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BIOSYNTHESIS OF SILVER NANOPARTICLES USING AQUEOUS EXTRACT OF *BUCHHOLZIA CORIACEA* (WONDERFUL KOLA) SEEDS AND THEIR ANTIMICROBIAL ACTIVITIES

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Abstract

The advent of biological method in the synthesis of nanoparticles had revolutionized the field of nanotechnology due to their advantages such as simplicity, cost effectiveness, eco-friendliness and broad applications over the conventional physical and chemical methods. In this study, we report a biosynthesis of silver nanoparticles (AgNPs) using aqueous extract of wonderful kola. The biosynthesized AgNPs were characterized by UV-visible spectroscopy, Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). Antimicrobial properties of the particles were evaluated against four clinical bacterial and three selected fungal isolates. The particles showed maximum absorbance at wavelength of 413 nm, while the distinct peaks at 1637 and 3319 cm^{-1} on FTIR spectrum indicate that protein molecules in the extract responsible for the coating and stabilization of the synthesized AgNPs. The AgNPs are dark brown in color by visual observation, spherical in shape with the size ranging from 15-50 nm as revealed by SEM. The particles showed remarkable antimicrobial activities against the four clinical bacterial and three selected fungal isolates. The results obtained in this study suggest that the biosynthesized AgNPs would be relevant in the development of a novel antimicrobial agents for numerous biotechnological applications. To the best of our knowledge, this is the first report on the use of wonderful kola for the synthesis of nanomaterials. Hence, the present study has extended the applications of wonderful kola to the area of nanotechnology.

Keywords: silver nanoparticles; wonderful kola; aqueous extract; antimicrobial agents; nanotechnology

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1. INTRODUCTION

Nanotechnology is a multidisciplinary field that encompasses diverse areas of science such as biology, physics, chemistry, and material science. It deals with the study of materials at nano scale. In the past, physical and chemical methods were used for the synthesis of nanoparticles but in recent times green method of synthesis is gaining immense attentions due to its advantages such as cheapness, simplicity, eco-friendliness, biocompatibility, and wide areas of applications over the conventional methods (Adelere and Lateef, 2016). The unique properties of nanoparticles like size, distribution, and morphology make them suitable for many applications than bulk materials. Syntheses and activities of various types of nanoparticle have been reported but silver nanoparticles (AgNPs) is receiving significant consideration because of its great

antimicrobial activities (Kim *et al.*, 2007) which make it suitable for many biotechnological applications. Authors have reported the syntheses of AgNPs using microorganisms and extract from plant materials (Lateef *et al.*, 2015a,b; Manikprabhu *et al.*, 2013), but due to the rich biodiversity of plants and their potential secondary metabolites, plants and plant parts have been well utilized more recently in their syntheses (Madhumitha *et al.*, 2013). Plant extracts are rich in natural compounds such as alkaloid, flavonoids, saponins, tannins, and other nutritional compounds. These products are obtainable from various plant parts including seeds, leaves, stems, roots, shoots, flowers, and barks. They act as reducing, stabilizing and capping agents in the biosynthesis of metallic nanoparticles (Adelere and Lateef, 2016). In spite of overwhelming reports on the utilization of diverse varieties of plants for the synthesis

of metallic nanoparticles, there exist no report on the biosynthesis of nanoparticles using any form of *Buchholzia coriacea*.

Buchholzia coriacea is a perennial plant belonging to the family of *Capparaceae* (Ibrahim and Fagbonun, 2013). It is an evergreen, small to medium-sized plant with the height up to 20 m and commonly found in Nigeria, Cameroon, Gabon, Central African Republic, Congo, Angola, Ghana, among others (Mbata *et al.*, 2009). The leaves are large and glossy between 15-25 cm long and 5-7.5 cm wide (Akinyele, 2010) with conspicuous creamy white flowers and edible seeds of medicinal value. The seeds are blackish, covered with purple aril (Fig. 1) and has a characteristic sharp pungent taste with hot spicy flavor when are fresh (Odebiyi and Sofowora, 1978). Different local names have been assigned to the seeds among Nigerians. It is called “Uworo”, “Owi”, and “Uke” by Yoruba, Edo, and Igbo people, respectively (Sofowora, 2008). The seeds derived its popular name “wonderful kola” due to its great potency to numerous diseases. It is also called memory nut because of its ability to enhance the memory (Ibrahim and Fagbonun, 2013). *B. coriacea* seeds have traditionally been used successfully for the treatment of diabetes, rheumatism, hypertension, cold, catarrh, and cough (Adisa *et al.*, 2011). Also, complications such as chest pain, wrist pain, irregular menstruation, malaria, premature ejaculation, dysentery among others have also been remedied with the use of these seeds (Ezeifeke *et al.*, 2004; Jaiyesimi *et al.*, 2011; Ibrahim and Fagbonun, 2013). Wonderful kola can be used to improve nervous system and as a blood cleanser. It is specially used to treat migraine in Africa (Jaiyesimi *et al.*, 2011). Traditionally, the plant is used for many purposes but very few were documented. Therefore, this study seeks to extent the frontier of applications of wonderful kola to the field of nanotechnology. This study aimed to investigate the use of wonderful kola for biosynthesis of silver nanoparticles and to evaluate the synthesized silver nanoparticles for their antimicrobial activities against some pathogenic strains of

bacteria and fungi. To the best of our knowledge, this report represents the first reference to the use of wonderful kola for the green synthesis of nanoparticles.



Fig. 1: *Buchholzia coriacea* seeds (Wonderful kola)

2. MATERIALS AND METHODS

Preparation of wonderful kola extract

Wonderful kola were purchased from Central market of Minna, Niger State, Nigeria. They were thoroughly washed in the laboratory to remove extraneous materials. The seeds were chopped into smaller pieces and dried at room temperature (30 ± 2 °C) for 2 weeks. The dried seeds were pulverized using an electric blender. Approximately 1 g of the powder was dispersed into 10 ml of deionized water and heated in water bath at 60 °C for 1 h. The extract was then filtered using Whatman No. 1 filter paper and stored at 4 °C for further studies.

Synthesis and characterization of AgNPs

The aqueous extract of wonderful kola was used to synthesize AgNPs according to Lateef *et al.* (2015a). The extract of 1ml was reacted with 10 ml of 1mM silver nitrate (AgNO_3) at ambient temperature (30 ± 2 °C). The reaction was allowed to stand for 1h. It was visually monitored for possible color change and measurement of absorbance characteristic through UV-vis spectrophotometry analysis as preliminary characterizations.

The AgNPs solution was centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the solid residue was dried at room temperature. The dried AgNPs mixed with KBr pellets was used for fourier transform infrared (FTIR) spectroscopy analysis using BUCK M530 Spectrophotometer (Buck, USA) as

described by Bhat *et al.* (2011). For scanning electron microscopy (SEM) analysis, the dried sample of AgNPs was mounted on specimen stub coated with copper and the micrography was taken by scanning electron microscope (JEOL, Model 6390).

Antimicrobial Activities of Synthesized AgNPs
The antibacterial activity of the synthesized AgNPs was evaluated against bacterial pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella Typhi* and *Pseudomonas aeruginosa* using disc diffusion method as described by Ahmed and Ikram (2015). The organisms were grown overnight in peptone water and incubated at 37 °C for 24 h. The 24-h old cultures were used to seed the plates of Muller Hinton agar with the aid of sterile cotton swab stick. The seeded plates were allowed to stand for 3-5 h. The sterile 6 mm paper discs prepared from Whatman No. 1 filter paper were impregnated with graded concentrations of the synthesized AgNPs (50, 100, and 150 µg/ml). The AgNPs impregnated discs were gently placed on the seeded plates and incubated at 37 °C for 24 h. The diameter of zone of inhibition was thereafter determined. In the evaluation of antifungal activity of the biosynthesized AgNPs, the method described by Khatami *et al.* (2015) was followed. In this method, each of the graded concentrations of AgNPs (50, 100, and 150 µg/ml) was used to treat about 45 °C cooled saboraud dextrose agar (SDA) (1:10, v/v). The AgNPs-enriched SDA plates were then bored using sterile cork borer (6 mm) to create wells, the wells were inoculated with mycelial agar plug (6 mm) of 48-h old cultures of fungal strains including *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*. In the control experiments, fungal plugs were inoculated on SDA plates without the AgNPs incorporation. All the plates were incubated under ambient conditions (30 ± 2 °C) for 72 h. The diameters of fungal growth in all plates were measured and used to determine the percentage of fungal growth inhibitions as follows:

$(D_{\text{control}} - D_{\text{test}}) / D_{\text{control}} \times 100 \%$ where D is the diameter of fungal growth on the SDA plates.

3. RESULTS AND DISCUSSION

Biosynthesis and characterization of AgNPs

The aqueous extract of wonderful kola was successfully used for the synthesis of dark brown AgNPs under ambient conditions. The color was stabilized after 15 min. of reaction and the control silver nitrate solution showed no color change (Fig. 2). Authors have reported different colors of AgNPs and this may be attributed to variations in the compositions of the biomolecules used for the synthesis. For instance, Lateef *et al.* (2016c) reported the synthesis of dark brown AgNPs solutions using pod extract of *Cola nitida* while AgNPs synthesized from Lantana camara leaf extract exhibited yellowish brown color (Thirumurugan *et al.*, 2011). The color formation in AgNPs had been ascribed to the excitation of surface plasmon resonance (SPR) in metallic nanoparticles (Selvi and Sivakumar, 2012).



Fig. 2: Biosynthesized AgNPs (dark brown) using aqueous extract of wonderful kola after 15 min of reaction and the control AgNO₃ solution (colorless).

The wonderful kola mediated AgNPs displayed maximum absorbance at the wavelength of 413 nm (Fig. 3), which falls within the range of 391- 460 nm absorbance characteristics of AgNPs earlier reported (Thirumurugan *et al.*, 2011; Zaki *et al.*, 2011; Kannan *et al.*, 2013; Priyadarshini *et al.*, 2013; Lateef *et al.*, 2015a, b; 2016d). The UV-vis absorbance characteristics displayed by AgNPs is also the function of their surface plasmon resonance (Creighton *et al.*, 1979).

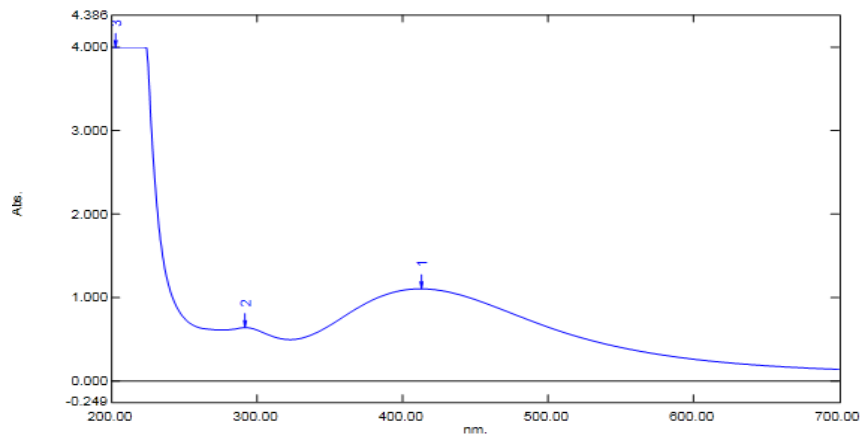


Fig. 3: UV-vis absorption spectrum of the synthesized AgNPs using aqueous extract of wonderful kola

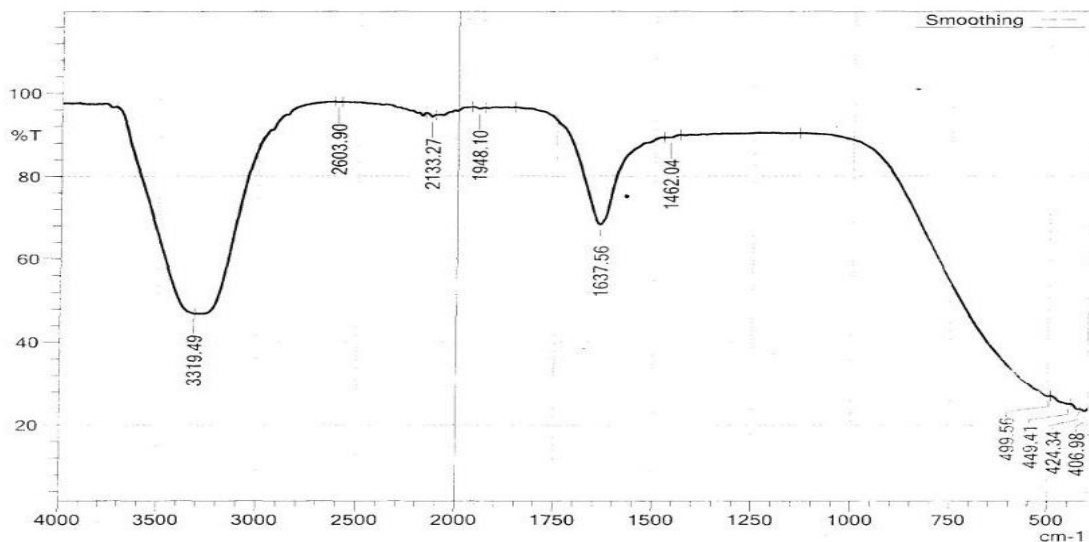


Fig. 4: FTIR spectrum of the synthesized AgNPs using aqueous extract of wonderful kola

The FTIR measurement was carried out to identify the possible biomolecules responsible for the capping and stabilization of the synthesized AgNPs. The FTIR spectrum showed peaks at 3319, 2603, 2133, 1948, 1637, 1462, 499, 449, 424 and 406 cm^{-1} (Fig. 4). The distinct peak at 3319 cm^{-1} corresponds to the bonding vibration of amine (N-H group) and 1637 cm^{-1} is indicative of C=C stretch of alkenes or C=O stretch of amides (Shankar *et al.*, 2014). Both indicate that protein molecules in the aqueous extract of wonderful kola were responsible for the capping and stabilization of the biosynthesized AgNPs.

The SEM image of the AgNPs synthesized from aqueous extract of wonderful kola is as shown in Fig. 5. The synthesized AgNPs are spherical in shape with the size ranging from 15-50 nm. Spherical shape of silver nanoparticles has been reported by Zaki *et al.* (2011) and Kannan *et al.*, (2013), while Lateef *et al.* (2016d) reported the synthesis of spherical AgNPs in the size range of 3-50 nm. It is well known that the physico-chemical properties of AgNPs contribute immensely to their versatility.

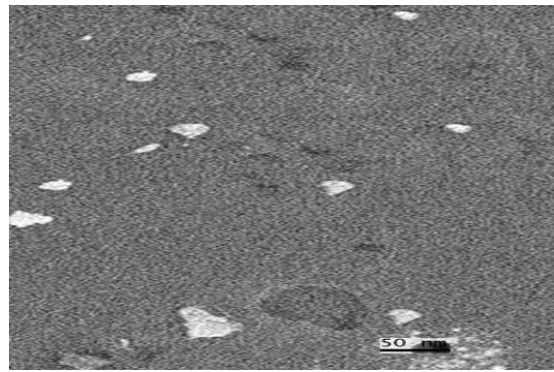


Fig. 5: SEM image of AgNPs synthesized using aqueous extract of wonderful kola

Antibacterial activities of the synthesized AgNPs at 50-150 $\mu\text{g/ml}$ concentrations are shown in Fig. 6. The particles demonstrated remarkable antibacterial effects against the clinical bacterial strains of *P. aeruginosa*, *S. aureus*, *S. Typhi* and *E. coli* by inducing 10-20 nm zone of inhibition against the test organisms (Table 1). These results corroborate the antibacterial activities of AgNPs reported in the previous studies (Priyadarshini *et al.*, 2013; Shankar *et al.*, 2014; Salem *et al.*, 2014; Augustine *et al.*, 2014; Lateef *et al.*, 2015a, b; 2016d). For instance, most recently Lateef *et al.* (2016a) reported that the cocoa pod husk extract-mediated AgNPs induced zone of inhibition of 10-14 mm against clinical isolates of bacteria while Salem *et al.* (2014) reported zones of inhibition of 7-19 mm for biosynthesized AgNPs using leaf and latex of *Ficus sycomorus* against some bacterial strains. Though, the exact mechanism of antibacterial activity of AgNPs is not yet known but researchers have suggested many possible effective mechanisms. For instance, the antibacterial activity of AgNPs was assumed to be related to their sizes (Morones *et al.*, 2005), the smaller the size, the larger the surface area to volume ratio. This property facilitates the interaction with microbial cells. Similarly, Pal *et al.* (2007) documented that the antibacterial activity of AgNPs is also shape dependent. AgNPs attack bacterial cells through the release of silver ions in the cells which induce antibacterial effects like denaturation of cell membrane, interference with DNA replication and respiratory chain finally leading to death (Feng *et al.*, 2000; Sondi and Salopek-Sondi, 2004; Morones *et al.*, 2005; Song *et al.*, 2006).

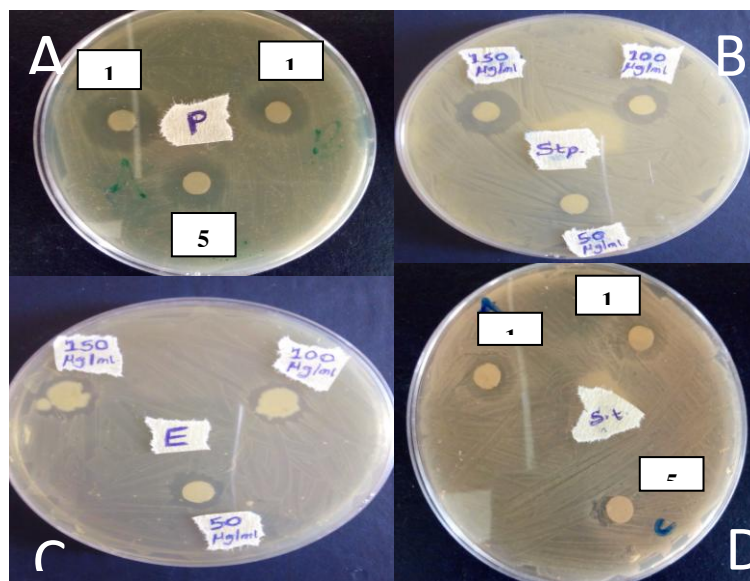


Fig. 6: Antibacterial activities of the synthesized AgNPs (150, 100, 50 $\mu\text{g/ml}$) against clinical bacterial isolates (A, *Pseudomonas aeruginosa*; B, *Staphylococcus aureus*; C, *E. coli*; D, *Salmonella Typhi*).

Table 1: Inhibition of growth of clinical bacterial isolates by the synthesized AgNPs

Organisms	Conc. of AgNPs ($\mu\text{g/ml}$)	Zone of inhibition (mm)
<i>P. aeruginosa</i>	150	20
	100	18
	50	15
<i>S. aureus</i>	150	16
	100	14
	50	10
<i>E. coli</i>	150	15
	100	14
	50	12
<i>S. Typhi</i>	150	15
	100	15
	50	10

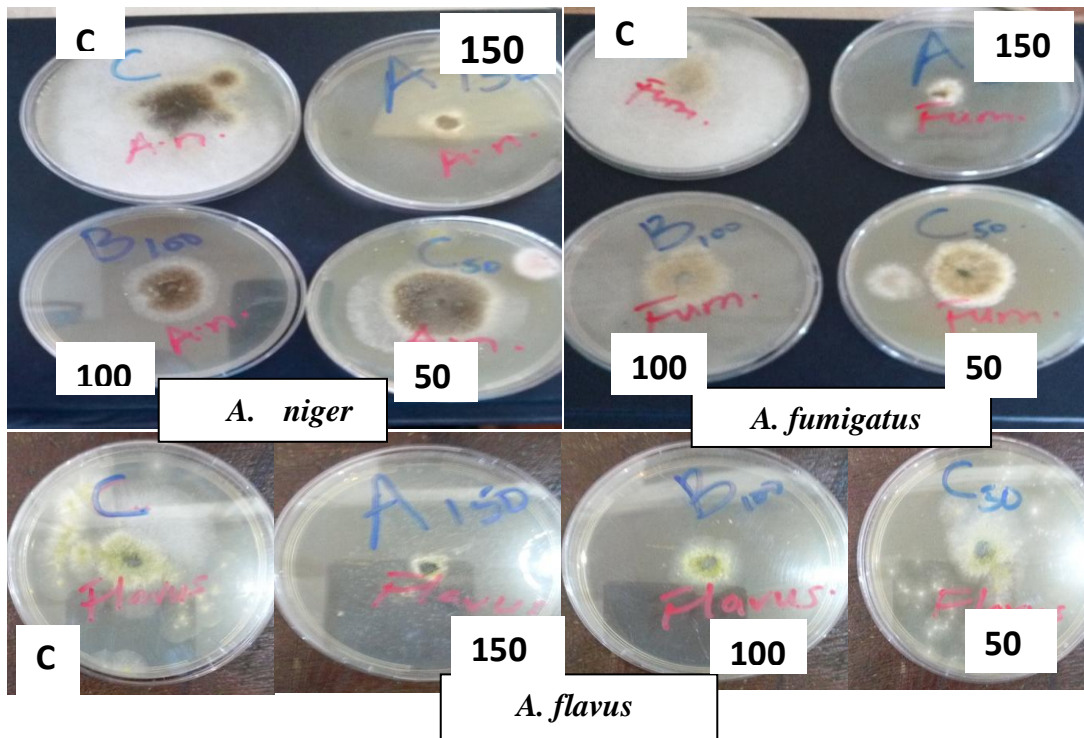


Fig. 7: Antifungal activities of the synthesized AgNPs (150, 100, 50 $\mu\text{g/ml}$) and the controls (C)

The synthesized AgNPs showed considerable antifungal activities against some fungal isolates including strains of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* at tested concentrations of 50, 100 and 150 $\mu\text{g/ml}$. (Fig. 7). The particles displayed excellent activities at concentration of 150 $\mu\text{g/ml}$ by producing over 80 % fungal growth inhibition against the tested fungal strains (Table 2).

Authors have reported similar antifungal activities of some biosynthesized AgNPs (Khatami *et al.*, 2015; Netala *et al.*, 2015; Ojo *et al.*, 2016; Lateef *et al.*, 2016b). The overwhelming results of antimicrobial activities of the AgNPs obtained in this study have shown that the particles have promising applications in the development of a novel antimicrobial agents.

Table 2: Fungal growth inhibition by the synthesized AgNPs

Organisms	Conc. of AgNPs (µg/ml)	Control (mm)	Percentage of inhibition (%)
<i>A. niger</i>	150	80	87.5
	100		75.0
	50		31.3
<i>A. fumigatus</i>	150	82	87.8
	100		73.2
	50		57.3
<i>A. flavus</i>	150	60	86.7
	100		75.0
	50		33.3

4. CONCLUSION

In this study, AgNPs were successfully synthesized using aqueous extract of wonderful kola in a cost effective, simple, and ecofriendly manner. FTIR analysis revealed that protein molecules in the extract responsible for the capping and stabilization of the synthesized AgNPs. The particles were spherical in shape with size ranging from 15-50 nm. The synthesized AgNPs demonstrated high potency against clinical bacterial isolates as well as effective antifungal activities on some selected fungal isolates. In view of these, the biosynthesized AgNPs can therefore be used in the development of novel antimicrobial agents for a wide biotechnological applications. To the best of our knowledge, this is the first report on the involvement of wonderful kola in the synthesis of nanoparticles.

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