Antioxidant and antimicrobial activities of nanosilver-mycomeat composite produced through solid state fermentation of tigernut waste and cassava pulp by *Pleurotus pulmonarius*

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Abstract

Bacterial deterioration is one of the most critical subjects in the production, processing, transport, and storing of food. The search for novel materials for the synthesis of nanoparticles is unending and this is likely to continue in the upcoming years. In this study, mycomeat samples were produced using cassava pulp (C) and tiger nut aggregate waste (T). The mycomeat extracts were used to produce nanosilver-mycomeat by catalytic conversion of Ag⁺ to Ag⁰. The antioxidant and antimicrobial activities of the nanosilver-mycomeat composites were determined using established methods. The nanosilver-mycomeat composites of both C and T showed increased antioxidant and antimicrobial activities compared to the ordinary mycomeat extract. This can find applications in the packaging of food, thereby preventing food samples from deterioration.

Keywords: Nanoparticles, nanosilver-mycomeat, antioxidant, antibacterial, medicinal mushroom, tiger nut

1 Introduction

Nanotechnology deals with materials ranging in size from 1 to 100 nm, mainly nanoparticles and nanostructures [1, 2]. It has found applications in multiple areas, including medical, pharmaceutical, textile, chemical sectors, paper conservation [3], removal of wastes such as dyes [4] and photocatalytic degradation [5]. The importance of nanomaterials was realized when researchers found that size can influence the physiochemical properties of a substance, including its optical properties. Nanoparticles can be synthesized at lowcost by using the extracts from plants and products from microbes [6]. The application of
nanotechnology in food is fairly recent [7], and has experienced a continuous upsurge since 2003 [2, 8].

Tested nanomaterials in food industry include natural product nanoparticles (NPs) and metal and metal oxide nanoparticles. Among all, silver nanoparticles have enjoyed increasing commercialization as antimicrobial compounds. On the other hand, gold nanoparticles are commonly used as sensors, while Titanium dioxide NPs are used as disinfecting agents, food additives and flavour enhancer [7]. Natural product nanoparticles are used as a delivery system, as a supplement in the food industry and in food contact materials. Nanoparticles are reported to be used in food and feed packaging industries as biosensors, antimicrobials and for extending the shelf-life of packaged foods [1, 9]. However, any material intended to be in contact with food and active and intelligent materials (AIM) must comply with the provision of the framework Regulation (EC) that defines the three requirements to ensure safe and quality food [8]. These requirements are that food contact materials must not transfer their components into food in quantities that could endanger human health, must not change the composition of the food in an unacceptable way and shall not deteriorate the taste, odour or texture of the food.

Tigernut (Cyperus esculentus) is commonly found in all continents except Antarctica [10]. They are considered as a weed in 15 countries [10], while they are highly nutritious, medicinal, but underutilized crops in other parts of the world [11]. Cyperus esculentus are a rich source of essential amino acids (oleic acid, leucine, methionine, glutamic acid and aspartic acid) and minerals (iron, calcium among others) [11, 12]. Medicinally, they are useful for treating indigestion, flatulence, dysentery and diarrhoea [11]. The tubers also function as a tonic, aphrodisiac, diuretic and as a stimulant [11, 13]. In addition, consumption of the tubers help in the treatment of poliomyelitis, boils, common cold, urinary tract infections and can prevent heart diseases [12, 14]. Cassava pulp is a rich source of energy and approximately 1.5 million tonnes of cassava pulp are produced annually [15]. Cassava pulp is a waste product from the ‘fufu’ (a cassava meal) processing industry and it constitutes about 30% of the original weight of cassava roots.

Food quality and safety are of great concern and as a result, preservation of food against spoilage by microbial agents and undesirable physical changes is of paramount importance. Therefore, this study is centred on the production of nanosilver-mycomeat as an antibacterial and antioxidant for food packaging materials.
2 Materials and methods

2.1 Sample Collection

The Spawn of *Pleurotus pulmonarius* also known as Oyster mushroom was purchased from Osogbo, Osun State, Nigeria. Cassava pulp was obtained from cassava processor in Ogbomoso, while tigernut was purchased from Sabo, Ogbomoso, Oyo State, Nigeria.

2.2 Preparation of the mycomeat extract

The tigernut aggregate waste and cassava pulp were each used to prepare mycomeat following the methods of Bamigboye *et al.* [16]. Briefly, the tigernut was manually sorted and cleaned to remove particles and unwanted materials. Dates together with coconut were added to the tigernut in the ratio 1:1:10 and then soaked in clean water for 5 h to soften the aggregate. The tigernut aggregate was milled into slurry in the ratio 1:3 of tigernut to water and then sieved using cheese cloth to extract waste from the aggregate. The cassava pulp was pounded using mortar and pestle. Precisely 100 g of each of the aggregate waste and cassava pulp was filled into four transparent polythene bags each. The bags were autoclaved at 121 °C for 15 min, allowed to cool and a set of two were inoculated with the spawn of *Pleurotus pulmonarius* and incubated till full ramification. The remaining two sets were left unfermented. After full ramification, it was dried at 80 °C for 8 h and milled to powder. Precisely 0.1 g of the milled powder for the fermented cassava pulp (FC), fermented tigernut aggregate waste (FK), non-fermented cassava pulp (C) and non-fermented tiger nut aggregate waste (K), were each suspended in 10 ml of distilled water in a transparent 50 ml bottle, labelled and heated in a digital dry bath (Labnet, USA) at 60 °C for 1 h. The extract was filtered using Whatman No.1 filter paper and then centrifuged at 4000 rpm for 20 min. The filtrate was collected and stored in the refrigerator at 4 °C for further use.

2.3 Biogenic synthesis of AgNPs and effect of varying the reaction mixture

For the preparation of nanosilver-mycomeat, 1 ml of each mycomeat extract (C, FC, K and FK) was added to 5, 10 and 20 ml of 1 mM AgNO₃. The reaction mixture was placed under sunlight exposure for the photo activation of the silver ions and identification of the concentration with the highest colour intensity. A change in the colour of the reaction mixture were visually observed, followed by the measurement of its absorbance spectrum using UV-visible spectrophotometer operating at the range of 200-800 nm wavelength range.

2.4 DPPH radical-scavenging activities of the nanosilver-mycomeat

The free radical-scavenging activity of the AgNPs was assayed according to Guntur *et al.* [17]. Precisely 1 ml of each nanosilver-mycomeat solution at different concentrations (1, 2, 5, 10, 20, 40 µg/ml) were mixed with 4 ml of methanol solution of DPPH in slant bottles.
Controls were set up comprising 4 ml of DPPH solution and 1 ml of the supernatant extract (for both the fermented and non-fermented) or AgNO₃ in transparent bottles, incubated in the dark at room temperature for 30 min. The setups were measured at 517 nm using a spectrophotometer. Antioxidant activity was estimated by calculating the % inhibition by following the formula [18]:

\[
\% \text{ DPPH radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100
\]

Where, \( A_0 \) is the absorbance of the control and \( A_1 \) is the absorbance of the extract.

2.5 Antimicrobial activities of the synthesized AgNPs

Four clinical isolates were obtained from LAUTECH Teaching Hospital, Ogbomoso, Oyo state, Nigeria. The synthesized nanosilver-mycomeat were evaluated as antimicrobial agent using point inoculation method and agar well diffusion technique [19]. Selected isolates included \textit{Staphylococcus aureus}, \textit{Staphylococcus epidermidis}, \textit{Escherichia coli} and \textit{Proteus} species.

3 Results and Discussion

3.1 Green synthesis and characterization of silver nanoparticles

The formation of AgNPs by reduction of the aqueous silver during exposure to the mycomeat supernatant showed dark brown colour, which suggested the formation of AgNPs in solution. The colour changed from colourless to dark brown and the reaction stabilized after 1 hour of exposure to sunlight (Figure 1). The colour changed due to excitation of surface Plasmon vibrations of silver nanoparticles, which gave rise to the surface Plasmon band [20]. This means that the mycomeat extract can be used in green synthesis of silver nanoparticles. The formation of nanosilver-mycomeat was confirmed by the UV-vis spectroscopy (Figure 2). The UV-vis spectra showed an absorption peak at a wavelength of approximately 410 and 420 nm for FK and FC respectively (Figure 2). Alsalhi et al. [21] prepared AgNPs which exhibited UV-vis absorption within the range 410-430 nm. In another study by Sriramulu and Sumathi [22], absorbance peaks were observed at 434 and 414 nm for AgNPs synthesized using extracts of \textit{Ganoderma lucidum} and \textit{Agaricus bisporus} respectively. Abdel-Aziz et al. [23] reported a broad absorption peak at 400 nm for a nanocomposite that was synthesized.
Figure 1. Visual observation of the nanosilver-mycomeat composites synthesized using fermented tigernut aggregate waste (FKNPs) and fermented cassava pulp (FCNPs) after 1 h of reaction.

Figure 2. UV-vis absorption spectra of nanosilver-mycomeat composites synthesized using tigernut aggregate waste (FKNPs) and cassava pulp (FCNPs)
3.2 Antioxidant Activities

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. Resulting DPPH produces purple colour in methanol solution and fades to shades of yellow colour in the presence of antioxidants. The scavenging efficacy of the nanosilver-mycomeat composites is presented in Figure 3. The present study showed that nanosilver-mycomeat composites can effectively scavenge DPPH radicals, with 1 µg/ml of FCNPs, and FKNPs scavenging up to 66 and 70% of DPPH respectively. This is higher compared to the activities obtained for the ordinary mycomeat extracts. Only 41.5 and 56.4% of DPPH were scavenged by FC and FK respectively when nanoparticles were not synthesized with the extracts. The study showed that the nanosilver-mycomeat composites exhibited better DPPH scavenging activities compared to the plain extracts. In an earlier study by Boonsong et al. [24], 500 µg/ml of ethanolic extracts of Lentinus edodes, Pleurotus eous and Auricularia auricula scavenged only 50, 58 and 22% of DPPH radicals respectively. Synthesized silver nanoparticles of Agaricus bisporus extract had antioxidant activity of 75% [22], while 70% was reported for aqueous extracts of Cymbopogon citratus [25]. Antioxidant activity can be linked with polyphenolic content, as well as flavonoids [26].

![Figure 3](image_url)

**Figure 3.** DPPH radical scavenging activities of nanosilver-mycomeat synthesized using tigernut aggregate waste (FKNPs) and cassava pulp (FCNPs)
3.3 Antimicrobial activities of the synthesized silver nanoparticles

The biosynthesized nanosilver-mycomeat composites displayed mild inhibitory activity against selected clinical isolates of bacteria. At 10 µg/ml, FK and FC mildly inhibited strains of *Escherichia coli*, *Proteus* spp and *Staphylococcus epidermidis* with the zone of inhibition being 14, 12 and 11 mm respectively. The point inoculation method strictly inhibited the growth of the organism to the point of inoculation (Figure 4). Interestingly, both silver nitrate and ordinary mycomeat had no inhibitory effect on the clinical isolates. The inhibitory activities displayed by AgNPs is attributed to its increased surface area at nanometer scale, thus the amount of silver ions released is directly linked to antimicrobial efficacy [27]. The antibacterial activities of AgNPs obtained in this study revealed the efficacy of the nanosilver-mycomeat composite to inhibit multi-drug resistant microbes in the environment. Other authors have demonstrated the antimicrobial effects of biosynthesized silver nanoparticles [28].

Main mechanism enabling the nanoparticles to act on bacteria includes the interaction of nanoparticles with membrane proteins and subsequent accumulation in the cell membrane, thereby affecting its permeability. Silver nanoparticles notably interact with the cell surface of some bacteria. This leads to structural changes and damage to cell membrane, thereby increasing the permeability of the bacteria [23]. It was also postulated that the uptake of free silver ions lead to a disturbance in ATP production and DNA replication [28, 29]. Biosynthesized AgNPs were reported to show 2 and 1.6 mm zones of inhibition against *E. coli* and *S. aureus* respectively [30]. This is similar to what was obtained in this report.

![Figure 4](image-url)

**Figure 4.** The antibacterial activities of nanosilver-mycomeat synthesized from the tigernut waste against the selected clinical bacteria isolates (*Escherichia coli*, *Staphylococcus aureus*, *S. epidermidis*, and *Proteus* sp) using pin point inoculation method.
4 Conclusion

AgNPs was successfully synthesized from aqueous extract of fermented cassava pulp and tigernut aggregate waste. This study has showed that the extract obtained from cassava pulp and tigernut aggregate which are important agro waste, can be used for the biogenic, green and eco-friendly synthesis of AgNPs. The particles showed mild activities against multi-drug resistant clinical bacteria strains. Fermented nanosilver-mycomeat extract (FCNPs) synthesized from cassava pulp exhibited potent higher antioxidant activities compared to that prepared from tigernut aggregate waste (FKNPs).

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