi S-4800 microscope attached with EDX at a voltage of 15 Kv. The sizes of the nanoparticles were determined with the help of transmission electron microscopy (TEM) measurements JEOL, TEM 1010 at 200 kV. The powder X-ray diffraction patterns were recorded on a Bruker D8 Advanced X-ray diffractometer with Cu-Kα radiation, having 2θ scale between 5 and 90°. Data intensity were collected and recorded by counting method (step: 0.014, and time; 189.5 s).

Antimicrobial studies

Antimicrobial susceptibility test was done in the bacteriology lab of the General Hospital Calabar, Cross River State. The agar diffusion test (disc diffusion method) was adopted (Prescott et al., 2005). Muller-Hilton agar was prepared from a dehydrated base according to the manufacturer's instruction. The medium was allowed to cool to 47°C and poured into petri-dishes that were arranged and labeled according to their microbial isolates, and allowed to set on a level surface to a depth of approximately 4 mm. When the agar had solidified, the plates were dried for 20 min at 35°C by placing them in an upright position in hot air oven with the lids tilted. A discrete colony of each of the isolate was picked with a sterile wire loop and streaked on the Muller-Hilton agar according to their names as labeled: *staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebseilla pneumonia* (bacteria) and *Candida albicans* (fungus). Filter paper discs carrying 2000 µg/ml AgNPs, CuAgNPs, plant extract and antibiotics; Ciprofloxacin (10 µg), Ofloxacin (30 µg), Augmentin (25 µg), Ceftriaxone (25 µg), Meropenem (30 µg) and Racinef (30 µg) were transferred to the appropriate locations on the agar plates with the help of sterile forceps. The plates were then incubated at 37°C for 18 h. At the end of the incubation, the zones of inhibition or no inhibition (for resistant strains) were measured and recorded in millimeters.

RESULTS AND DISCUSSION

Silver nanoparticles (AgNPs) and copper-silver bimetallic nanoparticles (AgCuNPs) were synthesized via green synthetic route by reduction of the metal ions with aqueous

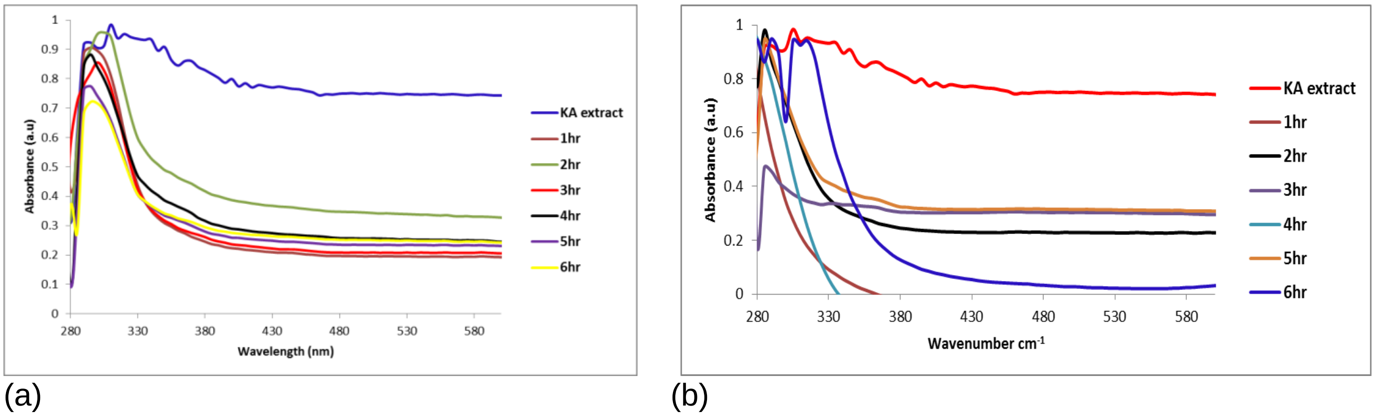

Figure 2. UV-Vis spectra of synthesized particles from *Kigelia africana* fruit extract at different heating time intervals: a) AgNPs b) Cu-AgNPs.

extract of *Kigelia africana* fruit. The main goal of the study is an evaluation of the potential of the plant extract as a reducing agent, which can replace the chemical reducing agents that are toxic to the environments. The bioreduction of the metal ions to nanoparticles was accompanied by colour change, which was monitored with the help of UV-Vis spectroscopic technique. Figure 1(b, c and d) shows the photographs of samples of aqueous extract of *k. africana* fruit, the AgNPs and AgCuNPs. The dark brownish colouration confirms the reduction of Ag^+ ions to Ag^0 and in the case of bimetallic mixture, gray precipitates with dark greenish colouration at the top was observed. In order to study the effect of temperature and heating time on the colloidal dispersion of the nanoparticles, some portions were taken for UV-Vis spectroscopic analyses.

In Figure 2, the UV-Vis spectra of the particles synthesized in aqueous plant extract are presented for both the AgNPs (Figure 2a) and AgCuNPs (Figure 2b). The spectra shows absorption maxima in the range of 285 to 350 nm for 1 to 6 h of heating, which shifts to higher wavelength with reduced intensity as the heating time increases. In literature, the surface plasmon resonance arising from the interactions of the electron cloud on the particles' surface and the electromagnetic radiation is in the range of 380 to 480 nm (Elumalai et al., 2014; Mukherjee et al., 2008; Kahrilas et al., 2014; Okafor et al., 2013; Fourough and Farhadi, 2010; Dare et al., 2014). The absorption bands in the range 285 to 350 nm observed in the present study is a clear indication of sufficient amount of reductive biomolecules in the *K. africana* fruit extract. This represents a discrete nucleation event with molecules of the active components of the extract being adsorbed on the surface of the nanoparticles. The nucleation is followed by slower controlled growth, which on heating, the particles acquired enough surface energy that promotes dissolution and second growth phase (Ostwald ripening) with the formation of uniform mono- dispersed particles. Thus there is rapid reduction, nucleation and growth with the formation of smaller particles which are stabilized throughout the reaction period. The increased temperature resulted in increased rate of synthesis and also promoted the synthesis of smaller size particles (Fayaz et al., 2009; Kim et al., 2011; Li et al., 2011; Elemike et al., 2014; El-Rafie et al., 2011).

The phytochemical screening reported on the aqueous extract of the plant revealed the presence of some phenolic compounds, alkaloids, and flavonoids (Gabriel and Olubunmi, 2009; Picerno et al., 2005; Gouda et al., 2006; Mobark et al., 2015). The anions of these compounds interact and are adsorbed on the surface of the nanoparticles, thus stabilizing the particles formed. To ascertain these interactions, FTIR spectroscopic measurements were carried out on the synthesized nanoparticles.

Figure 3 gives the FTIR spectra of the AgNPs and Ag-Cu bimetallic nanoparticles grown in the plant extract. The infrared spectra shows common absorption bands in the samples isolated even after centrifugation and washing, indicative of the adsorption of the secondary metabolites on the surface of the particles. A broad intense band at 3444 cm^{-1} in the spectrum of AgNPs can be attributed to the N-H stretching vibration arising from the peptide linkages present in the proteins of the extract (Mondal et al., 2014; Anuj and Ishnava, 2013; Mubayi et al., 2012). This broad band in the case of Ag-CuNPs, split into shoulders at 3427, 3502 and 3564 cm^{-1} which can be assigned to the overtone of the amide-II band and the stretching vibration of the O-H group, possibly arising from the carbohydrates and/or proteins present in the sample. The absorption band at 1622 cm^{-1} for AgNPs and 1625 cm^{-1} for Ag-CuNPs are due to amide-I bond of proteins, indicating predominant surface capping species having C=O functionality which are mainly responsible for

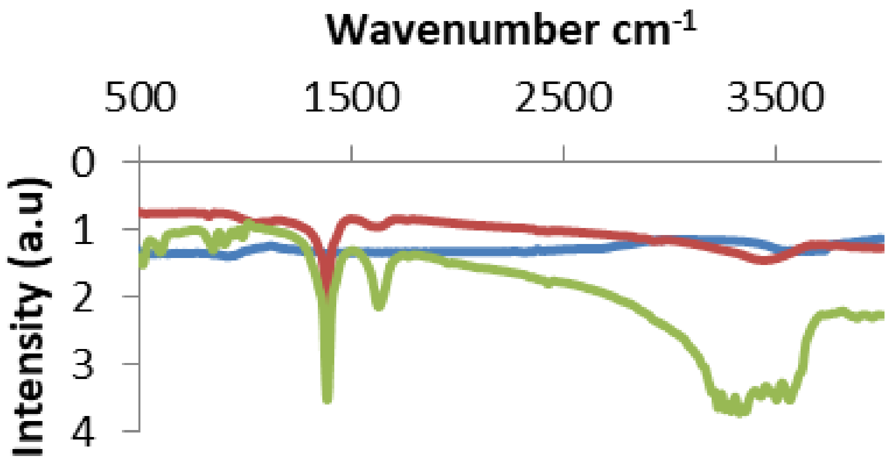

Figure 3. FTIR spectra of aqueous extract of *Kigelia africana* (blue), AgNPs (red) AgCu NPs (green).

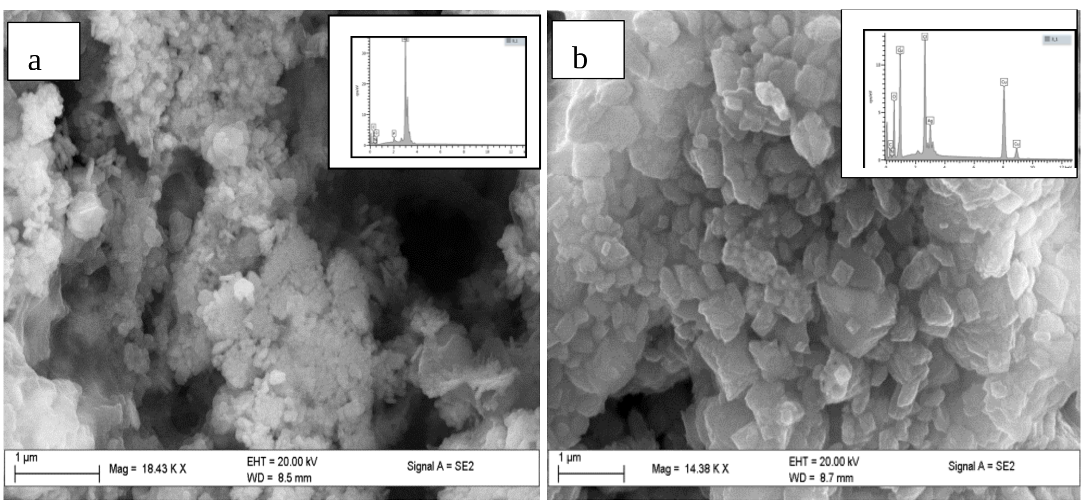

Figure 4. Representative SEM image (a) AgNPs (inset EDX spectrum) b) AgCuNPs (inset EDX spectrum) synthesized from the extract of *Kigelia africana* fruits.

stabilization (Mubayi et al., 2012; Mukherjee et al., 2008). The bands between 3016 and 2349 cm^{-1} can be assigned to C–H stretching and in-plane vibrations of the phenolic ring of plants metabolites. The band at ~1045 cm^{-1} largely might be due to the -C-O- groups of the polyols viz. flavones, terpenoids and the polysaccharides present. The band at 1382 cm^{-1} is attributed to the v(C-N) stretching vibration present in both AgNPs and Ag-CuNPs. The various assignments are in agreement with similar compounds reported in literature.

The representative scanning electron micrographs of the AgNPs and Ag-CuNPs synthesized in the extract are, respectively shown in Figure 4a and b. The AgNPs consists of monodisperse microspheres, while the Cu-Ag consists of anisotropic microplates. The energy dispersive X-ray (EDX) overall scans of both mono (inset of Figure 4a) and bimetallic nanostructures (inset of Figure 4b) showed the presence of carbon, nitrogen, oxygen and phosphorus originating from the secondary metabolites in the plant extracts, which acts as stabilizers. The peak around 3.0 keV correspond to the binding energies of AgNPs. This peak is reduced in intensity in the case of bimetallic particles with additional copper peak at about 8.0 keV. The weight percent of Ag is 80.64 and 8.69, respectively, for AgNPs and AgCuNPs .

Figure 5 shows the representative TEM micrograph of

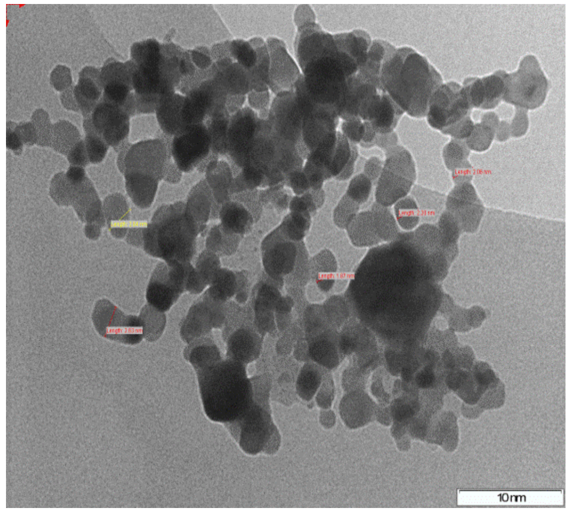

Figure 5. TEM micrograph of Cu-AgNPs

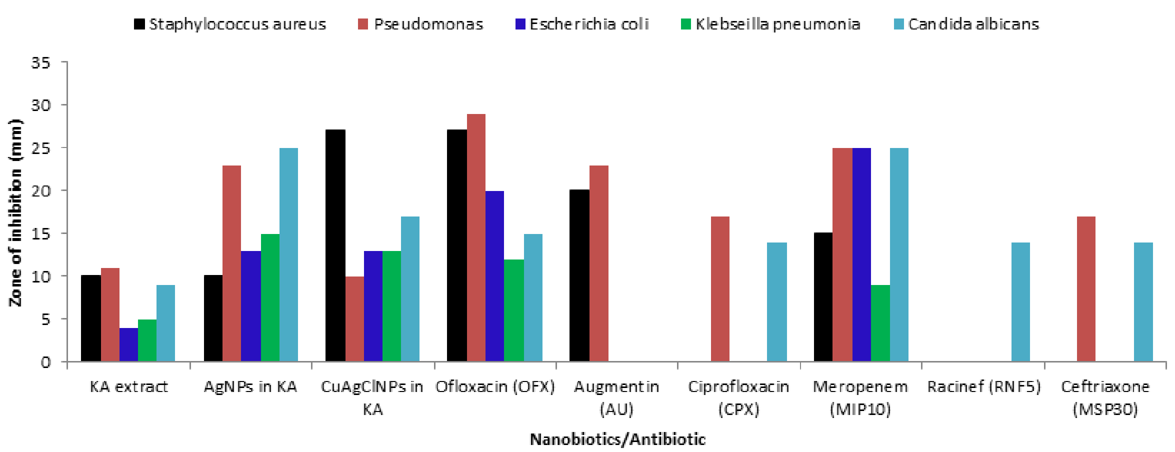

Figure 6. Antimicrobial activities of green synthesized nanoparticles using aqueous extract of *Kigelia africana* fruit.

the copper-silver nanoparticles. The particles are in the size range of 10 nm. Antimicrobial activities of the synthesized AgNPs and AgCuNPs were evaluated by using standard assay. The nanoparticles showed inhibition zone against all the bacteria (*K. pneumoniae, E. Coli, S. aureus* and *P. aeruginosa*) and fungus (*C. albicans*) under study as presented in Figures 6 and 7. The zone of inhibition of AgNPs obtained against *C. albicans* and *P.* aeruginosa are 25 and 23 mm, respectively, and are found to be higher than those of AgCuNPs. Whereas ZOI of AgCuNPs for *S. aureus* is 27 mm compared with 10 mm of AgNPs. This is indicative of the fact that the antibacterial sensitivity of the gram-positive *S. aureus* is lower for AgNPs as previously reported (Anuj and Ishnava, 2013; Sista et al., 2016; Mukherjee et al., 2008; Kahrilas et al., 2014; Okafor et al., 2013; Forough and Farhadi, 2010; Dare et al., 2014; Elemike et al., 2014; El-Rafie et al., 2011; Fayaz et al., 2009; Kim et al., 2011; Li et al., 2011; Soo-Hwan et al., 2011), but higher for AgCu bimetallic nanoparticles.

It is interesting to note that AgCu bimetallic nanoparticles synthesized from *K. africana* are crystalline in nature and have been found to be very active in inhibiting *S. aureus*. When compared with the standard antibiotics such as ofloxacin, augmentin, ciprofloxacin, meropenem or Racinef used as control in the present study, both the AgNPs and AgCuNPs have shown higher ZOI (15 mm for AgNPs and 13 mm for AgCuNPs) against *K. pneumoniae.* This inhibitory effect is also higher than those reported by Mubayi et al. (2012). The bacterial strain

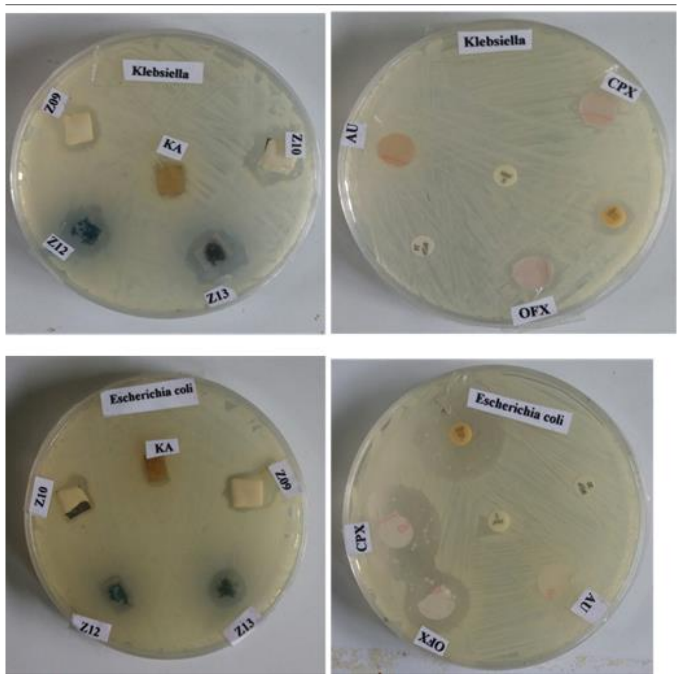

Figure 7. Representative plates showing zones of inhibition: Z09= AgNPs, Z13 = AgCuNPs, KA = *Kigelia africana* fruit extract, OFX = ofloxacin, CPX = ciprofloxacin, AU = augmentin.

of *E. coli* showed inhibition zone of 13 mm for both AgNPs and AgCuNPs. The nanoparticles obtained from *K. africana* fruit extract have been found to be more effective against both grams-negative and grams-positive bacterial strains.

Conclusion

Silver nanoparticles and copper-silver bimetallic nanoparticles have been successfully synthesized using aqueous extract of *K. africana* fruits. The SEM/EDX analyses confirm that nanostructures have been synthesized, while XRD shows that the particles are crystalline in nature. TEM reveals average particle size of 10 nm. UV-Vis and FTIR spectroscopic analyses showed that molecules of the active components of the plant extract are adsorbed on the surface of the particles, thus serving as stabilizing agents. The nanoparticles inhibit the growth of both Gram-negative and Gram-positive bacteria. The present nanoparticles synthesized from aqueous extract of *K. africana* fruits inhibits *K. pneumoniae* more than any of antibiotics tested in this study. It competes very well with augmentin against *P. aeruginosa* and with meropenem against *C. albicans* with inhibition zones of 23 and 25 mm, respectively. The bimetallic nanoparticles have demonstrated effectiveness against *S. aureus* with maximum ZOI of 27 mm. These results will motivate further investigation of the cytotoxicity of the synthesized nanoparticles on cancerous cells.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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