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MYCOSYNTHESIS OF SILVER NANOPARTICLES USING *Lentinus squarrosulus* AND ITS ANTIBACTERIAL ACTIVITIES

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Abstract

In this research, mycosynthesis of silver nanoparticles (AgNPs) using *Lentinus squarrosulus* extract was reported. Characterization of the biosynthesized AgNPs were carried out using FT-IR, UV-vis, EDX and FESEM. Antibacterial efficacy of the biosynthesized nanoparticles was carried out against selected bacteria of clinical importance. The UV-vis spectrum peaked at 418 nm, while EDX analysis revealed strong silver peak at around 1.5 KeV. The nanoparticle's size and morphology as captured by FESEM mostly showed particles with spherical shapes ranging from 18.6 nm to 78.5 nm. The nanoparticles induced a maximum zone of 13.00 mm for both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, while a zone of 10.00 mm was recorded for *Escherichia coli*. This study established the fabrication of AgNPs from fungus extract and the antibacterial activities of the synthesized nanoparticles; thus its possible usefulness in pharmaceutical industries.

Keywords: *Lentinus squarrosulus*, antibacterial, silver nanoparticles, mycosynthesis

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Introduction

Over the years, the field of nanotechnology has enjoyed tremendous development, with different researchers from various disciplines contributing to this fast growing field (Bhakya *et al.*, 2015; Carmona *et al.*, 2017; Aina *et al.*, 2018a; 2019). Its vast applications in various disciplines and its impact on

several facets of human daily life opens doors for corresponding career opportunities. Metallic nanoparticles are being focused on, as they display properties notably different from their bulk materials (Abdel-Mohsen *et al.*, 2012). These metallic particles have been produced via a variety of methods – chemical

(Evanoff *et al.*, 2004; Wiley *et al.*, 2005; Merga *et al.*, 2007), physical (Magnusson *et al.*, 1999; Kruis *et al.*, 2000; Jung *et al.*, 2006) and biological method (Klaus *et al.*, 1999; Panáček *et al.*, 2006; Adelere and Lateef, 2016; Lateef *et al.*, 2016a-d). While chemical and physical methods have been used to successfully fabricate well-defined particles, their cost and the potential danger they pose to the environment are major setbacks (Makwana *et al.*, 2015).

This has led several researchers to opt for a rather eco-friendly method; the biological method. In the biological method, biomaterials such as plant biomass, plant extract or microorganisms are used in nanoparticles production. Synthesizing AgNPs by microorganisms is eco-friendly and the synthesized particle has been reported to possess efficacious antimicrobial property against bacteria, fungi and other microorganisms. Jaidev and Narasimha (2010) produced AgNPs from *Aspergillus niger* which displayed antimicrobial activities against *Aspergillus niger* and *E. coli*. Other fungi whose metabolites have been used in the biosynthesis of nanoparticles include *Aspergillus fumigatus* (Bhainsa and D'Souza, 2006), *Aspergillus flavus* (Vigneshwaran *et al.*, 2007), *Aspergillus niger*, *Trichoderma longibrachiatum* (Elegbede *et al.*, 2018a, b, 2019) and *Fusarium oxysporum* (Ahmad *et al.*, 2003).

Among the numerous fungi used in nanoparticles synthesis, the use of higher fungi, mushroom, and its product is becoming increasingly important, a field tagged Myconanotechnology, which is an interface between nanotechnology and mycology. In a recent report by Lateef and Adeeyo (2015), the enzyme laccase was harvested from the mushroom *Lentinus edodes* and used in fabricating silver nanoparticles, which was

then evaluated against some clinical bacterial isolates.

Lentinus squarrosulus is a common edible mushroom collected in the wild for consumption in Nigeria; its local name in the South-East Nigeria being “*Ero atakata*” (Ayodele and Idoko, 2011). It is a saprophytic white rot fungus that is mostly found in the forest on dead or deteriorated wood. It has been reported to possess good antimicrobial (Borokini *et al.*, 2016), antioxidant (Omar *et al.*, 2015) and anti-ulcer (Omar *et al.*, 2011) activities.

Asides its numerous medicinal importance, it has also been known to be nutritionally rich, possessing dietary fibres, folic acid, riboflavin, thiamine, minerals (iron, phosphorus, calcium, magnesium and potassium) and high protein content. In this study, *Lentinus squarrosulus* was used to synthesize silver nanoparticles and its antibacterial effectiveness on some pathogenic organisms was evaluated.

Materials and Methods

Sample Collection

Lentinus squarrosulus fresh fruiting body developing on a rotting *Mangifera indica* tree stump were collected from Odogbolu town in Ogun state, Nigeria. Identification was done using the method of Alexopoulos *et al.* (1996). The samples of the mushroom were dried, blended to powder, stored in sealed shut amber container and refrigerated at 04°C.

Collection of Test Organisms

Clinical isolates that the AgNPs will be tested against were procured from LAUTECH Teaching Hospital, Ogbomosho, Nigeria. They included *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and *Staphylococcus aureus*.

Extract Preparation

For this study, aqueous extraction of the mushroom sample was done. 1 g of the lyophilized mushroom was suspended in 100 ml of distilled water, heated at 60° C for an hour, filtered and centrifuged at 4000 rpm for 20 minutes. Supernatant was then collected (Lateef *et al.*, 2018).

Synthesis of the Nanoparticles

The biological synthesis of AgNPs from *Lentinus squarrosulus* extract involved introducing 1 ml of the extract into 40 ml of 1 mM AgNO₃ followed by colour changes observation. This synthesis, which took approximately 2 h, was performed using the techniques of Lateef *et al.* (2018).

Characterization

The AgNPs biosynthesis was tracked for colour change through visual observations in the reaction vessel. After stabilization, the absorption spectra were monitored at 190-900 nm using the UV-visible spectrophotometer (Cecil, USA). The vibrational frequencies of the nanoparticle were gotten from FT-IR transmission spectra by KBr method using FTIR model 8400S (Shimadzu, Japan). The presence and ratio of the elements in the particles was analyzed using the EDX Silicon-drift detector (X-MaxN, Oxford Instruments, UK).

For the size and morphological properties of the synthesized nanoparticle, the particle was analyzed using the FESEM. Powder samples were sonicated in ethanol medium in ultrasonic probe sonicator to have maximum de-agglomeration of the particles before imaging. The AgNPs' surface morphology was noted with a FESEM (Lyra 3, Tescan, Czech Republic), accelerating voltage of 20 up to 30 kV. The above procedure is according to standard method.

Antibacterial Activity

AgNPs' activity against the test organism was evaluated using the agar well diffusion method adopted from Perez *et al.* (1990). Mueller Hinton agar was seeded with an 18 h old culture of each organism. Gradient concentrations of 100 µl of the AgNPs were then dispensed into each of appropriately labelled holes bored on the plate. At 37 °C for 18 h, the plates were incubated after which the computation of the zones of inhibition followed suit. The test was run in triplicate. Control experiments with distilled water as the negative control and the mushroom aqueous extract and silver nitrate as positive control were set up.

Results and Discussion

Synthesis and Characterization

The reduction of AgNO₃ to AgNPs was evidenced by a change in colour in the solution, which after 10 min changed from being initially colourless into a stable dark brown colour as shown in Figure 1, an observation that has been attributed by various scientists to the excitation of surface plasmonic vibrations of AgNPs (Ojha *et al.*, 2017; Rao and Tang, 2017; Thirumurugan *et al.*, 2010).

Figure 1: *L. squarrosulus*-mediated AgNPs (a) at initiation of synthesis; (b) after 10 min

The UV-vis spectrum showed peak at 418 nm (Figure 2). This is in great correlation with previous findings from various researchers. Kannan *et al.* (2014) reported a similar peak at 430 nm when the fungus *Ganoderma lucidum* extract was utilized in AgNPs synthesis. Basavaraja *et al.* (2008) also reported a peak of 420 nm when the fungus *Fusarium semitectum* was employed in AgNPs synthesis.

Figure 2: UV-vis spectrum of the AgNPs

Figure 3 represents the FTIR spectrum of the AgNPs. The synthesis of NPs with plants and fungi extracts introduces organic functional groups on the NPs surface. These groups are usually characterized using FTIR analysis. The NPs presents characteristic peaks at 3450 cm^{-1} assigned to stretching vibration of -NH_2 (amide I) and/or -OH of phenolic compounds (Raghunandan *et al.*, 2011). The bending of alkenes and aromatics are reported as a sharp peak at 1384 cm^{-1} (Rahmaniyan *et al.*, 2015),

Figure 3: The FTIR spectrum of the AgNPs

while the 1637 cm^{-1} peak is that of -N-H bend (Lin *et al.*, 2005). The NPs surface carbonate vibrational mode is at 871 cm^{-1} and Ag-O stretching modes were noted at 723 cm^{-1} and 418 cm^{-1} (Taghavi Fardood *et al.*, 2017). The FT-IR findings thus showed that NPs were surrounded by biomolecules such amino acids thereby conferring some functional groups on them. These functional groups may contribute to the bioactivities of the NPs.

The EDX spectrum is as shown in Figure 4. It was used in the synthesized AgNPs to verify the existence of elemental silver. A strong silver signal peak was observed around the 1.5keV. Some other researchers (Aina *et al.*, 2018b; Islam *et al.*, 2017; Puchalski *et al.*, 2007) have similarly reported observing peaks within this range (1.5 to 5.0 keV). In the spectrum, elemental peaks of Ca, S, C, N, Na, Si and O were also present. While the C and O are perceived to be from the extract, the others are impurities.

The AgNPs' size and morphology were captured by FESEM (Figure 5). The particle size varied between as low as 18.6 nm to 78.5 nm, most of them being sphere-shaped. Comparably, some other researchers, in their use of fungal extract in the biofabrication of AgNPs, gave reports of similar size ranges (Afreen *et al.*, 2011; Birla *et al.* 2009; Karbasian *et al.*, 2008; Ahmad *et al.*, 2003; Ingle *et al.*, 2008).

Figure 4: EDX spectrum of the AgNPs

Figure 5: Field emission scanning electron micrograph of the biosynthesized AgNPs at (a) 100 kx magnification (b) 50 kx magnification

Antimicrobial Activity

The biosynthesized silver nanoparticles exhibited noticeable antimicrobial activity when evaluated against some clinical isolates (Figure 6; Table 1). The nanoparticles induced a maximum zone of 13.0 mm which was displayed against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* while the lowest zone was recorded for *Escherichia coli* with a zone of 10.0 mm. The mechanism of action of the biofabricated AgNPs has been suspected to be as a result of its large surface area to volume ratio which allows for stronger

adherence to the bacterial cell wall and easy penetration into the cytoplasm, thus modifying the cell wall and hence inhibiting the organism (Ahmed *et al.*, 2016). As expected, previous reports on the efficacy of mycosynthesized AgNPs against bacterial isolates fully support this result (Afreen *et al.*, 2011; Birla *et al.*, 2009; Nithya and Ragunathan, 2009; Verma *et al.*, 2010).

As remarkable as the AgNPs' antimicrobial activity can be, some organisms have been recently reported to be resistant to their activity. In this study, *Staphylococcus*

aureus was resistant to all the concentrations of the synthesized AgNPs as evidenced from Figure 6d. In another study by Jagtap and Bapat (2013), some of the Gram negative and Gram positive isolates were reported to be resistant to AgNPs synthesized from *Artocarpus heterophyllus* seed extract.

While the mechanism of resistance of bacteria to AgNPs is not yet fully understood, Panáček *et al.* (2018) hypothesized resistance in flagellated bacteria to be as a result of flagellin, a component of bacterium flagellum which

Table 1: Zone of inhibition obtained by agar well diffusion method

Isolate	Mean Zone of Inhibition (mm) $\pm$ standard deviation (SD)							LSE	Distilled H ₂ O
	AgNPs						AgNO ₃		
	10μg/ml	20μg/ml	40μg/ml	60μg/ml	80μg/ml	100μg/ml			
<i>Staphylococcus aureus</i>	NZ	NZ	NZ	NZ	NZ	NZ	6.2±0.2	NZ	NZ
<i>Klebsiella pneumoniae</i>	NZ	NZ	NZ	11±0.1	12±0.2	13±0.1	6.4±0.1	NZ	NZ
<i>Pseudomonas aeruginosa</i>	NZ	NZ	NZ	11±0.1	13±0.1	13.0±0.2	6.1±0.1	NZ	NZ
<i>Escherichia coli</i>	NZ	NZ	NZ	10±0.1	11±0.2	12±0.1	6.3±0.1	NZ	NZ

*NZ, no zone of inhibition

Figure 6: Antibacterial activity of AgNPs against some clinical bacterial isolates (a) *Escherichia coli* (b) *Klebsiella pneumoniae* (c) *Pseudomonas aeruginosa* (d) *Staphylococcus aureus*

clumps the AgNPs together and invariable hinders its activity against the bacteria.

Conclusion

In a world where antimicrobial resistance is the talk of the day, the need for new antimicrobial substances is becoming increasingly pertinent. This study has shown eco-friendly biosynthesis of AgNPs using *L. squarrosulus* extract. The synthesized nanoparticles showed activity on the bacterial isolates. Thus the silver nanoparticles biologically synthesized from the extract of *L. squarrosulus* could be of great importance in medical field for effectual antimicrobial activities; hence in the arsenal of weapons against antimicrobial resistance, a new eco-friendly weapon has been deposited.

Conflict of Interest

There are no conflicts of interest as regards this publication

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