

Biosynthesis of Silver nanoparticles from aqueous extract of unripe plantain peel and its antibacterial assay: a novel biological approach

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Abstract

Biological method of silver nanoparticles synthesis has now become a novel method to physical and chemical approaches. In the present study, silver nanoparticles (AgNPs) were synthesized from unripe plantain peel extract (UPPE). This study examined the aqueous extracts of unripe plantain peel (sundried and air-dried) for the synthesis of Silver nanoparticles and compared their possible antibacterial potency with standard antibiotics: Ofloxacin and Ampicillin against human pathogens such as *Escherichia coli* and *Salmonella typhi*. The silver nanoparticles were prepared by reducing AgNO_3 with unripe plantain peel extract which act as natural reducing agent. The antimicrobial assay was carried out using Mueller Hinton Agar well. Synthesis of AgNps was confirmed by UV-Visible spectroscopy and plasmon peak maxima was observed at 425nm and 475nm for sundried and airdried samples (SD-AgNps and AD-AgNps) respectively at 24h interval. The antibacterial assay of the samples revealed higher antimicrobial activity for SDAgNps against *Escherichia coli* than Ofloxacin while ADAgNps showed antibacterial activity against *E. coli* and ampicillin showed none. SDAgNps showed a synergetic effect with the standard antibiotics (ofloxacin) against *Salmonella typhi*. Hence, the antimicrobial activities of the AgNps synthesized from aqueous extract of unripe plantain could be utilized in the management of infectious diseases due to their antimicrobial potency.

Keywords; Antimicrobial Activity, Silver nanoparticle (AgNp), Unripe Plantain Peel, UV-Visible Spectroscopy

INTRODUCTION

Nanotechnology involves the synthesis, characterization, fabrication, and manipulation of devices, or materials that have at least one dimension (or contain components with at least one dimension) that is approximately 1–100 nm in length. Particle size below this threshold has been found to have physical and chemical properties that are significantly different from the properties of macroscale materials composed of the same substance¹. In recent years the biosynthetic method of employing plant and plant waste extracts for the synthesis of metal nanoparticle has received some attention as a simple, ecofriendly and viable alternative to chemical procedures and physical². Silver nanoparticles in comparison to other metal nanoparticles has been reported to be non-toxic to humans and most effective against bacteria³. A very important aspect of recent nanotechnology research is the development of consistent processes for the synthesis of silver nano materials, one of which is the green synthesis⁴. Crude extracts from fruits could act as green reactant for silver nanoparticles synthesis⁵. Extensive work has been carried out on the biological synthesis of nanoparticles

by using plant extracts^{6,7,8}. However very few studies are available on the biosynthesis of silver nanoparticles from peel extracts^{9,10}.

Plants are an important source of food and energy for man.¹¹ *Musa paradisiaca* which is in the family of *Musaceae*, genus *Musa* and specie *paradisiaca* is a mono herbaceous perennial crop¹² that is an important staple food in Nigeria¹³. It has several nutritional values because it is rich in phyto-nutrients¹⁴ hence several delicacies are made from plantain fruits. The unripe fruits for instance are processed into flour and thickened into paste by stirring in boiling water. The ripe or unripe mature fruits can also be consumed boiled, steamed, pounded, roasted or fried into chips while the overripe plantains are fried with palm oil¹². Peel is the main by-product of the plantain processing industry and it represents about 30 % of the fruit¹⁵. Because the demand for processed and unprocessed plantain fruit (ripe and unripe) in Nigeria is high, this results in abundant waste generation in the form of plantain peels thereby constituting a menace to the environment, processing industries and pollution monitoring agencies^{15,16}. This problem can be recovered by utilizing its high value compounds in the development of potential new drugs. However, due to poor permeability, poor solubility, low bioavailability, etc, the delivery of herbal therapeutic molecules as drugs is challenging. These limitations can be overcome by attaching or encapsulating the herbal drugs with suitable nanomaterials which can enhance their pharmacokinetics and improve their performance to a great extent¹⁷. Report has shown that unripe plantain peel contains polymers such as lignin, hemicellulose and pectins⁴ which can be utilized in the synthesis of silver nanoparticles. Drug resistance among human pathogens as well as undesirable side effects of certain antimicrobial agents have been reported¹⁸. Products that can prevent pathogens from growing and shows only slight toxicity on host cells are being looked at as good candidates for the development of new antimicrobial drugs¹⁹.

2. Materials and Methods

2.1. Materials

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

2.2 Sample Preparation

The unripe plantain peels were removed using table knife, and the peels were diced into small pieces and washed thoroughly with distilled water. A portion of the peels were air-dried for 1 day while the other part was sundried for 5 days. The air-dried peels were chopped into smaller pieces using a clean knife whereas the sundried peels were ground into fine powder using an automatic electric blender.

2.2.1 Extract preparation

Chopped Air-dried (25g) and Powdered (25g) unripe plantain peel was weighed into separate beakers containing 100ml distilled water. The mixtures were boiled in a water bath at 60°C for 30min, filtered and centrifuged at 1000 rpm for 10 min¹⁰. The samples were labeled as sun-dried (SD) and air-dried (AD) and stored at -20°C

2.3 Synthesis of silver nanoparticle

Aqueous solution of 1mM silver nitrate (AgNO_3) was prepared and used for the synthesis of silver nanoparticles. AD (5 ml) and SD (5ml) unripe plantain peel extracts were added into 95 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag^+ and kept for incubation period of 24hr at room temperature. Here the filtrate acts as reducing and stabilizing agent for 1 mM of AgNO_3 to bring about the synthesis of AD-AgNps and SD-AgNps for air-dried and

sun-dried nanoparticles respectively⁵. One tube each was kept as a control without addition of 1mM AgNO₃ solution for both extracts.

2.4 Characterization of silver nanoparticles

The reduction of pure Ag⁺ ions was monitored by measuring the UV spectrum of the reaction medium after overnight incubation after diluting a small aliquot of the sample in distilled water. The silver nanoparticles synthesized were analyzed using UV-Visible spectrophotometer. The scanning range for the samples was 300-600 nm.

2.5 Assessment of antibacterial activity of silver nanoparticles/Extracts

Antibacterial activity of extracts and synthesized silver nanoparticles was tested against *Salmonella typhimurium*, *Escherichia coli*.

2.5.1 Test Pathogens

The bacterial pathogens employed in this work were obtained from the Molecular Biology Laboratory of Covenant University after proper identification by a certified Microbiologist, the chief technologist, Mr Emmanuel Omonigbeyin.

2.5.2 Preparation of Sample for Antimicrobial Assay

All four (4) samples were kept in the oven at 60^oC and allowed to dry completely¹⁹. The amounts of dried metabolites obtained from all samples were dissolved in Distilled water making extracts of different concentrations.

Table1-Different concentration of samples

S/No	Sample	Weight of Empty Bottle + Extract(g)	Weight of Empty Bottle(g)	Extract(g)	Distilled water (ml)	Concentration (mg/ml)
1	AD	224.60	224.40	0.20	15.00	13.33
2	SD	213.80	213.60	0.20	15.00	13.33
3	AD-AgNps	223.41	223.40	0.01	15.00	0.67
4	SD-AgNps	351.81	351.80	0.01	15.00	0.67

2.5.3 Antibacterial Assay

The antimicrobial potency of the samples was checked using Agar well diffusion method as described by Abhay et al ¹⁰, with appropriate modifications. In brief, Muller Hinton agar was poured into petri dishes. After solidification of nutrient agar plates, 20 µl of standardized inoculum of the test organism was seeded on respective plates and wells of 5mm diameter were bored using a cork borer. About 50 µl of extracts, synthesized AgNPs, a disk of standard antibiotic; Ampicillin and Ofloxacin, a quinolone (25µg) was used. Plates were incubated at 37 °C for 24hrs and was inspected for zone of inhibition. Ampicillin and Ofloxacin were used as positive controls in this experiment

3.RESULT AND DISCUSSION

Development of reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology⁹. The synthesis of silver nanoparticles through unripe plantain peel was confirmed by the color change of the solution after 24hrs of incubation viz., light yellow in air-dried sample and brownish-orange color in

sundried sample (Figure 1-2). Report has shown that silver nanoparticles exhibit striking color of yellow to brown due to the excitation of surface plasmon vibration in the particles and that the peak value between 400 -500 nm ranges indicates the presence of silver nanoparticles by UV- Visible spectrophotometer analysis²⁰. The peak value for SD-AgNps and AD-AgNps was found to be 427nm and 488nm respectively as shown in (Figure 3-4).

Synthesis of nanosized particles with antibacterial properties is of great interest in the development of new pharmaceutical products²⁰. The silver nanoparticles synthesized from aqueous extract of unripe plantain peel showed good antibacterial activity against the two bacterial strains used. The antibacterial activity was confirmed on the basis of zone size exhibited by standard antibacterial agent Ofloxacin and Ampicillin against *E.coli* and *Salmonella typhi* are gram -ve bacteria and are food borne pathogens. Many common bacterial infection such as cholecystitis, cholangitis, bacteremia, urinary tract infection, traveler's diarrhea and other clinical infections such as neonatal meningitis and pneumonia are caused by *E.coli*²¹. *Salmonella* like *E.coli* causes the life threatening infection called typhoid fever in humans²². Ofloxacin is a quinolone which is known to be active against gram +ve and gram -ve bacteria whereas Ampicillin is a gram -ve antibacterial agent. Report by Abhay and Rupa¹⁰, confirmed that infectious diseases which is the leading cause of death worldwide is as a result of multidrug resistant strain of bacteria. Our findings showed that no zone of inhibition was observed for AD and SD unripe plantain peels (normal control) against the pathogens used whereas SD-AgNps had a zone of inhibition of 13mm and 15mm against *E.coli* and *Salmonella typhi* respectively while Ofloxacin had a zone of inhibition of 10mm and 22mm against *E.coli* and *Salmonella typhi* respectively. The result also revealed a synergetic effect between SD-AgNps and the standard antibiotics (Ofloxacin) showing a zone of inhibition of 44mm against *Salmonella typhi*. Ampicillin did not inhibit the growth of *E.coli* whereas the AD-AgNps had a zone of inhibition of 12mm against *E.Coli* (Figure 5). This result confirmed the report by Kim et al.,²³ that AgNPs has a broad antibacterial effect on a range of Gram-negative and Gram-positive bacteria and antibiotic-resistant bacteria strains.

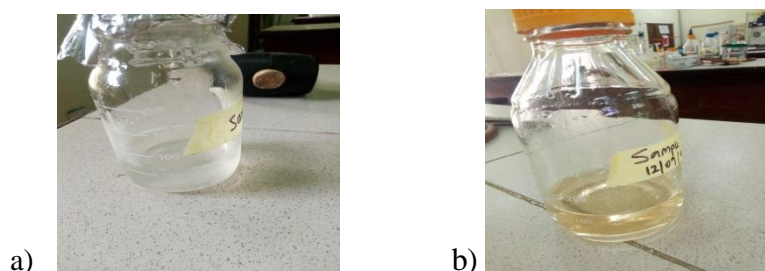


Figure 1- a) Clear Aqueous extracts of air-dried unripe plantain peel extract (AD) and b) Solution of synthesized air-dried unripe plantain peel nanoparticle (AD-AgNps)

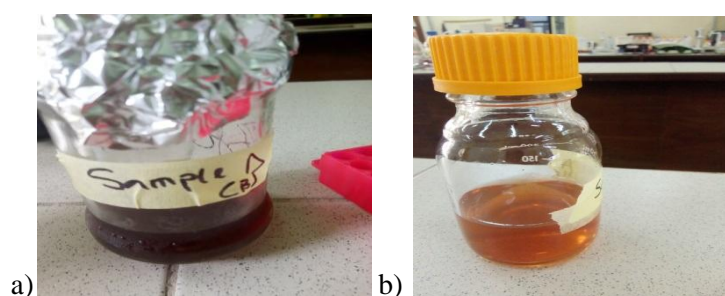


Figure 2- a) Clear Aqueous extract of sundried unripe plantain peel extract (SD) (b) Solution of synthesized sun-dried unripe plantain peel nanoparticle (SD-AgNps)

Figure 3:UV-Vis Characterization of AgNPs:airdried unripe plantain peel extract

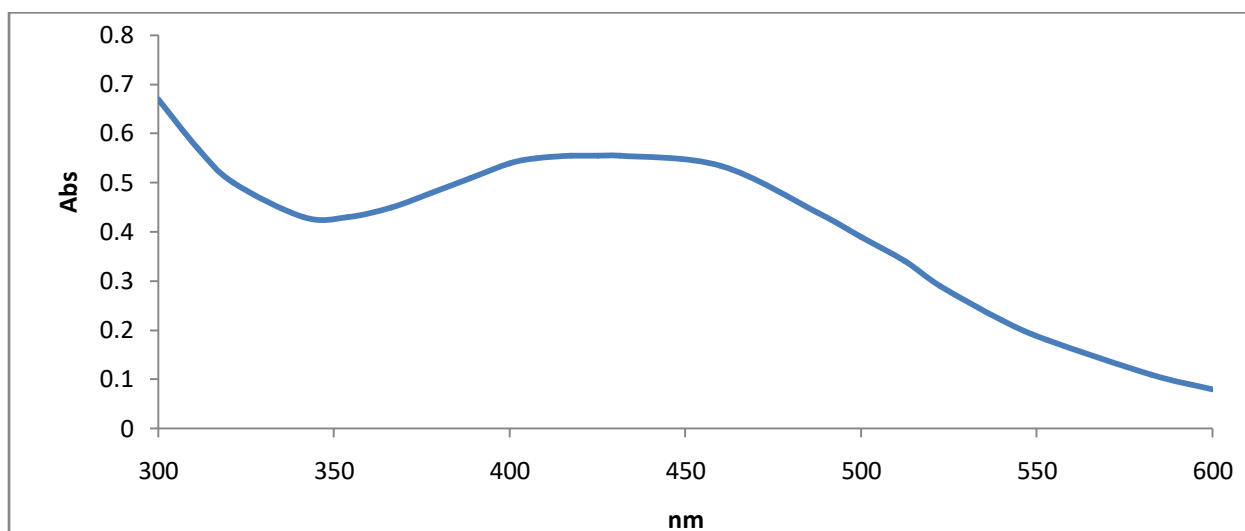


Figure 4:UV-Vis Characterization of AgNPs :sundried unripe plantain peel extract

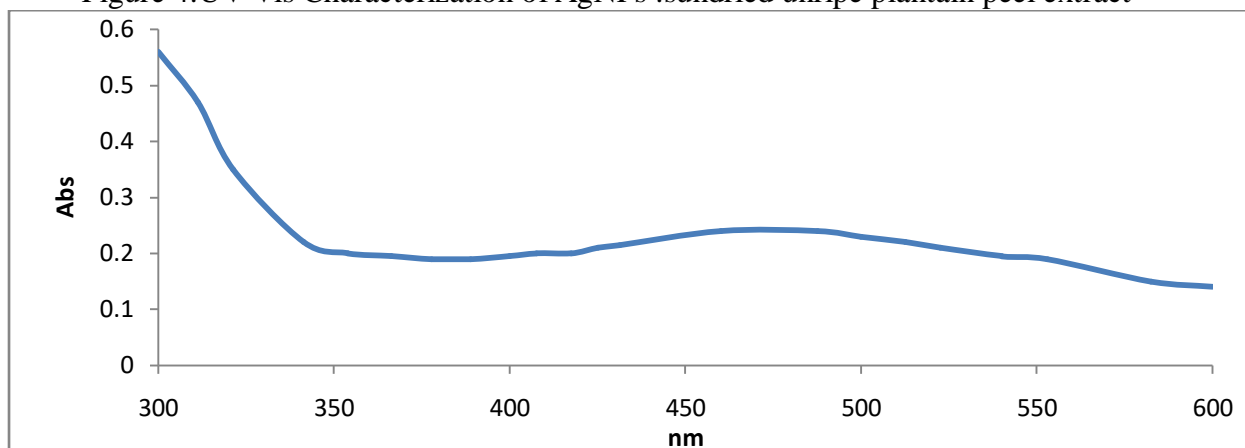
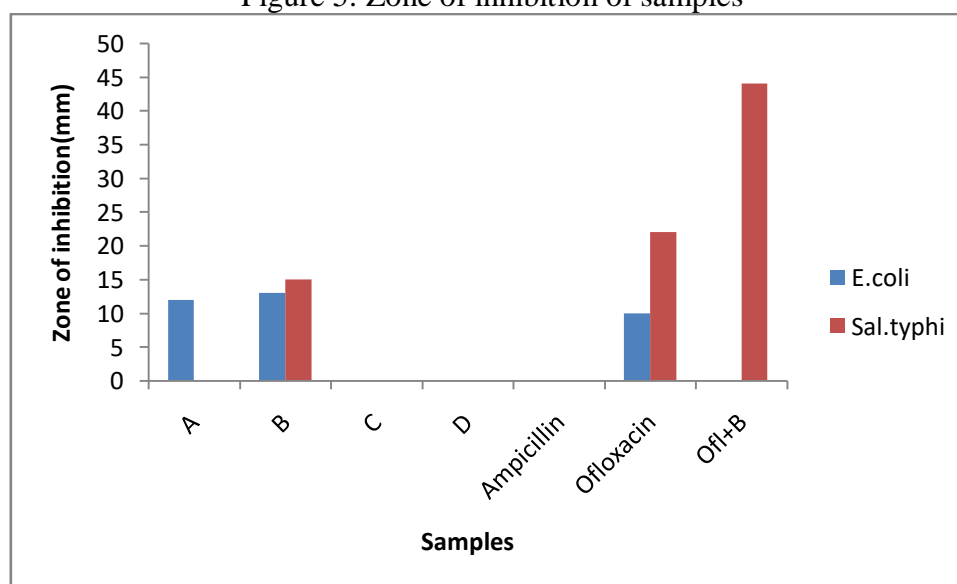


Figure 5: Zone of inhibition of samples



A-AgNps; Airdried unripe plantain peel extract, C-Airdried Sample extract
B-AgNps; Sundried unripe plantain peel extract, D-Sundried sample extract

CONCLUSION

The present study is an initial work on the biosynthesis of silver nanoparticles from aqueous extracts of airdried and sundried unripe plantain peel. Based on the above research work it can be concluded that AgNps synthesized from aqueous extract of unripe plantain peel could be used as an effective drug against pathogenic diseases after proper pharmacological evaluation.

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