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Original Research Article

Biogenic Synthesis and Primary Characterization of Silver Nanoparticles Using Aqueous Extract of Unripe Banana Peel; *In Vitro* Assessment on Antioxidant and Antibacterial Properties

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ABSTRACT

The incidence of many life-threatening diseases has been linked to oxidative stress and infectious organisms. The present work investigated the synthesis of silver nanoparticles (AgNPs) using unripe banana peel aqueous extract (UBPE) and assayed for its antioxidant property and antibacterial activity. AgNPs were synthesized from 1 mM $AgNO_3$ solution through the aqueous extract of unripe banana peel. The primary characterization of synthesized silver nanoparticles was done using UV-Visible spectroscopy. The antioxidant and antibacterial properties of the synthesized AgNPs were carried out using standard methods of analysis. The colourless reaction mixture of the $AgNO_3$ solution and UBPE turned yellowish brown and displayed UV-Visible spectra of 421 nm which is characteristic of silver nanoparticles. The antioxidant assay revealed that the synthesized AgNPs have a total phenol content of 70.64 ±0.27 mg GAE/g of extract and showed maximum scavenging activity against DPPH when compared to the standard with IC_{50} of 59.93 µg/mL. The present study also revealed that the AgNPs prepared from UBPE had an equal zone of inhibition as the standard antibacterial drug used against *E.coli* but showed a greater zone of inhibition of 37 mm than the standard drug with a zone of inhibition of 31 mm against *S. aureus*. AgNPs synthesized from UBPE could therefore be utilized in the management of life-threatening diseases caused by oxidative stress and harmful pathogens.

Keywords: Unripe banana peels, Silver nanoparticles, UV- visible spectroscopy, Antioxidant property, Antibacterial activity

Introduction

Oxidative stress is a consequence of the normal metabolic processes; generated during anabolic and catabolic processes such as oxidation of glucose, xenobiotic metabolic processes, immunologic control, etc.[1] Improvements in the methods used for sensing and determining the course of a disorder have identified that severe ailments progress from infectious agents and oxidative stress.[2,3] The incidence of conditions such as heart disease, disorder of kidney, lung and nerve including cancer has been related to oxidative stress.[4]*Staphylococcus aureus*and *Escherichia coli* are opportunistic pathogens of humans and food degradation implicated to cause menacing conditions such as osteomyelitis[5]and gastrointestinal disease,[6] respectively. In the increasing rates of life-threatening disease as a result of oxidative stress and infectious organisms, it is, therefore, necessary to find more natural agents or products of natural agents with antioxidant ability.

The investigation of substances on an infinitesimal and molecular scale is called nanotechnology. It entails processing of objects referred to as nanoparticles or devices sized between 1 to 100 nanometer in at least one dimension.[7]Nanoparticles are usually used as an important tool for *in vivo* drug delivery due to their microscopic nature.[8]Recent researches on the production, specifications, and usage of nanoparticles are provided by metal nanoparticles.[9-11]Amidst severally used metal nanoparticles, AgNPs have an impressive role due to their distinct physical and chemical features. Numerous methods have been developed to produce AgNPs such as physical, chemical and biological methods.[12]However, the physical and chemical methods require the use of toxic materials that pose danger to life.[13,14]These limitations posed by the physical and chemical synthesis can be sufficed by the biological approach. Many biologic systems, including algae, bacteria, yeast fungi, and the plant can convert inorganic metal ions into metal nanoparticles via the diminution effects and capping potentials of the proteins and metabolites present in these organisms.[15] However, the use of microorganisms and enzymes as possible natural alternatives for nanoparticle synthesis[16] is not without limitations as they involve an elaborated process of culturing and maintaining the cell.[17,18]Using extracts from plant for AgNPs synthesis is therefore preferable to the use of fungi and bacteria as plants are easily available, with little or no toxicity, eco-friendly, and are rapid in the reduction of silver ions.[19]In furtherance to the establishment of a green, valid and achievable process, the use of industrial wastes are encouraged to substitute plant parts.[20,21] Fruit peels (ripe and unripe) which are the by-product of fruit processing are usually discarded into the environment as agro-waste causing a menace to the environment. However, bioactive compounds present in these peels utilized with authentic methods can lead to the identification of potentially useful molecules which could be used to develop new, safe, and environmentally friendly drug product.Very few studies are available on the biosynthesis of silver nanoparticles from peel extracts[22] even though a lot of studies have been done on the

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biological synthesis of nanoparticles by using plant extracts.[23]In this study, we have reported the *in vitro* antioxidant and antibacterial ability of AgNPs synthesized from the aqueous extract of unripe banana peel.

Materials and Methods

Silver nitrate ($AgNO_3$, 99.995%) was purchased from Sigma Aldrich, USA. Unripe plantain was obtained from the local market.

Sample preparation
Fresh unripe banana peels were removed from the pulp using table knife. The peels were diced into small pieces and washed thoroughly with distilled water.

Extract preparation
About 25 g of the unripe banana peel was put in a beaker containing 100 ml distilled water. The peels were boiled in a water bath at 60℃ for 30 min, the aqueous extracts were separated by filtration with Whatman No. 1 filter paper (pore size 0.45 µm) and then centrifuged at 1000 rpm for 10 min.[24]

Synthesis of Silver nanoparticle
An aqueous solution of 1mM silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 mL of peel extracts of each sample was added into 90 mL of the aqueous solution of 1 mM silver nitrate for reduction into Ag^+ and kept for an incubation period of 24 h at 25°C.[25] Here the filtrate acts as reducing and stabilizing agent for 1 mM of $AgNO_3$.

Primary Characterization of Silver nanoparticles
The silver nanoparticle was characterized using a UV visible spectrophotometer. The scanning range for the sample was 300 – 600 nm.

Antioxidant Assay
The antioxidant activity was evaluated by determining the total phenol content and 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging activity of the synthesized AgNPs.

Total phenol content
Total phenol content was determined quantitatively using the FolinCiocalteu reagent with Gallic acid as the standard.[26]The phenolic content was calculated as gallic acid equivalents GAE/g of biosynthesized AgNPs based on a standard curve of gallic acid. All determinations were carried out in triplicate.

1,1-diphenyl-2-picryl hydrazyl radical scavenging assay
DPPH radical scavenging ability of the biogenic AgNP was evaluated following the method of Gloria *et al.*,[27]. The DPPH radical scavenging ability of the nanoparticles and standard (Ascorbic acid) were calculated as follows:
DPPH scavenging activity (%) =
[(ABScontrol – ABSsample)/(ABScontrol)] × 100

Where, ABScontrol is the absorbance of DPPH + methanol and ABSsample is the absorbance of DPPH + sample (Nanoparticles/standards).

Antimicrobial assay
The comparative antibacterial activities of the AgNPs from unripe banana peel and standard drug Gentamycin was assessed against *E.coli* and *S.aureus*. These organisms were obtained from the Molecular Biology Laboratory of the Department of Biological Sciences, Covenant University. The antimicrobial activities were carried out using Agar well diffusion method as described by Olugbemi.[28] Muller Hinton agar was poured into Petri dishes and allowed to solidify after which about 0.1 mL of standardized inoculums of the test organism was seeded on respective plates and wells of 9 mm diameter were bored using a cork borer. About 1000 µL of synthesized AgNPs and a disk of standard antibiotic; Gentamycin was used. Plates were incubated at 37ºC for 24 h and were inspected for the zone of inhibition. Gentamycin was used as positive control in this experiment.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Biosynthesized Silver Nanoparticles
The microtiter broth dilution method was done to evaluate the minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).
Statistical analysis
All experiments were done in triplicate and the data were expressed as the mean± S.E.M. IC_{50} values were determined by interpolation and Microsoft Excel 2007 was used for the statistical and graphical evaluations.

Results and Discussion

Silver nanoparticles (AgNPs) have received large interest for several uses especially as antimicrobial and antioxidant agents.[29-31]Nanoparticles employed for medicinal applications must be such that is biocompatible and is produced via protocols that are either non-toxic or of low-toxicity.[32] Silver nanoparticles was biosynthesized from aqueous extract of unripe banana peels. The synthesis was confirmed by colour changes from colourless to light brown due to the reduction of silver ions to silver nanoparticles.[33]
A lot of researchers have reported similar color changes ranging from light yellow, yellowish-brown, to dark brown to confirm the formation of AgNPs.[27,34-36] AgNPs appear brown in aqueous medium as a result of surface Plasmon vibrations.[28,37]

Characterization of Silver nanoparticles
The ultraviolet-visible (UV-Vis) spectrum of AgNPs (Figure 2) was recorded for the reaction medium at 24h interval and was found to be 421nm confirming the synthesis of silver nanoparticles.
It has been revealed that the plasmon absorption band due to the combined vibration of electrons of AgNPs in resonance with lightwave shows absorption spectra between 400-490 nm confirming the formation of spherical AgNPs.[38]This confirms further the synthesis of silver nanoparticles from aqueous extract of unripe banana peel. A similar absorption spectrum of 430 nm was reported by Aungkana*et al.*,[39] for the banana mediated synthesis of AgNPs.

Antioxidant activity of biosynthesized AgNPs
To determine the antioxidant activity of the biosynthesizedAgNPs (bAgNPs), the total phenol (TPC) content and DDPH radical scavenging assay were carried out.

Total phenol content
The mean total phenolic content of the biosynthesized AgNPs (bAgNPs) using unripe banana peel was found to be 70.64 ±0.27mg GAE/g of bAgNPs. This result is more than the TPC reported for AgNPs biosynthesized using ripe peel aqueous extract of the bananapeel which was discovered to be 66 mg GAE/g as reported by Susan *et al.*[40]

DDPH radical scavenging activity of bAgNPs
The bAgNPs showed a maximum scavenging activity against DPPH with a concentration-dependent scavenging activity with IC_{50} of 52.98 µg/mL when compared to the standard (Ascorbic acid) with IC_{50} of 59.93 µg/mL.

Antimicrobial activity of biosynthesized AGNPs
The antimicrobial activity of the biosynthesized AgNPs and standard drug (Gentamycin) against the test pathogens (*E.coli* and *S.aureus*) employed for this study is shown below; the antimicrobial activityresults revealed that AgNPs synthesized from aqueous extracts of unripe banana peels and Gentamycin, the standard drug used hadequal zones of inhibition of 30 mm against *E.coli.*

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However, a greater zone of inhibition of 37 mm was observed for the synthesized AgNPs than gentamycin with a zone of inhibition of 31 mm against *S. aureus*. The zones of inhibition observed in this study for AgNPs biosynthesized from the unripe banana peel is greater than the zones of inhibition observed for AgNPs of ripe banana peel and AgNPs of unripe plantain peel against *E.coli*and *S.aureus*as reported by Kokila *et al.*,[41] and Olugbemi,[34] respectively.

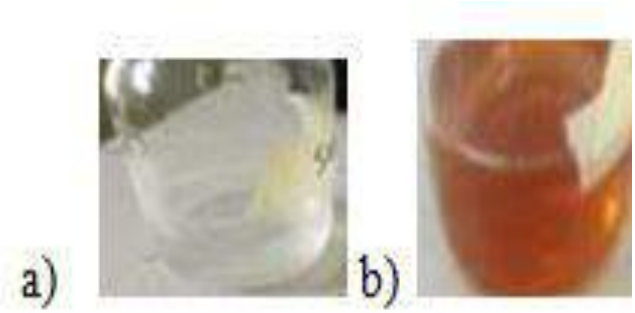

Figure 1: Unripe Banana peel extract **(a)** before and **(b)** after reaction with silver nitrate

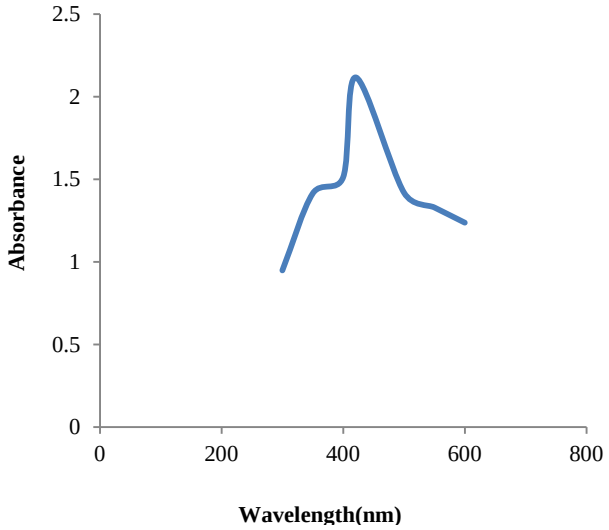

Figure 2: UV-Vis absorption spectra of silver nanoparticles fabricated from unripe Banana peel

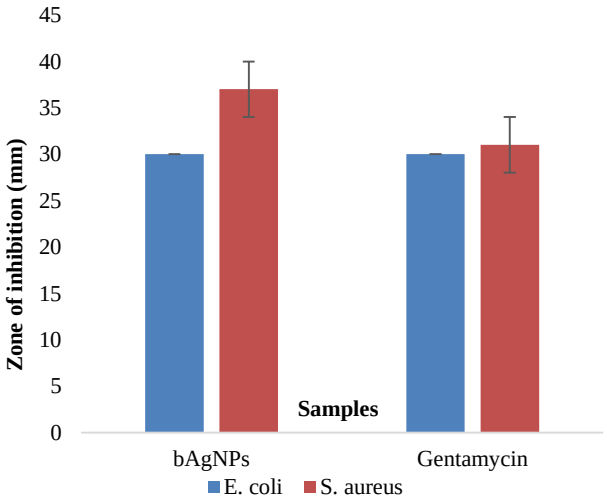

Figure 3: Zone of inhibition of samples (bAgNPs-Biosynthesized Silver nanoparticles)

Table 1: Table showing MIC and MBC of bAgNPs against *E. coli and S. aureus*

	E. coli	*S. aureus*
MIC($\mu g/ml$)	12.5	12.5
MBC $\mu g/ml$)	25	25

The result of MIC and MBC revealed that bAgNPs has the same inhibitory action and bactericidal potency against *E.coli* and *S.aureus*.

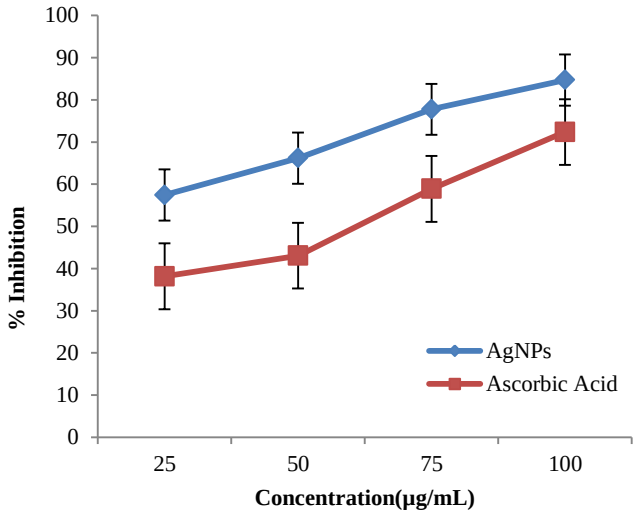

Figure 4: DPPH free radical scavenging power of synthesized AgNPs from Unripe Banana peel.

The data represent the percentage of inhibition of DPPH free radical scavenging. Each point represents the values obtained from three experiments, performed in triplicate (mean± S.E.M).

Conclusion

Based on the characterizations done, the biogenic method of synthesis employed in this study was successful for the preparation of AgNPs. The method utilized unripe banana fruit peels as a reductant and a capping agent in the synthesis of AgNPs through green chemistry. The biogenic method adopted for the synthesis of AgNPs in this study is considered economical, eco-friendly and should be utilized for the commercial synthesis of AgNPs with antioxidant and antimicrobial properties after proper pharmacological evaluation.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by her.

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