

Full Length Research Paper

## Biosynthesis of silver nanoparticles using *Garcinia kola* and its antimicrobial potential

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Received 11 August, 2015; Accepted 5 November, 2015

We have investigated the green synthesis and antimicrobial activity of silver nanoparticles using *Garcinia kola*. Aqueous extract of *G. kola* was used to reduce  $\text{AgNO}_3$  solution to obtain black nanocrystal of silver nanoparticles. Antimicrobial activity of the silver nanoparticles was tested against *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Candida albican*. The synthesized silver nanoparticles was characterized using UV-vis spectroscopy, X-ray diffraction (XRD), Transmission electron microscopy (TEM), and Fourier transform infrared (FTIR) spectroscopy. UV-visible exhibits prominently the characteristic surface plasmon resonance at around 427 nm. A remarkable particle growth onset of 10 min was observed while the reaction ended within 30 min. The XRD analysis shows that the synthesized silver nanoparticles are crystalline in nature and well-dispersed silver nanoparticles with an approximate size of 10.4 nm were observed on TEM. The synthesized silver was found to possess good antimicrobial activity against all the tested microbes. The application of the green synthesized nanoparticles can be used in many fields such as foods, beverages, cosmetics and medicine.

**Key words:** Silver, nanoparticles, green synthesis, *Garcinia kola* seed extract, transmission electron microscopy, antimicrobial activity.

### INTRODUCTION

Nanotechnology is a growing field that has drawn the attention of researchers in different disciplines. Metal nanoparticles with controlled size and shape form the basis for advanced functional materials for electronic,

sensor, optical devices (Gurunathan et al., 2009). Metal nanoparticles synthesis is achieved majorly using physical, chemical and biological approaches. Laser ablation (Tsuji et al., 2003), Arc discharged method (Tien

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et al., 2008), irradiation (Yong et al., 1999), polyol process (Sun and Xia, 2002), polymers (Hebeish et al., 2011; Chengcai et al., 2005; Narendra and Khanna, 2007), co-precipitation (Yang et al., 2003), sol-gel (Lu et al., 2002), bacteria (Korbekandi et al., 2012), fungi (Ahmad et al., 2003; Balaji et al., 2009) have been used in the synthesis of nanoparticles. The use of plant extracts, enzymes, microorganisms, polymers, sugars and vitamins in nanoparticles formation has made nanotechnology a promising area of research (Oxana et al., 2013). Among the noble metals (e.g., Ag, Pt, Au and Pd), silver (Ag) is the metal of choice for potential applications in the field of biological systems, living organisms and medicine (Jain et al., 2009). The use of plant extract in synthesizing silver nanoparticles is eco-friendly, energy saving and can be easily scaled up. Biologically synthesized silver nanoparticles using medicinal plants or plant extracts include locust bean gum (Chandrakant et al., 2013), *Andrographis paniculata* (Venkata et al., 2014), lingonberry and cranberry juices (Judita et al., 2014), *Morinda citrifolia* (Gnanasekar et al., 2012), *Alternanthera dentata* (Deenadayalan et al., 2014), guava leaves extract (Shinde et al., 2014), *Gardenia jasminoides* (Lü et al., 2014), Jatropha seedcake (Anjali et al., 2013), *Mimusops elengi* (Hoskote et al., 2014), *Abutilon indicum* (Ashokkumar et al., 2015), *Ziziphora tenuior* (Babak and Gholamhoseinpoor, 2015), *Ficus carica* (Bulent et al., 2015), *Acacia auriculiformis* (Pradnya et al., 2014), *Cocos nucifera* (Mariselvama et al., 2014). Pineapple leave extract has also been used for the green synthesis of silver nanoparticles and its high antibacterial activities have been reported (Elemike et al., 2014). Recently our research group studied the growth kinetic of silver nanoparticles under the influence of plant biodiversity (Dare et al., 2015) for pharmaceutical and biological applications have been reported. *G. kola* Heckel (Clusiaceae), commonly known as bitter kola (English) and orogbo (Yoruba) is a widespread tree of evergreen forest valued in Nigeria for its medicinal nuts which has led to its exploitation in the natural forests in recent times (Ibikunle and Emmanuel, 2011). The phytochemicals obtained from *G. kola* as documented in literature include biflavonoids such as kolaflavonone, and 2 hydroxyflavonoids, xanthenes, kolanone, amekoflavone, 2,4-methylenecyclartenol, coumarine and prenylatebenzophenones, oleoresin, the chromanols, garcioic and garcinal (Adesuyi et al., 2012). Others are tannin, saponins, alkaloids, and cardiac glycoside. The proximate analyses showed that the sample has high level of carbohydrate, little amount of crude fibre and protein respectively and negligible amount of ash content and crude fat. This composition shows that the sample could be a good source of carbohydrate, dietary fibre and protein (He et al., 2007). Biomolecules with carbonyl, hydroxyl, and amine functional groups have the potential for metal ion reduction and capping the newly formed particles during their growth processes (Adesuyi et al.,

2012). In this work, we report a simple, nontoxic, and eco-friendly green synthesis of silver nanoparticles using aqueous seed extract of *G. kola* and no toxic chemicals are used as reducing and stabilizing agent during the synthesis.

## METHODOLOGY

### Materials

*G. kola* seeds and Silver nitrate (AgNO<sub>3</sub>) of analytical grade was purchased from Sigma-Aldrich.

### Organisms

*Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus* (*S. aureus*), *Aspergillus niger* (*A. niger*), *Rhizopus stolonifer* (*R. stolonifer*), and *Candida albican* were collected at the Department of Microbiology, University of Ibadan.

### Plants and preparation of extract

*G. kola* seeds were obtained from the local market in Lafia, the capital of Nasarawa State, Nigeria. 10 g of seeds was milled using an ordinary coffee grinder and ground kernel were boiled with 100 mL triply distilled/ deionized water for 10 min. After filtration through Whatman No 1, filter clear seed extract was obtained for further use. All the aqueous solutions were prepared using triply distilled deionized water and stored at 4°C for further use.

### Synthesis of silver nanoparticles

10 ml of aqueous seed extract was added to 40 ml of 1 mM aqueous silver nitrate solution; the mixture was heated at 70°C in time intervals ranging from 2 to 30 min. Reduction of the Ag<sup>+</sup> ions was monitored by measuring the UV-visible spectrum of the solution on a spectrophotometer (T60 UV-vis spectrophotometer) operating at a resolution of 1 nm.

### Characterization

The crystallinity and phases of the Ag nanoparticles were characterized by X-ray diffractometer (XRD-6000, Shimadzu, Japan) with CuK $\alpha$  radiation ( $\lambda = 1.5412 \text{ \AA}$ ) in the range of 30°–90° with 2°/min scanning rate. The functional groups of Ag nanoparticles were characterized by Fourier-Transform Infrared (FTIR, Perkin Elmer, Spectrum BX) spectroscopy in the range of 4000–350 cm<sup>-1</sup>. In addition, time-resolved absorption spectra of prepared Ag nanoparticles was analyzed via UV-visible Spectrophotometer (T60 UV-Vis spectrophotometer). The morphology and size of the prepared Ag nanoparticles was observed by Transmission Electron Microscopy.

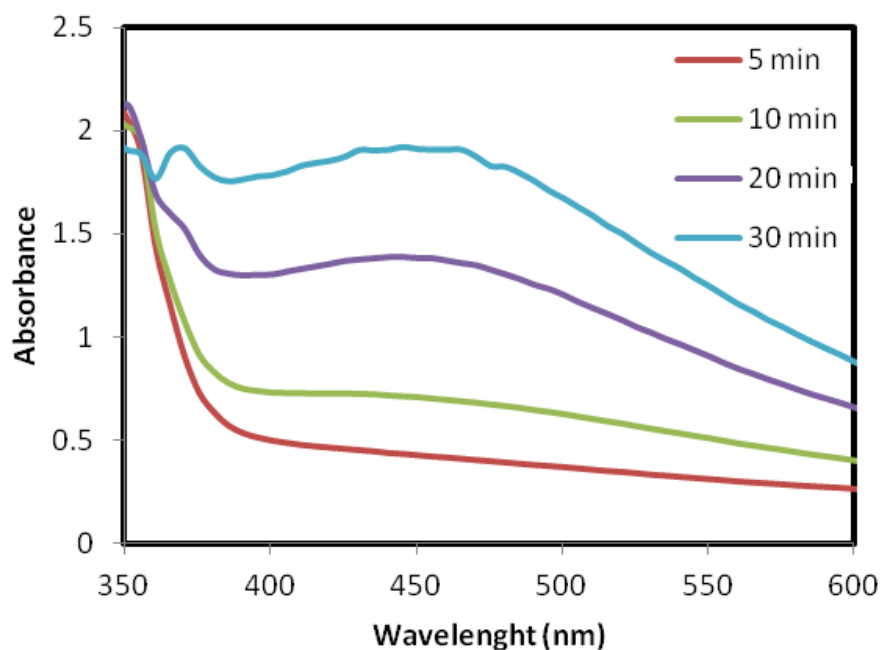
### Evaluation of antibacterial activity

The antimicrobial screening of the silver nanoparticles was carried out against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Candida albican* using Agar-well diffusion method.

**Table 1.** The variations in zone of inhibitions (mm).

AgNp conc. (mg/ml)	Zone of inhibition of tested pathogenic bacteria (mm)				Zone of inhibition of tested pathogenic fungi (mm)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>K. pneumonia</i>	<i>A. niger</i>	<i>C. albican</i>	<i>R. stolonifer</i>
500	26	25	20	13	18	17	ND
250	24	21	19	11	15	14	ND
125	21	17	14	10	12	12	ND
62.5	18	14	13	ND	10	9	ND
31.25	12	10	10	ND	ND	ND	ND

ND=Not detected.



**Figure 1.** UV-visible spectrum of the silver nanoparticles synthesized with aqueous seed extract of *G. kola*

Gentamycin and Tioconazole were used as control for bacteria and fungi respectively. Using a micropipette, 50  $\mu$ L of nanoparticle solution was poured onto each of the 8mm diameter well made on the prepared agar plates. On incubation at room temperature for 72 h, the diameter of zone inhibition was measured in millimeter; the values are shown in Table 1.

## RESULTS AND DISCUSSION

### UV- Vis spectra analysis

The color change was noted by visual observation in the Erlenmeyer flask that contains  $\text{AgNO}_3$  solution with *G. kola* extract. The color of the  $\text{AgNO}_3$ /seed extract solution changed from light yellow to light brown after 5 min and eventually to dark brown. This color change indicates the

formation of Ag nanoparticles in the solution. Seed extract without  $\text{AgNO}_3$  did not show any color changes. The formation of Ag nanoparticles was further confirmed by using X-ray diffraction (XRD), Fourier-Transform infrared spectroscopy (FTIR) and Transmission electron microscopy (TEM).

Figure 1 shows the UV-vis absorption spectrum of the synthesized Ag nanoparticles. Silver nanoparticles have free electrons, which give surface plasmon resonance (SPR) absorption band. This is due to the combined vibration of electrons of silver nanoparticles in resonance with light wave. A broad absorption peak was observed at 440 nm, which is a characteristic band for silver nanoparticles. No other peak was observed in the spectrum which confirms that the synthesized products are  $\text{Ag}^0$  only.

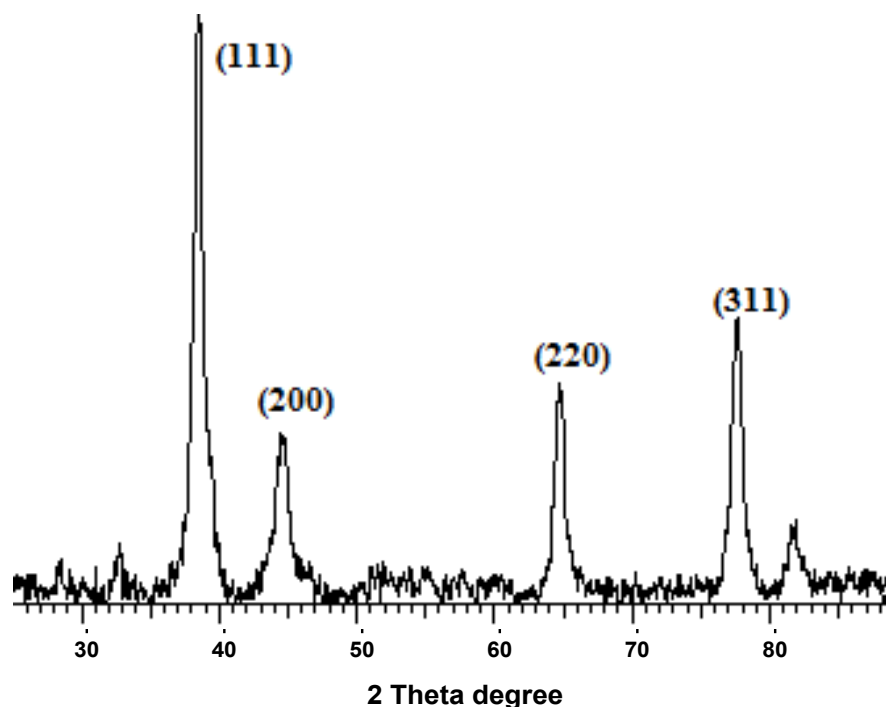


Figure 2. XRD patterns of Ag-NPs synthesized with *G. kola*.

### XRD analysis

Figure 2 shows XRD patterns for Ag nanoparticles synthesized by *G. kola* seed extract. Four peaks at  $2\theta$ , 38.4, 44.5, 64.8, and 77.7 corresponding to (111), (200), (220) and (311) reflect planes of face-centred cubic (fcc) structure of Ag. These peaks are consistent with the standard data file JCPDS No 04-0783 JCPD. The broadening of X-ray peaks observed is primarily due to the small particle size. The mean size of silver nanoparticles was calculated using the Debye-Scherrer's equation (Sheny et al., 2011). The average mean size of AgNPs was 10.40 nm. No peaks from any other phase were observed showing that single phase Ag with cubic structure nanoparticles has been obtained directly.

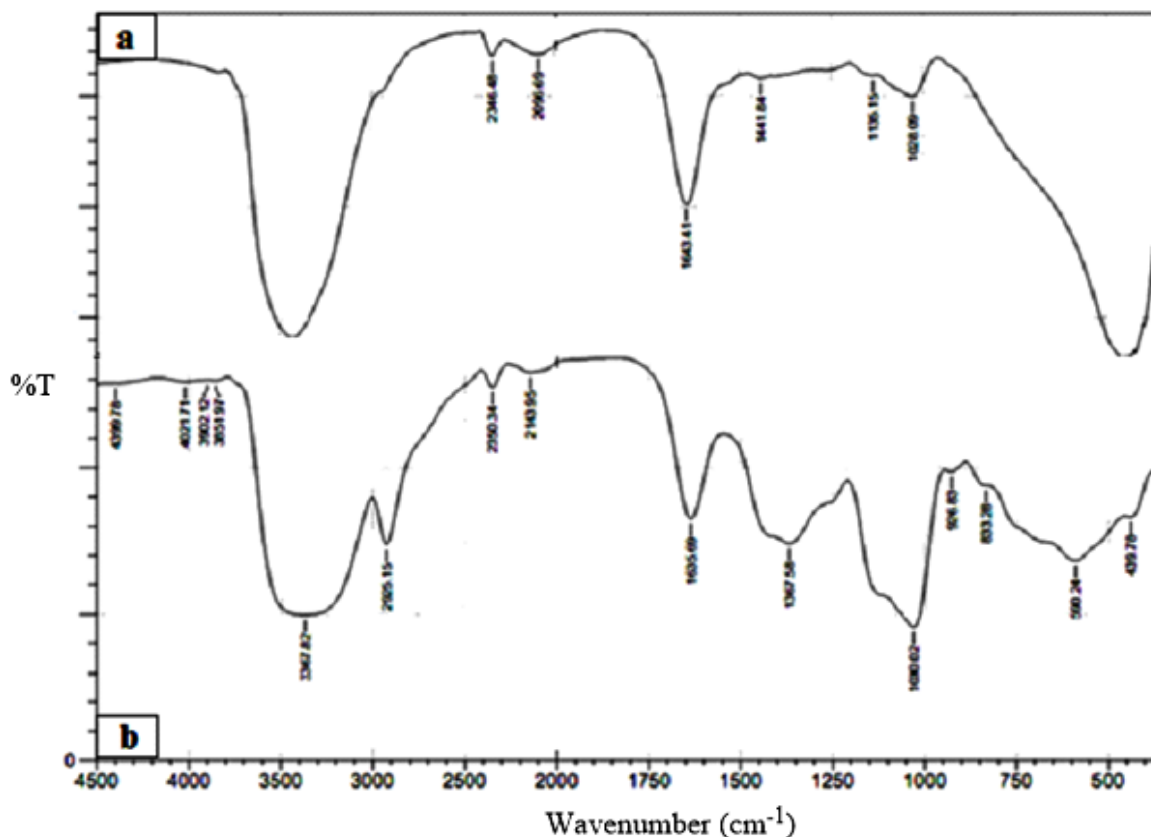
### FTIR spectra analysis

FTIR measurements were carried out on both the extract and the silver nanoparticles from *G. kola*. This was done to identify the biomolecules for reduction, and efficient stabilization of the metal nanoparticles synthesized by *G. kola* seed extract. The FTIR spectrum of silver nanoparticles is shown in Figure 3b. The presence of peaks at 3434, 2346, 2096, 1643 and 1028  $\text{cm}^{-1}$  was observed for the crude extract of *G. kola* (Figure 3a), which indicates that the silver nanoparticles may be surrounded by hydroxyl group, because the peaks indicate -OH symmetric stretching and C-O bonds in

aliphatic or carboxylic compounds (Ghosh et al., 2012). The band at 3434  $\text{cm}^{-1}$  corresponds to O-H stretching H-bonded alcohols. The peak at 1643  $\text{cm}^{-1}$  corresponds to C=O of ketone or aldehyde group and was shifted to lower frequency of 1635  $\text{cm}^{-1}$  in the spectra of the silver nanoparticles from *G. kola*. The peak at 1028  $\text{cm}^{-1}$  was assigned to the stretch of the C-O bond (Renquan et al., 2012). Therefore, the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids, biflavonoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Mukherjee et al., 2001). This shows that the aqueous seed extract of *G. kola* is a very good and robust bioreductant for the synthesis of silver nanoparticles.

### TEM and EDX analysis

The TEM image gives the morphology and size of the synthesized silver nanoparticles. A spherical nanoparticle which conforms with the SPR band in the UV-Vis spectrophotometer and to that of the XRD analysis was obtained with average particle size of 10.40 nm (Figure 4). Table 2 shows the EDX profile of the synthesized silver nanoparticle from *G. kola* extract. The selected area electron diffraction SAED pattern of *G. kola* silver nanoparticles was recorded at different scales. The



**Figure 3.** FTIR spectra of (a) extract of *G. kola* leaf extract (b) silver nanoparticles synthesized using *G. kola*.

**Table 2.** EDX elemental analysis of the silver nanoparticles of *G. kola*.

Element sigma	Weight (%)	Weight %
C	57.23	0.57
O	16.94	0.20
Ag	25.82	0.73

observed spot resulting into a circular ring confirmed the polycrystalline nature and face centered cubic structure of GSNP. The circular spot corresponds to the (111), (200), (220) and (311) Plane of Ag<sup>0</sup>.

### Antibacterial assay

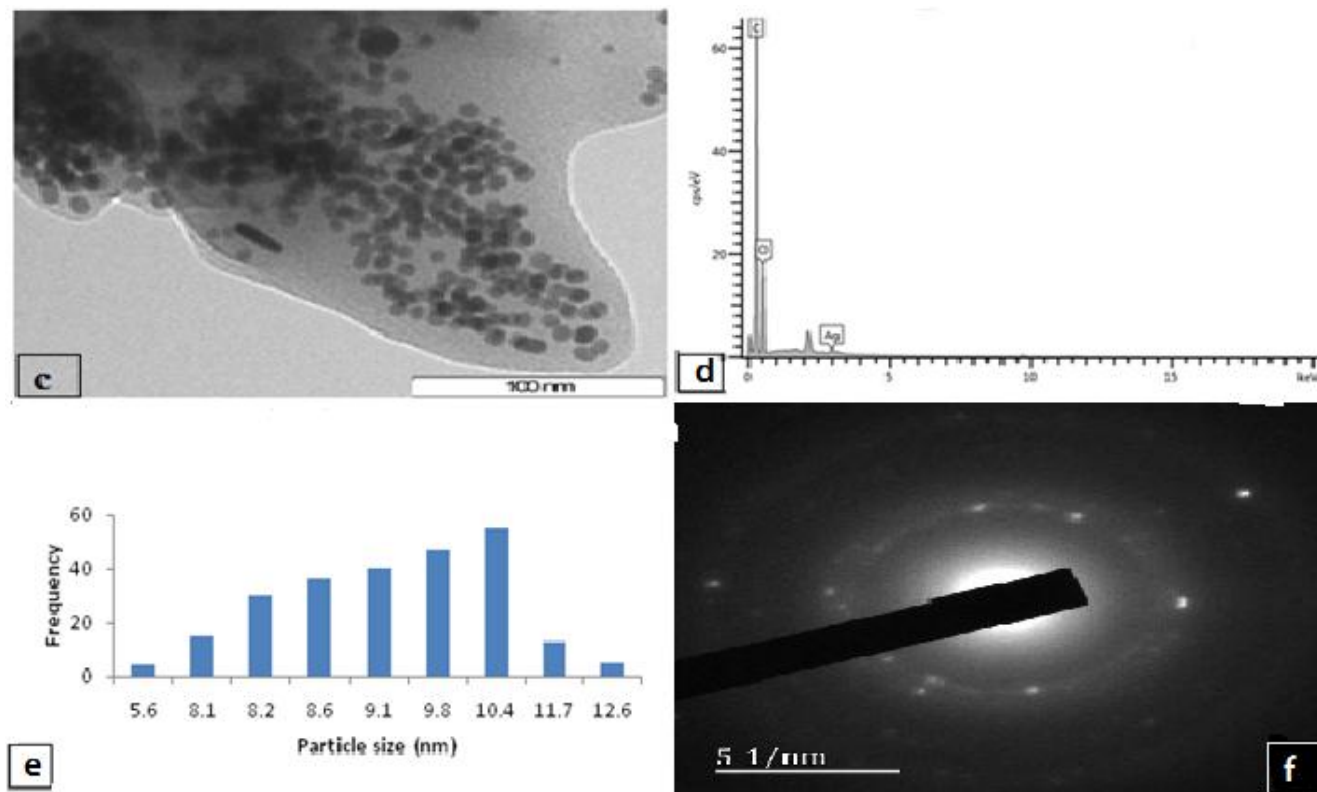
The bio-reduced silver nanoparticles from *G. kola* show a clear and high zone of inhibition as a result of the attachment of the silver ion to the negative charged cell wall. This led to structural changes in the cell wall, permeability and subsequently death of the bacteria (Sondi and Salopek-Sondi, 2004) which prevent the multiplication of *Staphylococcus aureus*, *Escherichia coli*,

*Klebsiella pneumoniae* and *Bacillus subtilis*; likewise on fungal pathogen namely *Aspergillus niger*, *Rhizopus stolonifer*, and *Candida albican*. *S. aureus* shows the highest zone of inhibition at 500mg/L compared to Gentamycin while *K. pneumoniae* shows the lowest. For fungal pathogen, both *A. niger* and *Candida albican* show same efficacy compared to Tioconazole; *R. stolonifer* shows no zone of inhibition (Table 1).

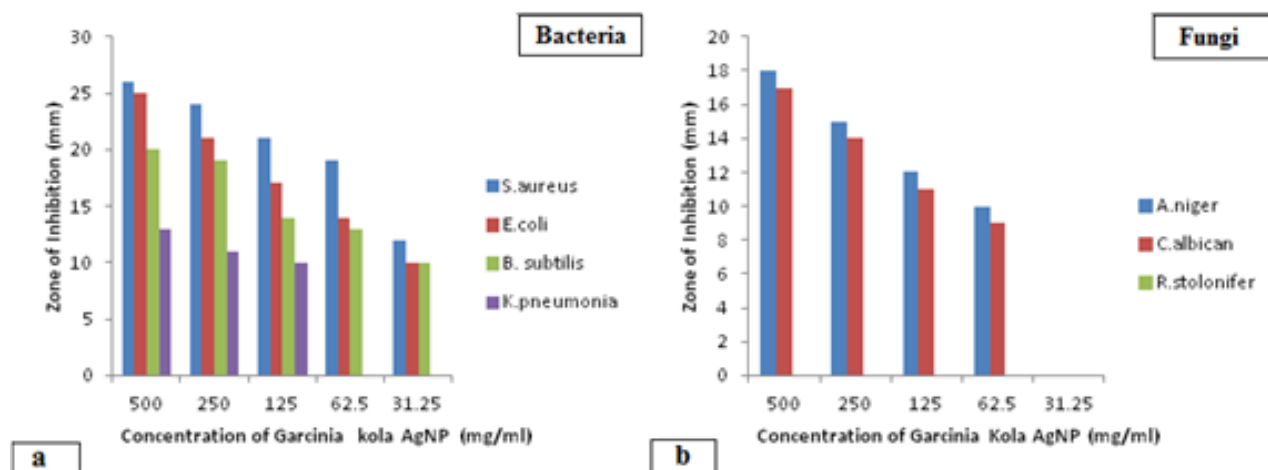
Also due to high zone of inhibition exhibited by *S. aureus* and *A. niger* followed by *C. albican* in Figure 5, the silver nanoparticles from *Garcinia kola* possess high antimicrobial activity which can be used in household appliances like clothes washer and refrigerator which can kill bacteria and reduce odour

### Conclusion

In this paper, we have reported a green method to synthesize silver nanoparticles using the aqueous seed extract of *G. kola*. The aqueous seed extract of *G. kola* helped in reducing and stabilizing the silver nanoparticles with remarkable growth onset of 10 min. This makes our green approach superior to conventional methods. The average size was found to be 10.4 nm with high



**Figure 4.** (c) TEM micrograph of the Ag nanoparticles synthesized using *G. kola* (d) EDX graph (e) the particle size distribution of the synthesized silver nanoparticles from *G. kola*. (f) selected area electron diffraction (SAED) pattern of the synthesized silver nanoparticles from *G. kola*.



**Figure 5.** Antimicrobial activity of silver nanoparticles synthesized from *G. kola*.

susceptibility against tested pathogenic bacteria and fungi.

#### Conflict of interests

The authors have not declared any conflict of interest.

#### ACKNOWLEDGEMENTS

The authors acknowledge the Vice Chancellor of Federal University Lafia, Prof. Ekanem Ikpi Braide, Dean Faculty of Science, Prof. Martin Ogbe and the entire staff of Chemistry and Physics Department for their support during the course of this research work.

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