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ANTIMICROBIAL ACTIVITIES OF *ALLIUM CEPA*-MEDIATED SILVER NANOPARTICLES AGAINST SOME CLINICAL BACTERIAL ISOLATES

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Abstract

The synthesis of nanoparticles is a research area that is growing as a result of the potential applications for the development of new ideas and novel technologies. This study revealed the convenient, fast and economic method to synthesize silver nanoparticles (AgNPs) using onion (*Allium cepa*) bulb extract. Onion bulbs were obtained from the open market in Osogbo, Osun State. The characterization of the synthesized AgNPs was done using UV-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The antimicrobial effect of synthesized silver nanoparticles using aqueous, methanolic and fresh extract of *Allium cepa* were assessed using clinical isolates of *Staphylococcus aureus* and *Escherichia coli* by agar well diffusion method. It was observed that the crude extract + silver nitrate solution changed from light yellow to dark brown in colour after incubation in the dark for 3 h. The mixture of onion extract and silver nitrate solution produced particles with maximum absorbance at the wavelength of 535 nm using UV-vis spectroscopy. The FTIR spectra revealed that many of the functional groups obtained using crude plant extract were repeated in FTIR spectrum of AgNPs with some changes in how they are positioned and also in the absorption bonds intensity. The particles were spherical in shape. From the experiment, the AgNPs was observed to have antimicrobial potentials as it inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* with zones of inhibition ranging from 18-26 mm at concentration of 125µg/ml.

Keywords: *Allium cepa*, Silver nanoparticles, *Staphylococcus aureus*, *Escherichia coli*

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Introduction

Onion (*Allium cepa*) which can also be called 'garden onion or 'bulb' onion belongs to the family *Alliaceae*. It is known in history as one of the oldest cultivated vegetables, and the bulbs have been used as a food source for a long period of time. The bulbs are large, fleshy and firm with leaves bluish-green and hollow (Azu *et al.*, 2006). The three common varieties of onions are available in red, white and purple skinned (Wang *et al.*, 2009).

Onions which are easily propagated, transported and stored are known to be effective against several infections and diseases including common cold, heart disease, diabetes, coughs and sore throat (Augusti, 2008). They can also be bacteriostatic in nature (Saulis *et al.*, 2002). Certain chemical compounds that have anti-inflammatory, anticancer and antioxidant properties such as quercetin are believed to be present in onions (Wilson and Demmings-Adams, 2007). The outer layer of the flesh has higher concentration of flavonoids compared with

other part of the onions (Nemeth and Piskula, 2007). Onions are also known to contain high polyphenols than other *Allium* vegetables (Wang *et al.*, 2009).

Nanoparticles are particles that range from 1-100 nm in size. Silver nanoparticles have attracted attention and become researchers' focus due to a wide variety of potentials in applicable areas like catalysis, optics, antimicrobials, biomedical and electronic fields (Abdullah and Hamud, 2013; Awwad *et al.*, 2013). AgNPs can be synthesized using methods like thermal decomposition, chemical and photochemical reactions in reverse micelles, electrochemical, sonochemical and also by biological methods (Nethradevi *et al.*, 2012).

Biological methods that require bio-derived chemicals, bacteria, fungus and plant extracts are considered safe and economically sound for the fabrication of nanomaterial among other methods (Mason *et al.*, 2012; Abdullah and Hamud, 2013). Green synthesis of AgNPs has become a better alternative to conventional physical and chemical methods which require the use of chemical compounds or organic solvents as reducing agents, not environmental friendly and also expensive (Mason *et al.*, 2012). A range of various plant parts including the leaf, flower, seed, tuber and bark extracts have been investigated for their ability to synthesize silver nitrate efficiently.

Bioreduction of Ag^+ ions to yield silver nanoparticles are caused by combinations of biomolecules such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides found in the extracts (Abdullah and Hamud, 2013). Silver has effective inhibition on microbes in medical and industrial processes. One of the most important applications of silver and silver nanoparticles in medical industry is topical

ointments which can be used to prevent infection against open wounds and burn (Ip *et al.*, 2006).

Several reports have studied antimicrobial activity of AgNPs synthesized using chemical and biological processes. This study is aimed at investigating the phytochemical composition of *Allium cepa* and to biosynthesize AgNPs using aqueous and methanolic extract of *Allium cepa*. The AgNPs was characterized and the antimicrobial activities of extracts and biosynthesized AgNPs were compared against clinical pathogens.

Materials and Methods

Collection of Plant Samples

The sample (*Allium cepa*) was obtained from Oja-oba market in Osogbo, Osun state.

Preparation of Aqueous Extract of *Allium cepa*

Allium cepa extraction was carried out thus: The onion was washed with sterile distilled water and kept at room temperature to air dry for some minutes before the outer covering of the onion was peeled manually. Exactly 200 g of onion was blended and soaked in 200 ml of distilled water for 24 h. The pulp obtained after blending was shaken vigorously in a sterile glass container for enhancement of proper extraction; it was then filtered using sterile cotton cloth after which the extract was obtained. The extract obtained was stored in a bottle below ambient temperature (Trease and Evans, 2009).

Methanolic Extraction of *Allium cepa*

Onion bulb was weighed; 200 g of it was blended and soaked in 200 ml of methanol for 24 h. The pulp obtained after blending was shaken vigorously in a sterile glass

container for enhancement of proper extraction. It was filtered using a filter paper and funnel. The extract obtained was stored in a bottle below ambient temperature (Trease and Evans, 2009).

Fresh Extraction of *Allium cepa*

Onion bulb was weighed and 200 g of it was blended, the pulp obtained was filtered using a sterile filter paper and the extract obtained was stored in a bottle below ambient temperature (Trease and Evans, 2009).

Phytochemical Screening

The extract of onion (*Allium cepa*) were subjected to phytochemical screening to determine the presence of saponin, carbohydrate, cardiac glycoside, tannins, flavonoids, alkaloid and steroid (Obadoni and Ochuko, 2001; Trease and Evans, 2009).

Synthesis and Characterization of Silver Nanoparticles

For biosynthesis of nanoparticles, 5 ml of aqueous, methanolic and fresh extracts of *A. cepa* were added to 10 ml of 1 mM silver nitrate and was incubated in the dark for about 3 h (Saxena *et al.*, 2010). It was observed for color change. The synthesized AgNPs were characterized using UV-vis spectroscopy for the verification of reduction of AgNO₃ to Ag⁺ by the onion extract. The Fourier-transform infrared spectroscopy (FTIR) analysis of the extracellular biosynthesized AgNPs was done. Scanning electron microscope (SEM) was used to observe the shape of synthesized nanoparticles.

Antimicrobial Activity

The antimicrobial property of the *Allium cepa* extract and the synthesized AgNPs was determined using agar well diffusion

method, against some clinical isolates of *Escherichia coli* and *Staphylococcus aureus*. The test pathogens were collected from University of Ilorin Teaching Hospital (UITH), Ilorin. The isolates were sub-cultured on nutrient agar plates and incubated for 24 h. Pure colonies were picked and stored in slants for further use.

Results

Phytochemical Profile of Onion Extract

Table 1 shows the phytochemical profile of aqueous and methanolic extracts of onion. Saponin, carbohydrate, cardiac glycoside, tannins, flavonoids and alkaloids were present in both aqueous and methanolic extracts.

Synthesis of AgNPs

There was a change in colour during the incubation of the extract and AgNO₃, from pale yellow to dark brown due to reduction of silver ions. The brown colour stabilized after the incubation. Plate 1 shows colour change before and after synthesis of AgNPs.

Characterization of AgNPs

UV-visible spectrophotometer was used to measure the maximum absorbance produced during reduction of AgNO₃ to Ag⁺ by the onion extract. As shown in Figure 1 UV-vis absorption spectrum of the produced AgNPs showed the peak maxima at 535.00 nm due to excitation of surface plasmon vibrations in nanoparticles. This is characteristic of silver nanoparticles and the peak indicated that the synthesized nanoparticles were dispersed in the aqueous solution with no evidence of aggregation.

Table 1: Phytochemical profile of *Allium cepa* extracts

S/N	Constituents	Tests	Aqueous	Methanolic
1	Carbohydrates	Reducing sugar	+++	++
		Molisch	+	+
2	Cardiac glycosides	Keller-Kiliani	++	+++
3	Saponin	Frothing	+	+
		Foaming	+	+
4	Triterpene/steroids	Lieberman Bucchard	+	++
5	Tannins	Ferric Chloride	+	++
		Lead sub-acetate	+	+
6	Flavonoids	Sodium Hydroxide	++	++
7	Alkaloids	Dragendorff	+	-
		Wagners	+	

+++ , Present in high concentration; ++, moderately present; +, present; -, absent

Figure 1: UV-vis spectrum of fresh silver nanoparticles

FTIR Spectroscopic Analysis

FTIR analysis was carried out to identify the possible biomolecules in onion extract responsible for capping leading to efficient stabilization of the AgNPs and to determine the chemical functional groups in the sample. The observation confirms the infrared fingerprints spectroscopy

spectrum of the AgNPs as the absorption band located at 1661.7 cm^{-1} , which may be as result of C=O or C=C stretching vibration. For the higher wave numbers, the peak at 1356.5 cm^{-1} is due to C-N or C-O stretching of proteins and O-H stretching of carbohydrates and water, the bands at both 800.6 and 876.7 cm^{-1} were assigned to the left-handed helix DNA.

After synthesis, the absorption peaks at 2607, 2371 and 1865 cm^{-1} corresponding to CH_3 and CO observed in plant extract get narrower and shifted to higher

frequency regions, while those at 3539, 3255 and 3209 cm^{-1} corresponding to NH

Figure 2: FTIR spectra of aqueous extract

Figure 3: FTIR spectra of AgNPs

and OH decreased in intensity and shifted to low frequency regions (Figures 2 and 3).

Antimicrobial Analysis

The growth of *Staphylococcus aureus* and *Escherichia coli* was inhibited by the extract at 100 µg/ml. All the AgNPs have antimicrobial properties as they inhibited the growth of the organisms at 125 µg/ml. The synthesized nanoparticles using fresh extract showed the highest zone of inhibition with 26 mm against *E. coli* and 24 mm against *S. aureus* as shown in Table 2. Methanolic extract-mediated AgNPs had zones of inhibition of 20 mm on *E. coli* and 18 mm on *S. aureus* and aqueous extract-mediated AgNPs yielded zones of inhibition of 20 mm on *E. coli* and 18 mm on *S. aureus* (Table 2). Some selected groups of Gram positive and negative antibiotics were used as positive control. Ofloxacin had no inhibition against the test organisms, while gentamycin, ciprofloxacin and amoxicillin inhibited the growth of the test organisms with higher zones of inhibition (Table 2).

Discussion

The phytochemical screening of *Allium cepa* in this study has shown the presence of flavonoids, tannins and other chemical constituents which explains why they are known to have antibacterial, antitoxigenic, and anticarcinogenic properties. Flavonoids were reported to be present in both aqueous and methanolic extract in the study as reported by Geetha *et al.* (2011). Flavonoids are known to be good antimicrobial agent over a wide range of microorganisms which may be due to the ability of flavonoids to form complexes with extracellular soluble proteins and bacteria cell walls. The reduction of silver ions to silver using green synthesis was monitored by measuring the UV-vis spectrum of the reaction mixture. After the sample was observed under the UV-vis

spectrophotometer for its maximum absorbance and wavelength, the maximum peak was found to be at 535 nm for onion extract-mediated AgNPs.

The FTIR spectra showed that many peaks obtained using crude plant extract were repeated in FTIR spectrum of AgNPs with changes in the intensity of absorption bonds and position. The band at 813 cm⁻¹ reflects structural information about polyphenols caused by ring CH deformation (Nethradevi *et al.*, 2012). These are as a result of compound such as flavonoids, alkaloid, terpenoids and tannin present in onion plant. The biological components are known to interact with metal salts via the functional group and mediate their reduction to nanoparticles (Nethradevi *et al.*, 2012).

The structural changes indicated that the reduction and the stabilization of silver nanoparticles increase through these groups. This is related to changes reported by Abboud *et al.* (2013). The bands obtained between 3579 and 3209 cm⁻¹ and 1865 and 1448 cm⁻¹ in the AgNPs spectra correspond to the amide groups. Amide groups are the most important band for protein infrared spectrum which confirms that the phytoconstituents of plant (mostly protein) interact with silver nanoparticles (Abboud *et al.*, 2013; Lu *et al.*, 2011; Adebayo-Tayo *et al.*, 2016). This suggested that AgNPs synthesized from onion extract were surrounded by some protein bands through either free amine groups or cysteine residues which makes stabilization of AgNPs by coating of protein possible (Figure 3)

Scanning electron microscopy (SEM) is used to observe the size distribution and morphologies of the synthesized AgNPs. The AgNPs synthesized using onion extract were scanned by SEM. It reveals that silver nanoparticles seemed to be

Figure 4: SEM micrographs of AgNPs using onion extract

crystalline in form. The present study has shown that onion extract has the potential to reduce silver nitrate ions into AgNPs.

The result of this study revealed that the fresh, aqueous and methanolic extracts of *Allium cepa* had antimicrobial activity when the extracts were tested against *S. aureus* and *E. coli*. The clinical pathogens used in this study were more susceptible to the extract than silver nitrate, and higher zone of inhibition was observed for biosynthesized AgNPs, which agrees with the study of Azu *et al.* (2006) that reported antimicrobial activity of *Allium cepa* extracts on *S. aureus*, *P. aeruginosa*, *Streptococcus pneumoniae*, and *E. coli*. According to research carried out by

Gomaa *et al.* (2017), AgNPs formed by extract of *Allium cepa* had highest inhibitory effect which ranged from 27 to 29 mm on *P. aeruginosa* and *Bacillus subtilis*. In this study, the biosynthesized nanoparticles had activities on the clinical isolates which ranged from 18 to 26 mm in diameter, while the extracts had inhibitions of 7-14 mm. Some of the test pathogens which were susceptible to ciprofloxacin which is a broad spectrum antibiotic were also susceptible to the biosynthesized AgNPs. This shows that medicinal plant extracts and their AgNPs can also exhibit broad spectrum activities as reported by Adebayo-Tayo *et al.* (2016).

Plate 1: Before and after synthesis of AgNO_3 using fresh (F), aqueous (A) and methanolic (M) extracts of onion

Plate 2: Antimicrobial activities of AgNPs (A, aqueous; F, fresh; M, methanolic extract) on *S. aureus* (Left) and *E. coli* (Right)

The results obtained shown that *Allium cepa* is a potent medicinal plant as the juice extract contains biologically active substances that contribute to the antimicrobial characteristics of the plant.

Conclusion

This research has clearly demonstrated the antimicrobial potentials of *Allium cepa* and validates the antimicrobial properties of its fresh, aqueous and methanolic extracts. The study also showed that onion extract has the potential to reduce silver nitrate

ions into AgNPs. Further, AgNPs synthesized from *Allium cepa* possessed good antimicrobial activity and thus may serve as an alternative against the conventional antibiotics and drugs.

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Conflict of Interest

Authors declare no conflict of interest

Table 2: Antimicrobial activities of the extracts and biosynthesized AgNPs from *Allium cepa* against the test pathogens

Pathogens	<i>Allium cepa</i>				Control				
	Fresh extract (mm)	Fresh extract + AgNPs (mm)	Aqueous extract + AgNPs (mm)	Methanolic extract + AgNPs (mm)	AgNO ₃	Gen	Cip	Amo	Ofl
<i>S. aureus</i>	14	24	20	18	10	33	32	28	-
<i>E. coli</i>	10	26	18	20	6	32	30	20	-

Each value is an average of three readings

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