

Anti-Bacterial Effect of Biosynthesized Silver Nanoparticles Using *Kigelia Africana*

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Abstract

A green method of synthesizing silver nanoparticles (AgNPs) using *Kigelia Africana* leaves extract as reducing and stabilizing agent. The formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored by UV-visible spectrophotometer analysis at 445 nm. FTIR spectrum showed the functional groups that were present in the leaf extract were responsible for the reduction of AgNPs. XRD result shows that the particles are in crystalline nature and size ranging from 70 to 140 nm with spherical structure from SEM analysis. In addition, the characterized AgNPs has the potential antimicrobial effect against various pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The zone of inhibition of AgNPs was observed to be high for all pathogens.

Keywords

AgNPs, Antimicrobial Study, Bioactive Molecules, Bio-reduction, *Kigelia Africana*

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1. Introduction

Nanoparticles referred as particles within 100 nm in size [1, 2]. Nanoparticles are completely new or improved properties based on specific characteristics such as size distribution and morphology compared to that of the bulk material. Silver recognized for a long time as having an inhibitory effect toward many bacterial strains and microorganisms that were commonly affects the humans [2-3]. In recent years silver nanoparticles (AgNPs) plays a major vital role in medical field for oral and drug application that include creams and topical ointments containing AgNPs to prevent infection of burns and wounds [4], and implants prepared with silver-impregnated polymers [5].

Green synthesis methods of nanoparticles using plant extract [6], microorganism [7-9], enzyme [10] have been proposed as possible eco-friendly alternatives to that of chemical and physical methods where the prepared particles can be used as such without any modification. The use of plants for the

synthesis of nanoparticles can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures and other critical steps [11]. AgNPs are widely employed as potential drug carriers for the treatment of cancer and even started their place in treatment of AIDS were been reported too. Metal nanoparticles (AgNPs) are more effective, because of the presence of large surface to volume fraction so that a large proportion of silver atoms are in direct contact with their environment [12]. Due to these properties, metal nanoparticles have many applications in different fields like medicine, electronics, agriculture, etc.

Phytochemicals such as alkaloids, tannins, saponins, glycosides, phenolic compounds, steroids, proteins, amino acids and secondary metabolites like irridoids, flavonoids, naphthoquinone, and volatile constituents that inhibit the development of various microorganisms [13]. Moreover these, phytochemicals that were present in the plant extracts can act as reducing, capping and stabilizing agent in the

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preparation of nanoparticles [14] and the synthesized metal nanoparticles can be used in drug delivery [15], tissue / tumor imaging [16], bio-sensing [17], catalysis [18] and surface-enhanced Raman scattering-based sensors [19]. The synthesis of metal nanoparticles and nanostructured materials attracting much attention in recent research because of their valuable properties that makes them useful for catalysis [20], sensor technology [21] and the biological labelling of opto-electronic recorded media and optics [22].

Currently the biosynthesis of metal nanoparticles using plant extracts has received considerable attention as a suitable alternative method to existing chemical and physical methods [23, 24]. It is also very cost effective [25, 26] and valuable alternative for the production of large scale metal nanoparticles. Many researchers have reported the biosynthesis of metal nanoparticles by using various plant extracts [27, 28]. AgNPs were found to be effective against multi-drug resistant bacteria [29]. As a result of their small size, nanoparticles possess large surface area. This larger surface area enables AgNPs to interact more easily with other substances and therefore increases their antibacterial efficiency.

Gardea-Torresdey *et al.* [30, 31] for the first time, demonstrated synthesis of gold nanoparticles inside live plant. Similarly, alfalfa sprouts were also used for the synthesis of AgNPs. Biosynthesis of nanoparticles using different plant species like Aloe vera [32], Cinnamomum camphora [33], Capsicum annum L. [34], Azadiracta indica [35] and Cymbopogon flexuosus [36] has been successfully

carried out. Armendariz *et al.* [37] studied the synthesis of gold nanoparticles of different sizes and shapes using oat and wheat biomass. Mude *et al.* [38], for the first time, used in vitro-generated callus extract of Carica papaya for the synthesis of AgNPs. In 2009, Kumar and Yadav [39] reported the synthesis of silver and gold nanoparticles using different plants and also found that plant system was more advantageous over other biological processes. Song and Kim studied the extracellular synthesis of metallic AgNPs using five plant leaf extracts (Pine, Persimmon, Ginkgo, Magnolia and Platanus). They reported that the stable AgNPs were formed by treating aqueous solution of AgNO_3 with all the above plant leaf extracts as reducing agents of Ag^+ to Ag^0 . Magnolia leaf broth was reported to be the best reducing agent in terms of synthesis rate and conversion to AgNPs as compared to other plants. Bar *et al.* [40] developed an eco-friendly method for rapid synthesis of Ag NPs using aqueous seed extract of *Jatropha curcas*.

2. Materials and Methods

2.1. Materials

Silver nitrates (AgNO_3) of AR grade were purchased from Sigma Aldrich Chemicals, Mumbai, India. All glass wares have been cleaned well and dried in an oven before use. Double distilled water was used throughout the process. Fresh leaves of *Kigelia Africana* were collected from CEG Campus, Anna University, Tamilnadu, India.



Fig. 1. Full view of *Kigelia Africana* tree (A) and leaves(B).

2.2. Plant Description

Kigelia africana, belongs to the family of *Bignoniaceae*, always called as the sausage tree, is a spreading tree, which appears like a candelabra [41]. This is an evergreen in areas with abundant rainfall, but deciduous, losing its leaves at a particular time. *Kigelia africana* is also grown as an ornamental plant, specifically in tropical areas, for its remarkable flowers and fruits [42]. *K. africana* plants have many medicinal properties because of the presence of numerous secondary metabolites. These compounds include flavonoids, naphtha-quinones, irridoids, and volatile constituent's etc., [43]. Different solvent extract like ethanol, methanol etc., from the leaves of *Kigelia Africana* was used for the preliminary phytochemical identification [44].

2.3. Preparation of *Kigelia Africana* Leaf Extract

Collected *Kigelia Africana* leaves were washed using running tap water to remove adhering impurities and again using double distilled water. Later leaves were air dried under sunlight to remove the moisture completely. Then the leaves were chopped down and powdered using mechanical grinder. The obtained powders were then sieved to collect fine powder that was stored in an air locked covers for further process. 5 g of powder was taken along with 100 ml double distilled water and boiled at 60°C for 20 min. The obtained solution was then filtered through Whatmann no. 1 filter paper and the filtrate was stored for further biosynthesis process [45].

2.4. Biosynthesis of Silver Nanoparticles

Biosynthesis of silver nanoparticles includes, 5 ml of *Kigelia africana* leaf extract and 100 ml of aqueous solution of AgNO₃ (1mM). Aqueous solution of AgNO₃ was placed under stirring at 60°C temperature while drop by drop of leaf extract were added into it and left undisturbed for 30 min under same condition. After 30 min of stirring there was a color change was observed in the mixture. The initial colorless solution turned to dark brown color that confirm the formation of silver nanoparticles. Further the colloidal solution was centrifuged at 8000 rpm for 20 min and the supernatant was collected for further studies.

2.5. Characterizations

2.5.1. UV-visible Spectroscopy

Initial confirmation of as prepared silver nanoparticles were done using UV-visible spectral analysis that was carried out by UV-visible JascoV-550 spectrophotometer at the wavelength of 200–700 nm.

2.5.2. Fourier Transforms Infra-Red Spectroscopy

The synthesized silver nanoparticles were characterized by (FTIR) Jusco 5300 model with the wave number in the range of 400–4000 cm⁻¹ for identification of bioactive molecules that were present in the leaf extract of *Kigelia Africana*.

2.5.3. X-ray Diffraction

The crystalline nature of the synthesized AgNPs were confirmed by X-ray diffraction studies, X' Pert Pro X-ray diffractometer (PANalytical) was used for this analysis that was operated at a voltage of 40 kV and a current of 30 mA. The centrifuged AgNPs samples were air dried overnight and the powdered samples were used for analysis.

2.5.4. Scanning Electron Microscopy

Surface morphology and size of the synthesized AgNPs were analyzed using Scanning electron microscopy (SEM) that was carried out using Supra Zeiss with resolution of 1 nm at 30 kV with 20 mm Oxford EDS detector. Here the 1 µl of the sample were dropped on the sample holder and air dried before the analysis.

2.5.5. Particle Size Analyzer

Dynamic light scattering (DLS) was used for the measurement of average hydrodynamic diameters of the synthesized Ag nanoparticles and polydispersity indices (PDIs) of the particles, Malvern Zetasizer Nano-ZS, Malvern Instruments, UK. Sample is analyzed in triplicate at 20°C at a scattering angle of 173° and double distilled water is used as a references dispersing medium.

2.6. Antimicrobial Activity

The silver nanoparticles were tested for their antibacterial activity by the agar diffusion method. Four bacterial strains, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used for this study.

3. Results and Discussion

3.1. UV-visible Spectroscopy

The colorless mixture turned into dark brown color after 30 minutes, indicating the biotransformation of ionic silver reduced to silver nano, as a result of the surface Plasmon resonance phenomenon (SPR). Fig. 2a shows the UV spectrum of AgNPs and at 455 nm a peak was observed that was identified to be AgNPs. It is well known that AgNPs exhibit reddish brown [46]. The color change ensued as of the active molecules present in the leaf extract that owing to the excitation of SPR effect [47, 48].

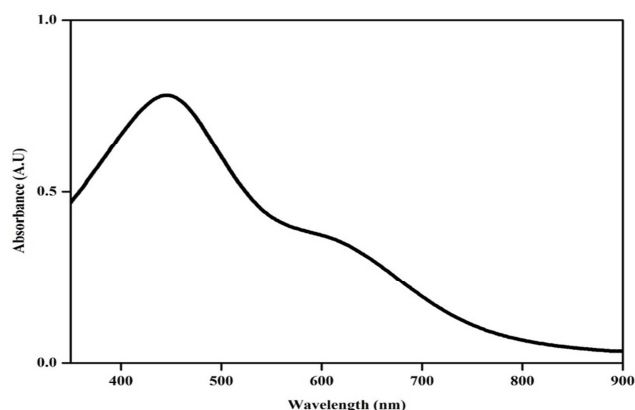


Fig. 2a. UV-Vis spectrum of AgNPs synthesized from *Kigelia Africana* leaf extract.

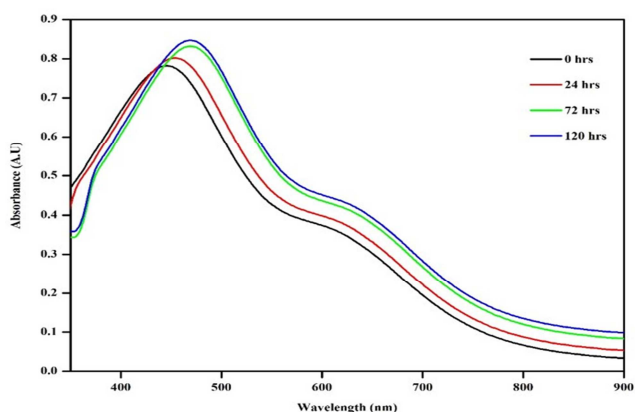


Fig. 2b. UV-Vis spectrum of AgNPs at different time interval.

The synthesized silver nanoparticles were thereafter analyzed at different time interval to find the stability of the particles. In this present work AgNPs were analyzed in UV- visible spectrometer at different time interval such as 24, 48, 72, 96, 120 hours as shown in Fig. 2b. It was found that the initial peak obtained at 455 nm were shifted to 485 nm after 120 hours. Shift in the peaks were also related to the size change of the AgNPs. The leaf extract of *K. Africana* provide less stability in the synthesized AgNPs. Thereafter the particles aggregates with

each other to form particles with larger size [46, 48].

3.2. FTIR Analysis

FTIR spectra of leaf extract (a) and synthesized AgNPs (b) shown in Fig. 3. There was a change in the following peaks: 3452.8 cm^{-1} represent O-H/N-H stretching, the peak shift from $3452.8\text{--}3455.4\text{ cm}^{-1}$ implicated that flavonoid (luteolin) group maybe involved in the process of nanoparticle synthesis (Happi Emaga, et al 2007). The peak located at 2368.5 cm^{-1} due to C-H stretching, H-C=O (aldehyde group) which indicate there is a chances of naphthoquinone derivative (kigelinone) present in the leaf extract. Shifting of peak occurs due to the breaking of bond between silver nitrate and kigelinone. The peak located at 2368.5 cm^{-1} assigned to N-H stretching. This peak was shift from $2368.5\text{--}2369\text{ cm}^{-1}$ because of the amino group present in the leaf extract which may reduce silver nanoparticle [40, 49]. Peak at 2345.9 cm^{-1} was assigned to the N-H stretching or the C=O stretching vibrations. The peak at 1638 cm^{-1} corresponds to N-H bend it represent primary amine [50]. The peak shift from $1638.3\text{--}1639.9\text{ cm}^{-1}$ indicated the possible involvement of primary amine groups which may be reduced the silver iron into silver nanoparticles [51]. A peak at 1019.2 cm^{-1} could be assigned to C-O stretch was suggestive the possible involvement of alcohols, carboxylic acids, esters, ethers. This may be involvement of ferulic acid in the leaf extract. The peak shifted from $689.9\text{--}690.6\text{ cm}^{-1}$ it represents C-H bend (alkyne group). These data indicated the involvement of amides, phenolic group, alcohol, amino groups and carboxylic groups present in leaf extracts that may be responsible for the reduction of nanoparticle [52]. The *K. africana* plant has many medicinal properties due to the presence of numerous secondary metabolites such as irridoids, flavonoids, naphthoquinone, and volatile constituents etc. Because of these secondary metabolites present in the plants, they may be involved in the reduction mechanism.

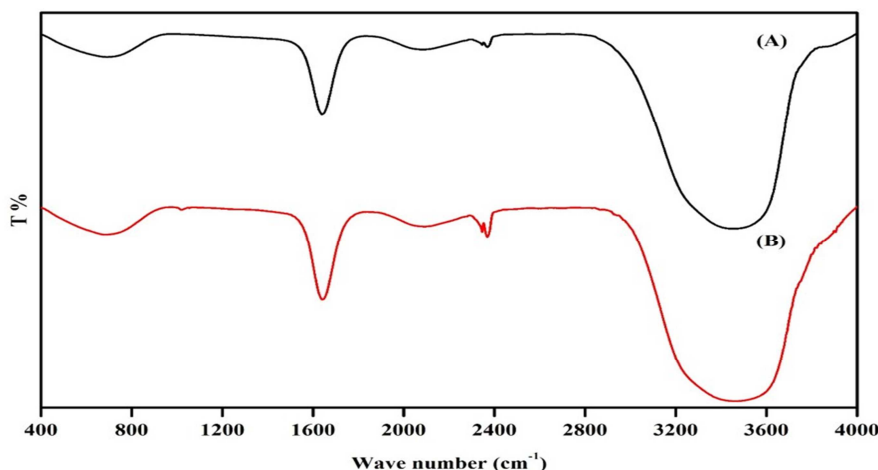


Fig. 3. FTIR spectrum of Leaf extract of *Kigelia Africana* (A) and AgNPs (B).

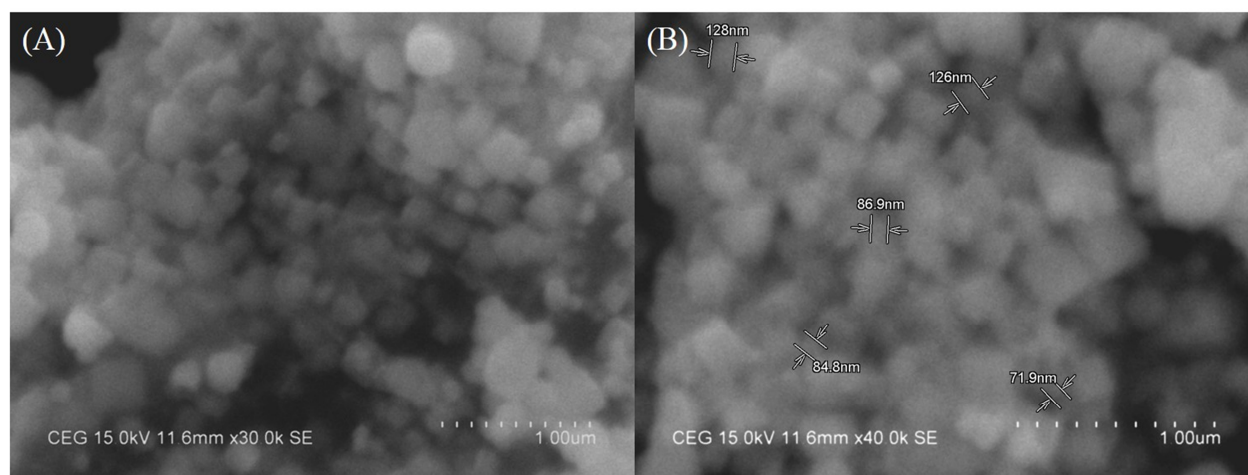


Fig. 4. SEM micrograph of AgNPs synthesized from *Kigelia Africana* leaf extract (A) and sized measured of AgNPs.

3.3. Scanning Electron Microscope

Fig. 3 shows representative SEM images of the Ag nanoparticles synthesized by treating AgNO_3 solution with *Kigelia africana* leaf extract. The resulting AgNPs were predominantly spherical and the size range from 40 – 75 nm. The SEM analysis of Ag nanoparticles from *Kigelia africana* supports the results of Chandran et al. in *Aloe Vera* [53]. Also, the rapid biosynthesis of silver nanoparticles of different shapes was observed and the sizes of nanoparticles were increased by high concentrations of *Kigelia africana* leaf extract [54].

3.4. X-ray Diffraction

The biosynthesized silver nanoparticle by employing *Kigelia africana* leaf extract was further demonstrated and confirmed by the characteristic peaks observed in XRD analysis. The

diffraction peak of silver nanoparticle at 2θ values 38.04° , 44.28° , 64.41° and 77.40° are assigned to plane (111), (200), (220) and (311) of lattice plane of face cubic center (FCC) for Ag nanoparticle respectively shown in Fig.5. It is confirmed that the nanoparticles were composed of pure crystalline Ag. These results are consistent with an earlier report on silver nanoparticle synthesis by the fungus *T. viride* [55]. The average Nano crystalline size has been estimated by using well known Debye–Scherrer formula,

$$D = K \lambda / \beta \cos \theta$$

Where, D is particle diameter size, k is a constant ($k=1$), λ is wavelength of X-ray source (1.5405 nm), and β is the full width at half maximum (FWHM). The average crystalline size according to Debye–Scherrer equation calculated is found to be 20 nm [28, 56].

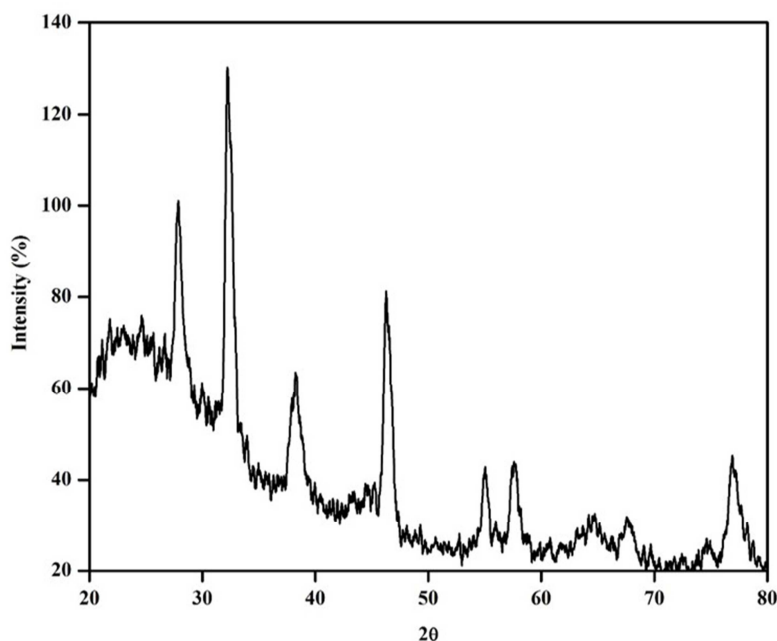


Fig. 5. XRD spectrum of AgNPs synthesized from *Kigelia Africana* leaf extract.

3.5. Particle Size Analysis

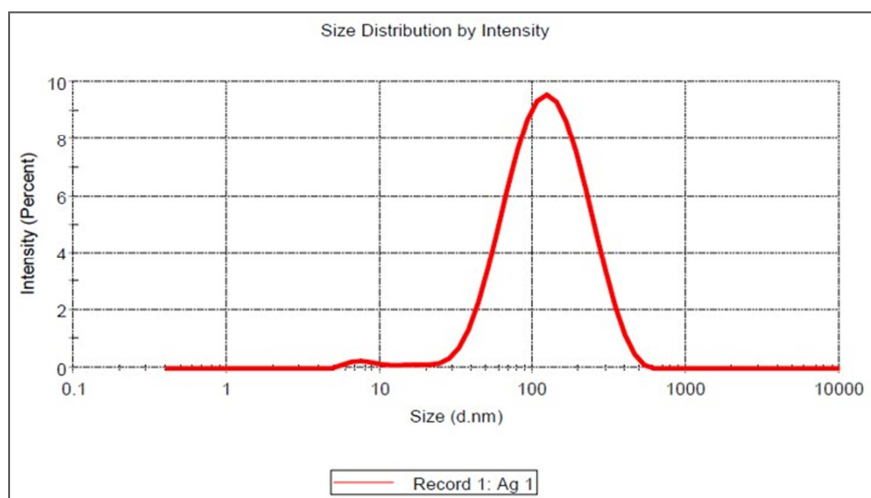


Fig. 6. Particle size distribution of AgNPs.

The average size of the particles, size distribution, and polydispersity index (PDI) of the synthesized AgNPs were determined by particle size analyzer, and the results are shown in figure 6. It shows the average particle diameter is 100.5 nm and Polydispersity index is 0.257. The average particle size and PDI revealed that the produced AgNPs were monodispersed [57].

3.6. Antimicrobial Activity

The behavior of the AgNPs synthesized from *Kigelia Africana* leaf extract against various bacteria was investigated by using Kirby- Bauer disc diffusion method. The antimicrobial studies were carried out on *Bacillus subtilis*, *E. coli* (*Escherichia coli*), *P. aeruginosa* (*Pseudomonas aeruginosa*) and *S. aureus* (*Staphylococcus aureus*). Table 1 shows the zone of inhibition of AgNPs on all four bacteria. The concentration of AgNPs was employed in various levels 25, 50, 75 and 100 μ l to bystander the prescription against the microbes. It was found at maximum prescription of 100 μ l the zone of inhibition was observed to be 21.3 mm for *Bacillus subtilis*, 24 mm for *S. aureus*, 23.7 mm for *P. aeruginosa* and 24.5 mm for *E. coli*. As the concentration of the AgNPs increases the zone of inhibition also increases.

Table 1. Zone of inhibition for biosynthesized AgNPs from *Kigelia Africana* leaves extract against different bacterial species (mm).

Name of the bacteria's	control	Different concentration of AgNPs			
		(25 μ l)	(50 μ l)	(75 μ l)	(100 μ l)
<i>Bacillus subtilis</i>	1	16 $\pm$ 0.5	18 $\pm$ 1.0	19 $\pm$ 1.3	21 $\pm$ 1.0
<i>Staphylococcus aureus</i>	1	17 $\pm$ 0.5	19 $\pm$ 1.0	23 $\pm$ 1.3	24 $\pm$ 1.0
<i>Pseudomonas aureoginosa</i>	1	15 $\pm$ 0.5	18 $\pm$ 1.0	22 $\pm$ 1.3	23 $\pm$ 1.0
<i>Escherichia coli</i>	1	16 $\pm$ 0.5	20 $\pm$ 1.0	21 $\pm$ 1.0	24 $\pm$ 1.3

4. Conclusion

The biosynthesized silver nanoparticles using *Kigelia Africana* leaves extract was successfully carried out with excellent antimicrobial activity. The FTIR spectrum shows that the leaf extracts act as an effective reducing agents that being responsible for the nano silver particles. The shape of silver nanoparticles was confirmed to be spherical by SEM analysis and crystalline nature through XRD. The larger zone of inhibition was obtained with AgNPs that proves AgNPs synthesized from the *Kigelia Africana* leaves extract have a very good property against microbes that can be employed for drug delivery applications.

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