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NEEM ROOT MEDIATED-BIOSYNTHESIS OF SILVER NANOPARTICLES: ANTIMICROBIAL ACTIVITIES AND APPLICATION AS DETERGENT ADDITIVE

By

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ABSTRACT

This study reports on the biosynthesis of Silver nanoparticles (AgNPs) using aqueous extract of neem root. The synthesis was carried out by mixing 1mM silver nitrate with the aqueous extract of neem root (10:1) under ambient conditions. Characterization was done by UV- vis spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, and Scanning Electron Microscopy (SEM). The antibacterial and algicidal activities of bio-synthesized AgNPs were investigated against some clinical bacterial isolates and a bloom forming cyanobacterial strain, respectively. Their potential application as an antimicrobial detergent additive was evaluated. The bio-synthesized AgNPs displayed maximum absorbance at wavelength 425 nm. The particles are predominantly spherical in shape with size ranging from 20 to 60 nm. Data obtained from FTIR indicate that the protein molecules in extract played a very active role in the reduction of silver ions to form AgNPs. The particles demonstrated considerable antibacterial activities against clinical isolates of Escherichia coli, Streptococcus sp, and Klebsiella pneumoniae as they induced inhibition zone of 12-23 mm. The algicidal activity displayed by the particles against the bloom forming cyanobacterial strain was appreciable. Similarly, the incorporation of AgNPs as additive in the locally made detergent led to the total inhibition of growth of Escherichia coli, Klebsiella pneumoniae, Candida sp, and Aspergillus flavus. Therefore, results obtained in this study suggest the promising applications of the particles as an antimicrobial agent in the water treatment and drug development. The particles exhibited potential application as an antimicrobial additive in detergent production.

Keywords: Silver Nanoparticles, Bio-synthesis, Antimicrobial Activities, Algicidal Activities, Detergent Additive.

INTRODUCTION

Nano biotechnology is one of the most fascinating technologies in recent times, which was developed due to the increasing need for developing efficient, reliable, and environmentally benign process in nano particles production and application. It is a process that involves the use of various biological resources for the production of nano particles. It offers advantages like energy efficiency, eco-friendliness, lower production cost, compatibility for very diverse areas of applications such as agriculture, environmental management, biomedical, catalysis, electronics, and in the manufacture of personal care products among others (Keat et al., 2015), as it does not require the use of high temperature, organic solvents, and toxic chemicals. Further, it allows the production of large quantities of nano particles with a defined size and morphology, which are free of contamination. Various biological resources that have been exploited for the synthesis of nanoparticles include bacteria, actinomycetes, fungi, plant extracts, and photosynthetic algae (Ahmad et al., 2003; Basavaraja et al., 2008; Asmathunisha & Kathiresan, 2013; Lateef et al., 2015; Adelere et al., 2017).

Plants are highly diverse in nature and their biodiversity has been harnessed for biotechnological applications. They are very rich in natural products like alkaloids, flavonoids, saponins, steroids, tannins and some other nutritionally important compounds. These metabolites are found in various plant parts such as stems, leaves, roots, flowers, barks, and seeds. They are used as a reducing and capping agents for the bio-reduction of metal ions to produce their corresponding metallic nanoparticles such as silver, copper, gold, palladium, platinum, zinc, and iron (Kuppusamy et al., 2016; Adelere & Lateef, 2016). The potent antimicrobial activities of AgNPs make it more important and have received unprecedented attention in recent times. Many authors have reported the synthesis of AgNP's with remarkable antimicrobial activity using a varieties of phyto-metabolites obtained from diverse plant sources (Madhumitha & Roopan, 2013; Devadiga et al., 2015; Lateef et al., 2016; Adelere et al., 2017).

Neem tree (Azadirachta indica) is a tropical evergreen tree belonging to Meliceae family, which grows rapidly in tropic and semi-tropic climate. This plant is capable of surviving in very dry and arid conditions (Liauw et al., 2008). Neem is a large tree growing up to 25 m height with semi-straight to straight trunk, 3 m in girth and spreading branches forming a broad crown. The plant parts such as fruits, seeds, leaves, bark and roots contain various bio-molecules with potent insecticidal, antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer andantifungal activities (Girish & Shankara, 2008; Dua et al., 2009). This study evaluates the use of aqueous extract of dried neem root for the synthesis of AgNPs and the antimicrobial activities of the synthesized particles were also investigated against some clinical bacterial isolates and a bloom forming cyanobacterial strain. Furthermore, the synthesized AgNPs were evaluated for their potential application as an antimicrobial additive in detergent production.

1. Materials and Methods

Collection of neem root and the preparation of extract is as follows: Fresh neem roots were collected from a farm in Gidan Kwano area of Minna, Niger, Nigeria. The roots were thoroughly washed with clean tap water to remove extraneous materials, chopped into smaller pieces, air dried at ambient conditions (30 ± 2 °C) for two weeks, and finally milled into powdery form using electric blender. The root aqueous extract was prepared by dispersing 1 g of the dried neem root sample into 10 ml of deionized water and heated in water bath at 60 °C for 1 h. The extract was centrifuged at 5000 RPM for 20 min and then filtered using Whatman No. 1 filter paper. The filtrate was collected and stored in refrigerator at 4 °C for further studies (Adelere et al., 2017).

1.1 Synthesis and Characterization of Silver Nanoparticles

The aqueous extract of neem root was used for the synthesis of AgNP's following a method described by Lateef et al. (2016) with little modification. Approximately 1 ml of neem root extract was mixed with 10 ml of 1 mM silver nitrate solution ($AgNO_3$) and allowed to stand at room temperature (30 ± 2 °C) for 3 h. The reaction was visually monitored by observing the possible color change due to the reduction of silver ion in the extract for the formation of AgNPs. The control experiment consisted of an aqueous solution of 1 mM silver nitrate was set for easy comparison with the test experiment. Preliminary characterization was carried out by determining the absorbance characteristic of the resulting product using UV-vis spectrophotometry analysis.

Fourier Transform Infrared (FTIR) spectroscopy analysis was carried out on a sample of AgNPs using CRY 630 Spectrophotometer (Agilent Technologies, USA) according to Bhat et al. (2011). The AgNPs solution was centrifuged at 10,000 RPM for 20 min, the pellet obtained was freeze dried and mixed with KBr pellets. The mixture was then used for FTIR measurement. Also, the sample was analysed using Scanning Electron Microscopy (SEM) method. A small quantity of AgNPs suspension was air died and the powder obtained was placed on the specimen stub coated with copper and imaged by Phenom ProX scanning electron microscope (PHENOMWORLD, NETHERLAND).

1.2 Antibacterial Activities of Synthesized AgNPs

Agar well diffusion method was used to evaluate the antibacterial activity of the AgNPs. Clinical bacterial isolates including Escherichia coli, Streptococcus Sp, Klebsiella pnuemoniae obtained from the General

Hospital, Minna, were used as a test organisms. These organisms were cultivated in peptone water and incubated at 37 °C for 18 h and then seeded on Mueller-Hinton agar plates. The seeded plates were allowed to stand for 1 h before creating wells on them by cork borer of 6 mm diameter. Each well was loaded approximately with 50 μl of graded concentration of AgNPs (50 μg ml-1, 100 μg ml-1 and 150 μg ml-1) prepared by dilution with sterile distilled water. The plates were then incubated at 37 oC for 24 h and examined for zone of inhibition which was measured in mm.

1.3 Evaluation of Algicidal Activity of the AgNPs

The algicidal property of the biosynthesized AgNPs was investigated using the modified method of Chaturvedi and Verma (2015). Freshwater algae collected from a water logged area of Federal University of Technology, Minna, Bosso Campus were transported in a clean plastic bottle directly to the laboratory for the study. Freshwater algae of 10 ml were measured in each Mc Cartney bottle and 1 ml of graded concentration of AgNPs (50 μg ml^{-1}, 100 μg ml^{-1} and 150 μg ml^{-1}) was added. A bottle containing only 10 ml of freshwater algae (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 the algal growth in each bottle was measured using UV-vis spectrophotometer on a daily basis for 5 days.

1.3.1 Evaluation of Antifungal Properties of Synthesized AgNPs as Additive in Detergent

The potential application of the synthesized AgNPs as antifungal additive in detergent was investigated by incorporating the AgNPs in locally made detergent. One litre of a locally made detergent soap purchased from a retailer outlet in Bosso Market, Minna was used for this study. Detergent of 19 ml was dispensed in McCartney bottles, autoclaved at 121 °C for 15 min and then incorporated with 1 ml (150 μg ml^{-1}) of biosynthesized AgNPs. After cooling, 0.5 ml of 48h old culture of fungal strains, including Aspergillus niger, Aspergillus flavus and Mucor sp were inoculated each into the bottles. The control experiments consisted of detergents and test organisms only. The bottles were incubated at 30 ± 2 °C for 48 h, after which 1 ml of the contents of each bottle was drawn and inoculated into freshly prepared potato dextrose agar plates using pore plate technique. The plates were then incubated at ambient temperature for 48 h and then observed for growth.

2. Results and Discussion

The aqueous extract of neem root mediated the synthesis of AgNPs within 15 min under ambient temperature (30 ± 2 °C). The bio-synthesized AgNPs exhibited a dark yellow coloration after 15 min of reaction, which later turned into stable brown after10 min (Figure 1) whereas, the control silver nitrate solution showed no color change. The authors reported that there are variations in the color of previously bio-synthesized AgNPs due to the complexity nature of bio-molecules. The color formation in the AgNPs synthesis is attributed to the excitation of Surface Plasmon Resonance (SPR) on the metallic nanoparticles (Selvi & Sivakumar, 2012). The AgNPs showed a maximum UV-vis absorbance at the wavelength of 425 nm (Figure 2), which is within the range of 391-460 nm AgNPs absorbance characteristics earlier reported (Thirumurugan et al., 2011; Zaki et al., 2011; Priyadarshini et al., 2013; Lateef et al., 2015; Adelere et al.,

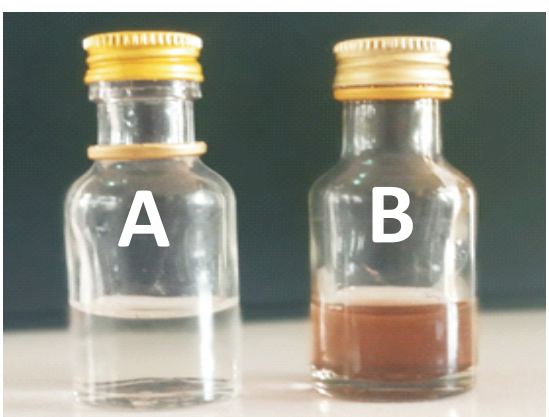


Figure 1. Biosynthesized AgNPs Using Aqueous Extract of Neem Root: A, Silver Nitrate Solution as Control; B, Synthesized AgNPs Within 25 min

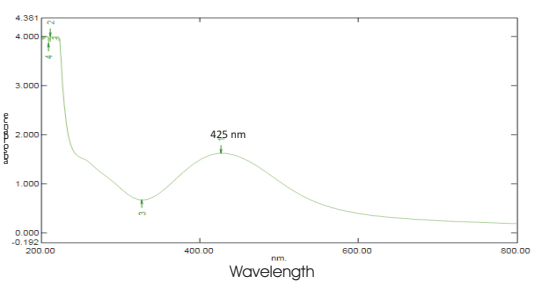


Figure 2. UV-vis Absorption Spectrum of AgNPs Synthesized from Aqueous Extract of Neem Root

2017). Creighton et al. (1979) affirmed that the UV-vis absorbance characteristics of AgNPs are a function of their surface plasmon resonance.

The FTIR measurement was carried out to identify the possible bio-molecules responsible for the capping and stabilization of the synthesized AgNPs. The FTIR spectrum showed peaks at 3268 cm^{-1} and 1636 cm^{-1} (Figure 3).The two bands are correspond to N-H bond of amines, and C=C stretch of alkenes or C=O stretch of amides, respectively (Shankar et al., 2014). This is an indication that protein molecules play a significant role in capping and stabilization of the AgNPs. Mandal et al. (2005) reported that proteins are involved in the stabilization and capping of nanoparticles by binding either through their free amine groups or cysteine residues.

The image obtained from microscopic analysis using SEM shows that the bio-synthesized AgNPs are predominantly spherical in shape (Figure 4) with size ranging from 20-60 nm and this corroborate some previously reported findings (Lateef et al., 2015; Adelere et al., 2017). The uniqueness of

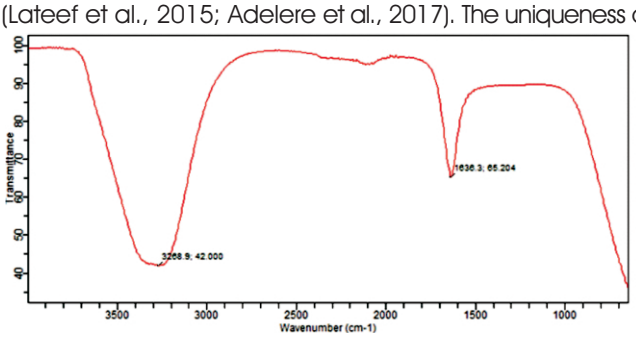


Figure 3. FTIR Spectrum of AgNPs Synthesized from Aqueous Extract of Neem Root

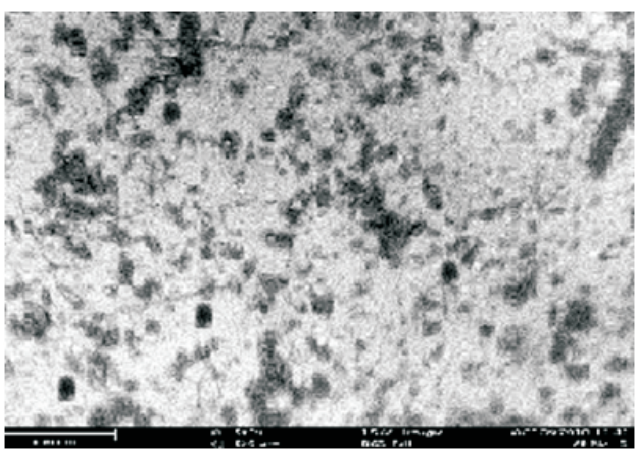
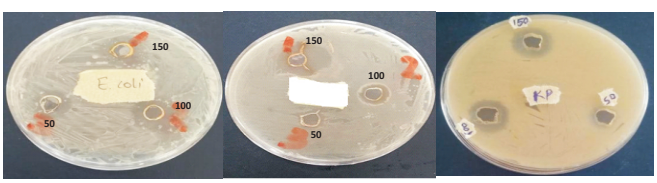


Figure 4. SEM Image of AgNPs Synthesized from Aqueous Extract of Neem Root

physico-chemical properties of nanoparticles such as size, morphology and chemical compositions make suitable for diverse areas of application.

The biosynthesized AgNPs exhibited considerable inhibitory activity against some clinical bacterial isolates (Figure 5). The AgNPs at concentrations of 150 and 100 $\mu g\ ml^{-1}$ remarkably inhibited strains of Streptococcus sp, E. coli, and K. pneumoniae. The maximum and minimum inhibitory activities exhibited were 22 and 10 mm, respectively (Table 1). It is obvious from the obtained data that the antibacterial activity of the synthesized AgNPs is dose dependent, as the activity increases linearly with increasing concentration. Moreover, the antibacterial activities as reported herein agree with some results obtained in the previous similar studies (Salem et al., 2014; Lateef et al., 2015; Adelere et al., 2017). Studies have suggested that size, morphology, and chemical compositions play an active role in the antimicrobial activity of nanoparticles. Moreover, silver ions release from dissociation of AgNPs make them most potent antimicrobial type of nanoparticles, as it attack and disrupt cell wall and cytoplasmic membrane through electrostatic attraction (Raffi et al., 2008). Also, its affinity for sulphur and phosphorus facilitates the interference with the electron transport chain and destruction of molecules like DNA, lipids and proteins (Feng et al., 2000; Song et al., 2006; Inbakandan et al., 2016). Since, AgNPs are capable of attacking many structures in the bacterial cell, hence, it can



(a) (b) (c)

Figure 5. (a) Antibacterial activities of biosynthesized AgNP against clinical isolates of Escherichiacoli, (b) Streptococcus sp and (c) Klebsiellapneumoniae

Organisms	Concentration of AgNPs ($\mu g\ ml^{-1}$)	Zone of Inhibition (mm)
Streptococcus sp	150	22
	100	20
	50	17
E.coli	150	19
	100	16
	50	14
K.pneumoniae	150	22
	100	21
	50	10

Table 1. Antibacterial Activity of Biosynthesized AgNPs and Zones of Inhibition (mm) Against Clinical Isolates

be suggested to be a suitable alternative for the treatment of infections caused by antibiotic resistance bacterial pathogens.

The biosynthesized AgNPs demonstrated algicidal property against a blooming forming cyanobacterial strain. The AgNPs effectively inhibited the growth of cyanobacterial strain in the test experiment while the control test was characterized with profuse algal growth as shown in Figure 6 and Table 2. This result corroborates the algicidal activity of a flame of forest mediated AgNPs reported by Chaturvedi and Verma (2015). Similarly, Roychoudhury et al. (2018) recently reported the algicidal activity and DNA binding affinity of AgNPs synthesized from another algal species. Consequently, the exploitation of nanoparticles in controlling algal growth will reduce their adverse environmental problems like odoriferous, unsightly scums, toxicity of water bodies, and eutrophication (Anusha et al., 2017).

The evaluation of bio-synthesized AgNPs for potential application as detergent additives showed the excellent result. The locally made detergent fortified with the AgNPs completely inhibits the growth of microbes, including Escherichia coli, Staphylococcus aureus, Aspergillus flavus, and Candida sp, while dense growths were recorded in the control experiments

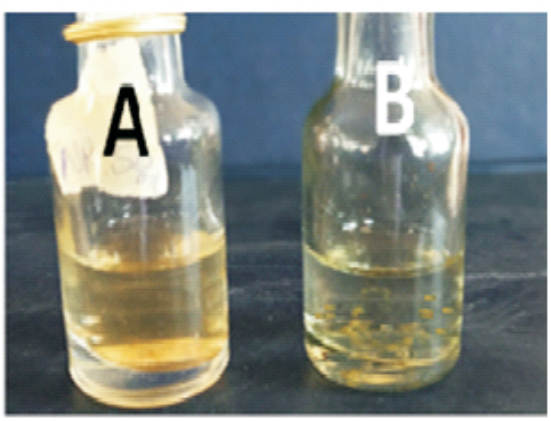


Figure 6. Algicidal Activity of AgNPs Against a Bloom Forming Cyanobacterial Strain. A, Inhibition of Algal Growth by the Synthesized AgNPs: B, Algal Growth in Control Experiment Without AgNPs After 5 days

Days	Control (OD_{600})	Test (Od_{600})
0	0.01	0.01
1	0.09	0.05
2	0.31	0.08
3	0.69	0.12
4	0.85	0.16
5	0.96	0.17

Table 2. Algicidal Activity of AgNPs

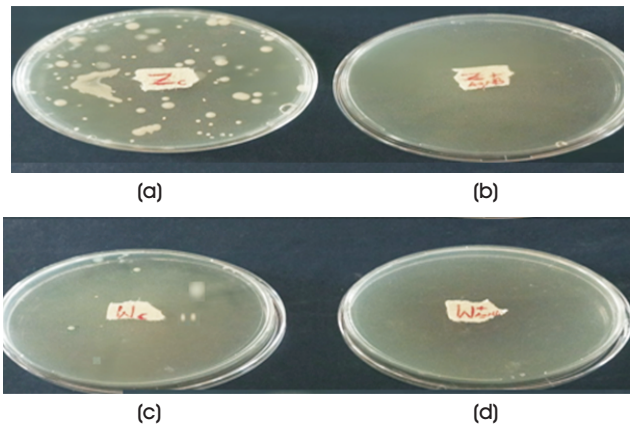


Figure 7. Antibacterial Activities of Synthesized AgNPs on Bacteria Inoculated into Liquid Detergent: (a), Control Plate of S. Aureus without AgNPs; (b) Complete Inhibition of S Aureus in the Detergent by AgNPs; (c), Control Plate of E. coli without AgNPs; (d), Complete Inhibition of E. coli in the Detergent by AgNPs.

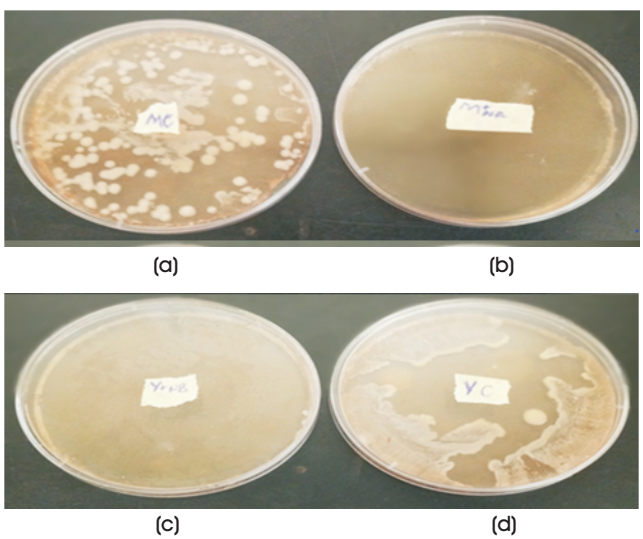


Figure 8. Antifungal Activities of Synthesized AgNPs on Fungi Inoculated into Liquid Detergent: (a), Control Plate of Candida sp Without AgNPs; (b), Complete Inhibition of Candida sp in the Detergent by AgNPs; (c), Complete Inhibition of A. Flavus in the Detergent by AgNPs; (d) Control Plate of A. Flavus without AgNPs

except the E. coli plate that has a very low growth (Figures 7 and 8). The bactericidal and fungicidal activities displayed in the detergent by AgNPs may be due to the attack of cellular metabolism and nucleic acid molecules by the silver ions released upon dissociation.

Conclusion

The green synthesis of AgNPs was achieved using the aqueous extract of neem root under ambient conditions. The bio-synthesized nanoparticles showed appreciable antibacterial and algicidal activities against some clinical

bacterial isolates and a bloom forming cyanobacterial strain, respectively. Also, the particles can successfully inhibit the microbial growth when used as additive in detergent. Hence, the excellent activities demonstrated by the AgNPs as obtained in this study suggest their potential application in consumer products, medical and environmental management.

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