ANTIBACTERIAL ACTIVITIES OF SILVER NANOPARTICLES SYNTHESIZED USING AQUEOUS EXTRACT OF GINGER (*Zingiber officinale*) RHIZOME

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Abstract
This study aimed to synthesize silver nanoparticles (AgNPs) from aqueous extract of ginger rhizome using $AgNO_3$ as precursor and to evaluate antibacterial activities of the synthesized AgNPs against some clinical bacterial isolates and bloom forming cyanobacterial strain. The synthesis was carried out by mixing 1mM silver nitrate solution with aqueous extract of ginger rhizomes (10:1). The synthesized AgNPs were characterized by UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The AgNPs were dark brown in color and have maximum absorbance at 429 nm. FTIR spectrum showed strong and distinct peak at 3356, 2855, 2129, and 1636 cm^{-1}, indicating the role of biomolecules in the capping and stabilization of the particles. They have spherical shape with size ranging from 30-90 nm. The particles demonstrated potent antibacterial activities against clinical isolates of Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli and bloom forming cyanobacterial strain. This study therefore, suggests the application of the synthesized AgNPs in the manufacture of antibacterial drug and as anticyanobacterial agent in freshwater treatment.

Keywords: Biosynthesis, Silver nanoparticles, Ginger rhizome, Antibacterial drug.

Introduction

The synthesis of nanoparticles using biological method is gaining considerable momentum in recent times because of the simple procedure required, cheap cost of production, low energy consumption, eco-friendliness, and biocompatibility that account for its diverse applications (Kumar *et al.,* 2019). The great biodiversity of nature also contributes immensely to the growing of this method as it allows the use of vast biomolecules for the fabrication of varieties of nanoparticles for wide applications (Adelere *et al.,* 2019). Many authors have reported the biosynthesis of nanoparticles using microorganisms and plant extracts (Khatami *et al.,* 2015; Lateef *et al.,* 2015; Kuppusamy *et al.,* 2016; Adelere & Lateef, 2016; Adelere *et al.,* 2017; Elegbede *et al.,* 2018; Adelere *et al.,* 2020). Nanoparticles have numerous applications in areas such as agriculture, environmental management, biomedical, catalysis, electronics, and in the manufacture of personal care products among others (Keat *et al.,* 2015). However, silver nanoparticles compare to other types of nanoparticles had been considered most important as they had found applications in many areas owing to their strong antimicrobial activity (Adelere *et al.,* 2019).

Plant mediated-synthesis of nanoparticles is sometimes preferred to other biological methods simply because of wide diversity of plant metabolites and simplicity in handling plant materials (Singaravelu *et al.,* 2007; Madhumitha & Roopan, 2013). Phytochemicals such as steroids, flavonoids, saponins, tannins, alkaloids and others have been reported to be responsible for the reduction, capping and stabilization of biosynthesized nanoparticles (Kuppusamy *et al.,* 2016). Several authors have reported remarkable antibacterial and antifungal activities of silver nanoparticles synthesized from varieties of plants (Madhumitha & Roopan, 2013; Devadiga *et al.,* 2015; Lateef *et al.,* 2016a,b; Adelere *et al.,* 2017; Elegbede *et al.,* 2018; Adelere *et al.,* 2020).

Ginger (*Zingiber officinale*) is a flowering perenial plant belonging to family *Zingiberiaceae*, whose rhizome (Fig. 1) is widely used as a food condiment and as a medicine to treat many diseases (Tapsell *et al.,* 2006). It consists of a complex mixture of pharmacological compounds such asgingerols, beta-carotene, capsaicin, caffeic acid, curcumin, shogaols, paradols, salicylate and zingerone (Iwaskai *et al.,* 2006; Altman & Marcussen, 2001). Ginger is widely used to treat various ailments like nausea, vomiting, pregnancy-associated morning sickness, motion sickness and indigestion (Ernst & Pittler, 2000; Ladas *et al.,* 2006; White, 2007).

Fig. 1: Ginger (*Zingiber officinale*) rhizome

In this study, the aqueous extract of ginger rhizome was used for the synthesis of silver nanoparticles and the particles were evaluated for antibacterial activity against some clinical bacterial isolates. Also, anticyanobacterial activity of the biosynthesized silver nanoparticles was also investigated against fresh water cyanobacterial strain for their application in freshwater treatment.

Materials and Methods

Collection of Sample

Zingiber officinale rhizome (Fig. 1) was procured from Bosso central market of Minna, Niger State, Nigeria. The rhizome was washed several times with distilled water, cut into pieces, air dried for 14 days under ambient conditions (30 ± 2℃) and then milled into powder form using electric blender.

Preparation of Ginger Extract

Approximately 1 g of the milled ginger rhizome was dispersed into 10 ml of deionized water and heated in water bath at 60 ℃ for 1 h. The extract was centrifuged at 4000 rpm for 20 min and then filtered using Whatman No. 1 filter paper. The filtrate was collected and stored in refrigerator at 4℃ for further studies.

Biosynthesis and characterization of silver nanoparticles

The synthesis of AgNPs was carried out by mixing 1 ml of aqueous extract of ginger rhizome with 10 ml of 1 mM silver nitrate ($AgNO_3$) and allowed to stand at room temperature (30 ± 2℃) for 1 h. The control experiment consisted of only aqueous solution of 1 mM silver nitrate. The progress of reaction was visually monitored to observe color formation and the preliminary characterization was done by determining the absorbance characteristic through Ultraviolet-visible spectrophotometry analysis (Lateef *et al.,* 2015).

The fourier transform infrared (FTIR) spectroscopy analysis was carried out by freeze drying the colloidal AgNPs and mixed with KBr pellet. The resulting mixture was then analyzed

using CRY 630 Spectrophotometer according to Bhat *et al*. (2011). Also, the scanning electron microscopy (SEM) analysis was carried out by mounting the freeze dried AgNPs sample on specimen stub coated with copper and imaged by PhenomProX scanning electron microscope.

Antibacterial Activity of Biosynthesized AgNPs

Antibacterial activity of the biosynthesized AgNPs was evaluated using agar well diffusion method. Clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* obtained from General Hospital, Minna were used as test organisms. Each of the isolates was grown in peptone water for 18 h and then seeded on the plates of Mueller-Hinton agar. Thereafter, cork borer of 6 mm diameter was used to create wells on the seeded plates. Each well was loaded with 50 µl of graded concentration of AgNPs (50µg ml^{-1}, 100µg ml^{-1} and 150µg ml^{-1}) prepared by dilution with sterile distilled water. The plates were then incubated at 37℃ for 24 h and thereafter examined for zone of inhibition which was measured in mm.

Evaluation of Anticyanobacterial Activity of the AgNPs

Anticyanobacterial activity of the biosynthesized silver nanoparticles was determined using the modified method of Chaturvedi and Verma (2015). A bloom foming cyanobacterial strain was collected in a clean plastic bottle from a water logged area in Federal University of Technology, Minna, Bosso Campus and was taken directly to laboratory for the study. Sample of 10 ml was measured into McCartney bottles and 1ml of graded concentrations of AgNPs (50 µg ml^{-1}, 100 µg ml^{-1} and 150 µg ml^{-1}) was added separately into each bottle. A bottle containing only 10 ml of freshwater containing cyanobacterial strain (OD_{600} 0.01) was used for the control experiment. The bottles were allowed to stand close to visible light in a well aerated place and growth in each bottle was measured using UV-vis spectrophotometer on daily basis for 5 days.

Results and Discussion

Biosynthesis and Characterization of AgNPs

The aqueous extract of ginger rhizome completely reduced silver ion to form dark brown AgNPs under ambient temperature (30 ± 2℃) within 10 minutes while the control silver nitrate solution showed no color change (Fig. 2). This is similar with the results of previous findings where literatures reported the synthesis of dark brown AgNPs using extracts obtained from different plant materials (Ahmed & Ikram, 2015; Lateef *et al.,* 2016b; Adelere *et al.,* 2017; Elegbede *et al.,* 2018; Adelere *et al.,* 2020). In some studies, slight changes in the color of plant mediated AgNPs synthesis were reported due to variations in phyto-molecules used. For instance, Thirumurugan *et al.* (2011) and Lalitha *et al.* (2013) reported the synthesis of yellowish brown AgNPs and brownish gray AgNPs from leaf extracts of *Lantana camara* and *Azhadirachta indica*, respectively. The development of color during the synthesis had been related to the excitation of surface plasmon resonance (SPR) in silver nanoparticles (Selvi & Sivakumar, 2012).

Fig. 2: Biosynthesized AgNPs using aqueous extract of ginger rhizome: A, synthesized AgNPs within 10 min; B, control silver nitrate solution

The UV-vis absorption spectrum of the biosynthesized AgNPs (Fig. 3) showed distinct and broad peak at 429 nm which falls within the characteristic absorption peak range of 391-460 nm earlier reported for AgNPs (Thirumurugan *et al.,* 2011; Zaki *et al.,* 2011; Kannan*et al.,* 2013; Priyadarshini *et al.,* 2013; Lateef *et al*., 2015; 2016a,b; Adelere *et al.,* 2017; Elegbede *et al.,* 2018; Adelere *et al.,* 2020). The FTIR spectrum of the AgNPs (Fig. 4) shows peaks at 3356, 2855, 2129 and 1636 cm^{-1}. The distinct peaks at 3356 cm^{-1} and 1636 cm^{-1} indicate the role of biochemical molecules in the capping and stabilization of the synthesized AgNPs. These bands correspond to N-H bond of amines, and C=C stretch of alkenes or C=O stretch of amides, respectively (Shankar *et al.,* 2014). In most studies, phenolic compounds are recognized as the active reducing compounds among other biomolecules in the synthesis (Kummara *et al.,* 2016).

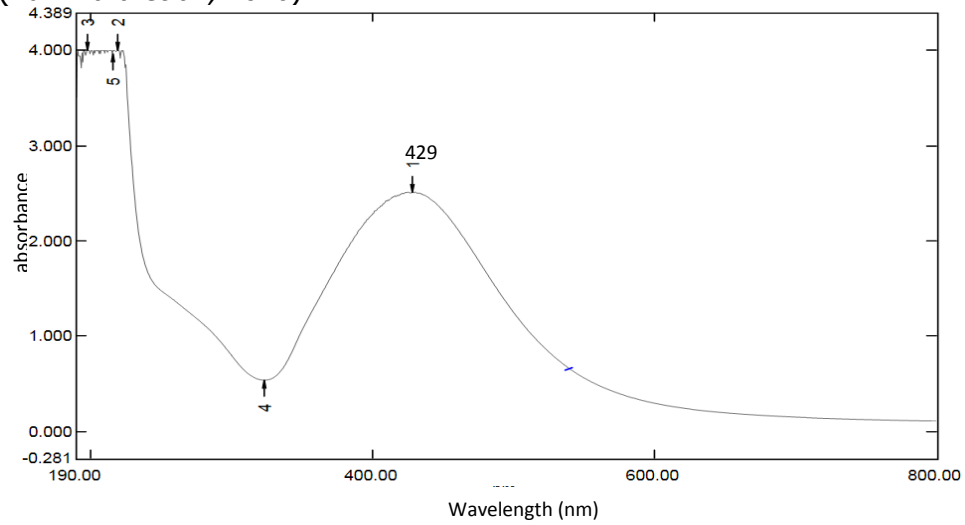

Fig. 3: UV-vis absorption spectrum of AgNPs synthesized from aqueous extract of ginger rhizome

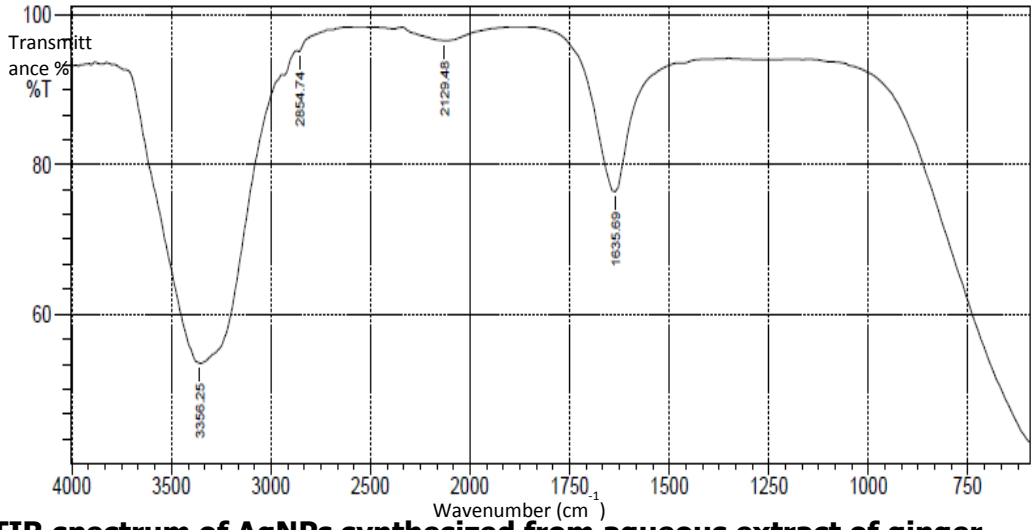

Fig. 4: FTIR spectrum of AgNPs synthesized from aqueous extract of ginger rhizome

The SEM image of the AgNPs synthesized from aqueous extract of ginger rhizome is as shown in Fig 5. The AgNPs were spherical in shape with the size ranging from 30-90 nm and this corroborates the results of previous studies (Zaki *et al.,* 2011; Kannan *et al.,* 2013; Lateef *et al.,* 2015; Adelere *et al.,* 2017). The uniqueness of physico-chemical attributes of nanoparticles such as size, morphology and chemical compositions contribute greatly to their versatile applications.

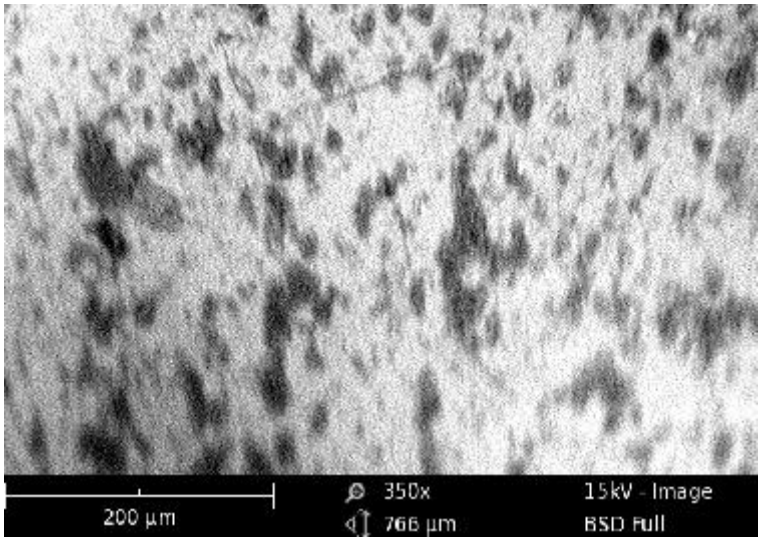

Fig. 5: SEM image of AgNPs synthesized from aqueous extract of ginger

Antibacterial Activities of Biosynthesized AgNPs

The biosynthesized AgNPs exhibited considerable antibacterial activities against the clinical isolates of *E. coli*, *P. aeruginosa* and *K. pneumoniae* (Fig. 6). The AgNPs at concentration of 50-150 µg/ml induced zone of inhibition ranged from 9 to 24 mm (Table 1). It was observed that the zone of inhibition increased with increasing concentration of the AgNPs. The highest antibacterial activity was obtained at concentration 150 µg/ml as the particles produced maximum zone of inhibition (24 mm) against the strains of *E. coli* and *K. pneumoniae.* The antibacterial activities obtained in this study conform to some of previous related studies (Salem *et al.,* 2014; Lateef *et al.,* 2015; Adelere *et al.,* 2017; Elegbede *et al.,* 2018; Aina *et al.,* 2019; Adelere *et al.,* 2020). Though details of mechanism of antibacterial activity of

AgNPs is not well known, however, studies have revealed that silver ion play a very active role in the process while size and morphology are also known to have great contribution. When AgNPs dissociates, it releases silver ion which attack many structures of bacterial cell. Silver ion attack and disrupt cell wall and cytoplasmic membrane through electrostatic attraction (Raffi *et al.,* 2008). Since AgNPs are capable to attack many structures of bacterial cells, therefore the biosynthesized AgNPs obtained in this study have promising application in the manufacture of new antibacterial drug to curtail the menace of antibiotic resistance.

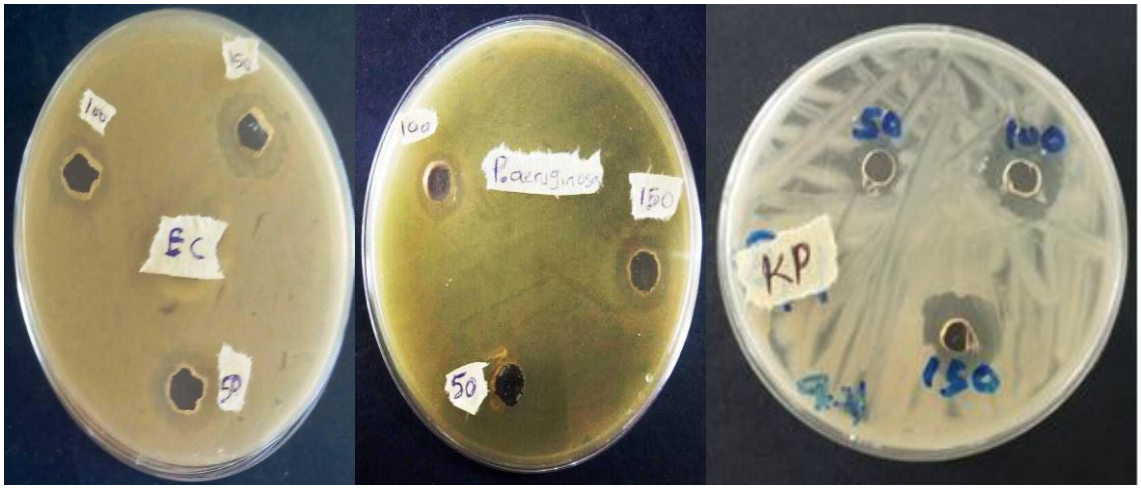

Fig. 6: Antibacterial activities of biosynthesized AgNP against clinical isolates of *Escherichia coli* (EC), *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (KP)

Table 1: Antibacterial activity of biosynthesized AgNPs and zones of inhibition (mm) against clinical isolates

Organisms	Conc. of AgNPs (µg/ml)	Zone of inhibition (mm)
P. aeruginosa	150	22 ± 0.01
	100	18 ± 0.03
	50	11 ± 0.08
E. coli	150	24 ± 0.06
	100	17 ± 0.02
	50	18 ± 0.01
K. pneumoniae	150	24 ± 0.03
	100	19 ± 0.04
	50	9 ± 0.02

Anticyanobacterial Activities of Biosynthesized AgNPs

The biosynthesized AgNPs demonstrated potent anticyanobacterial efficiency against a blooming forming cyanobacterial strain. The AgNPs (150 µg/ml) significantly inhibited the growth of the cyanobacterial strain in the test experiment as against the profuse growth observed in the control bottle (Fig. 7 and Table 2). The anticyanobacterial activity recorded in this study corroborates the study of Chaturvedi and Verma (2015), where the AgNPs synthesized from the leaf extract of *Butea monosperma* (Flame of forest) remarkably inhibited the growth of bloom forming cyanobacterial species. The results obtained for the optical densities (OD) in this work is similar to that of Adelere *et al.* (2019) where algicidal activity of AgNPs were determined against bloom forming cyanobacterial strains. Hence, it could be suggested that the biosynthesized AgNPs obtained in this study could be a better

and ecofriendly alternative to the conventional toxic copper sulphate in controlling cyanobacterial growth in freshwater treatment.

Fig. 7: Anticyanobacterial activity of AgNPs against a bloom forming cyanobacterial strain. C, profuse cyanobacterial growth in control experiment without AgNPs and T, inhibition of cyanobacterial growth by the synthesized AgNPs

Table 2: Anticyanobacterial activity of AgNPs

Days	Control (OD_{600})	Test (OD_{600})
0	0.01	0.01
1	0.06	0.03
2	0.21	0.04
3	0.48	0.04
4	0.56	0.05
5	0.57	0.06

Conclusion

In this study, the aqueous extract of ginger rhizome (*Zingiber officinale*) was used to reduce silver ion to form AgNPs under ambient temperature. It was revealed that the biomolecules present in the extract played prominent role in the reduction and stabilization of the biosynthesized AgNPs. The particles were dark brown in color, spherical in shape with size ranging from 30-90 nm. The AgNPs displayed potent antibacterial activities against clinical isolates of *E. coli*, *P. aeruginosa, K. pneumoniae* and a very remarkable anticyanobacterial efficacy against a bloom forming cyanobacterial strain. The results obtained in this study suggest the potential biomedical application of the biosynthesized AgNPs. Also, the particles could be a better alternative to the non environmental-friendly methods used in controlling cyanobacterial growth in freshwater.

Acknowledgement

AIA thanked authority of FUT, Minna for providing facilities to undertake this study.

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