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Comparative Antibacterial Activity of Biosynthesized Silver Nanoparticles and Aqueous Extract of Unripe Pawpaw Peels Against *E.coli*

Temitayo I. Adesipe[1,2]* and Abiodun H. Adebayo[1,3]

[1]*Dept. of Biochemistry, College of Science and Technology Covenant University, Ota, Ogun State, Nigeria*
[2]*Dept. of Science Laboratory Technology, Federal Polytechnic Ilaro,Ogun state, Nigeria*
[3]*Vice Chancellor, Covenant University ,Ota Ogun State, Nigeria*

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ABSTRACT

One of the most important issues of current medicine is bacterial drug resistance as well as uropathogenic tract infections and the primary factor of these issues are extraintestinal pathogenic *E. coli* strains. This study examined and compared the antibacterial activity of unripe pawpaw peel aqueous extract (UPPA), biosynthesized silver nanoparticles (b-AgNPs) and Ampicillin (Positive control) against *E.coli*. AgNPs was prepared by reducing 1mM aqueous $AgNO_3$ solution with UPPA. Primary characterization of synthesized AgNPs was carried out using UV-visible spectroscopy. The total phenol content (TPC) and antibacterial activities of the samples (UPPA and AgNPs) were carried out following standard analytical methods. Synthesis of AgNPs was confirmed by a color change of the reaction mixture to brown after 24hrs of incubation at room temperature, UV-visible spectroscopy also revealed an absorption maxima at 450 nm which is characteristic of AgNPs. The result of this study shows that the biosynthesized AgNPs contains a higher amount of total phenolics and consequently showed a greater antibacterial activity than the unripe pawpaw peel extract alone based on the zone of inhibition exhibited by both samples on the test organism used. However , no zone of inhibition was exhibited by the standard drug (Ampicillin) against the test organism. This study therefore indicates that the antibacterial performance of aqueous extract of unripe pawpaw peels in combating drug resistant pathogenic organisms could be improved to a great extent if incorporated for the synthesis of AgNPs.

Keywords: Silver nanoparticles, Unripe pawpaw peels, Total Phenol content, Uropathogenic *E. coli*.

Introduction

Uropathogenic *E.coli* (UPEC) is a common pathotype of extraintestinal pathogenic *E.coli* (ExPEC) which greatly emanates from intestinal microbiota.[1]ExPEC has been implicated to cause bacteremia in older adults, meningitis in neonates and the majority of urinary tract infections (UTIs) in young healthy women.[2] Estimation shows that 40-50% of women and 5% of men will develop a UTI in their life time.[3] However, an increase in morbidity and mortality rates caused by UTI are due to untreated or mistreated UTI which results in kidney and systemic infections .[1] Also the extended usage of last-line antibiotics as a result of recurrence of infection ultimately causes resistance to antibiotics.[4] This rise in antibiotics resistance of ExPEC has hindered the effective management of UTI.[5] The tactics used by this pathogenic bacteria to evolve antibiotics resistance includes active efflux of drugs, variation of target sites and degradation of enzymes .[6] Since medicinal plants contain a wide variety of secondary metabolites, they are being promoted as alternate source of antimicrobials and resistance modifying agents.[7-9] Recently, phenolic compounds found in plant has been implied to show significant antimicrobial activity,[10-12] and also have the ability to attack multidrug resistant bacteria.[13,14] For example, an aqueous extract of unripe pawpaw peel has been reported to contain phenolics[15] and consequently exhibit a broad range of antibacterial properties[16]However because of poor solubility, low bioavailability and poor permeability, the delivery of herbal therapeutic molecules as drugs is challenging. These limitations can therefore be overcome by attaching or encapsulating the herbal drug with suitable nanomaterials; nutrient delivery systems through bioactive nanoencapsulation,which will further enhance the pharmacokinetics of the medicinal plant and improve their performance to a great extent.[17-19]A nanoparticle (or nanopowder or nanocluster or nanocrystal) is a microscopic particle with at least one dimension less than 100nm.[20-22] The recent researches on the synthesis, characterization, and applications of nanoparticles are provided by metal nanoparticles,[23,24] especially silver nanoparticles because they have unique features and have been shown to be non- or less toxic towards human when compared to other types of metal nanoparticles.[25,26] Several methods of nanoparticle synthesis including physical, chemical and biological methods are either being developed or improved in order to enhance their properties and reduce the production costs .[27, 28]However, green synthesis; biological method of synthesizing nanoparticles which uses plants extract as reducing and capping agents are more advantageous than other methods of synthesis because it is a single-step method and it is human friendly.[29-31] Recently, as a further step towards the development of greener and more sustainable processes, attempts have been made to replace plant parts with agro industrial wastes.[32,33]Biogenic silver NPs has been reported to have a wide spectrum of applications as an antimicrobial agent and for drug delivery .[34]In the present study, aqueous extracts of unripe pawpaw peels were used for a simple, low-cost and green method of AgNPs synthesis. The prepared nanoparticles were characterized primarily by UV-Visible spectroscopy and the

*Corresponding author. E mail: iyanu.adesipe@federalpolyilaro.edu.ng
Tel: +2347066025250

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antibacterial activity against *E. coli* was compared to the aqueous extract of unripe pawpaw peels.

Materials and Methods

Unripe pawpaw was first obtained from local market in April 2018. All chemicals and reagent used were of analytical grade

Sample preparation

The peels were removed using table knife, washed thoroughly with distilled water and diced into tiny pieces.

Extract preparation

The aqueous extract of the peel was prepared according to the method of Abhay and Rupa[35] and was denoted as UPPA-unripe pawpaw peel aqueous extract. About 25 g of the unripe pawpaw peel was weighed into a beaker containing 100 mL distilled water and heated in a water bath at 60^{o}C for 30min.The aqueous extracts were separated by filtration with Whatmann No. 1 filter paper (pore size 0.45 µm) and then centrifuged at 1000 rpm for 10 min .

Synthesis of silver nanoparticles

The modified method of Nooshin*et al.*,[36]was adopted for the green synthesis of silver nanoparticles from UPPA. In brief 10 ml of aqueous unripe peel extract was added into a flask containing 90 ml of 1 mM silver nitrate ($AgNO_3$) solution and was allowed to incubate for 24hrs at room temperature .

Characterization of silver nanoparticles

After overnight incubation at room temperature, the reduction of pure Ag^+ ions was monitored by measuring the UV spectrum of the reaction medium within the range of 300 – 600 nm range.

Determination of total phenol content

Total Phenol Content was determined quantitatively using the Folin Ciocalteu reagent with Gallic acid as the standard .[37,38] The phenolic content was calculated as Gallic acid equivalents GAE/g of samples on the basis of a standard curve of Gallic acid. All determinations were carried out in triplicate.

Comparative antibacterial activity of extract and biosynthesized AgNPs

Preparation of Samples for Antimicrobial Assay

The test organism employed in this study was obtained from the Molecular Biology Laboratory of Covenant University. UPPA (unripe pawpaw peel aqueous extract) and biosynthesized Silver nanoparticles (b-AgNPs) were oven-dried at 60 ^{o}C.[18]The resultant dried extracts were dissolved separately in Phosphate Buffer Saline (PBS) to make a concentration of 4.7 g/mL solution each.

Antibacterial assay

The antibacterial potency of the samples were accessed using Agar well diffusion method as described by Abhay and Rupa,[35]with appropriate modifications. In brief, pre-sterilized Mueller Hinton agar was dispensed into petri dishes and allowed to solidify. After solidification of the agar plates, 20 µl of standardized inoculum of the test organism (*Escherichia coli.*) was seeded on agar plate and wells of 5mm diameter were bored using a cork borer. About 50 µl of UPPA and b-AgNPs were loaded into the wells.
Plates were incubated at 37 ºC for 24 hrs, and was inspected for Zone of inhibition. The zone of inhibition was compared with that formed by Ampicillin.

Statistical analysis

All experiments were done in triplicate and expressed as the mean± S.D. Graphical representation was done using Microsoft Excel 2007 package.

Results and Discussion

Addition of the UPPA to aqueous $AgNO_3$ solution resulted in a color change of the reaction mixture to colloidal brown indicating AgNP formation (b-AgNPs) as shown in Figure 1.
Previous studies have reported that AgNPs can be biosynthesized via peels such as Onion peels ,[39,40] Orange peels,[32] Black pomengranate peels ,[41]Canvedish banana peel ,[42]unripe plantain peel,[29] and ripe pawpaw peels .[43] In this study, AgNPs was synthesized by reducing aqueous silver ions using UPPA after incubation at room temperature for 24 h in the dark. The colour of the reaction mixture turned brown and this color change has been previously reported by several researchers to indicate the formation of AgNPs .[18,29,44,45,46]These authors mentioned that the color change is as a result of the surface plasmon resonance of precipitated AgNPs.

Characterization of Silver Nanoparticles

The ultraviolet-visible (UV-Vis) spectrum of AgNPs (Figure 2) was recorded from the reaction medium at 24 h interval. The AgNPs from the unripe pawpaw peel extracts gave absorbance peak of 450 nm. The peak due to silver ion at 300 nm was found missing.
The most useful characterization pertinent to the synthesis of silver nanoparticles has been established to be UV-visible spectroscopy.[47,48]
The biosynthesized AgNPs in this study was primarily characterized using UV-Vis spectrophotometer and an absorption peak of 450 nm was observed, which is typical of AgNPs as they usually display a surface Plasmon resonance band ranging from 400-550 nm .[18,49,50,51]An absorption spectra of 450 nm has been reported for several biosynthesized AgNPs.[44,52,53]

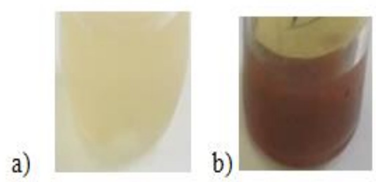

Figure 1: Unripe pawpaw peel aqueous extract before(a) and after (b) reaction with silver nitrate.

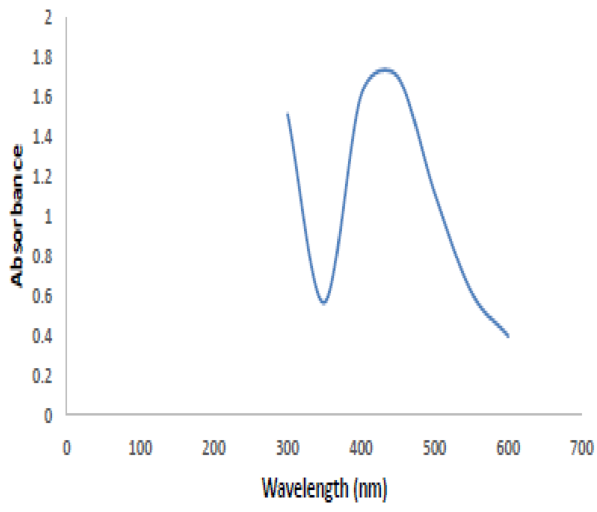

Figure 2:UV-Vis spectrum of AgNPs synthesized from aqueous extract of unripe pawpaw peels

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Total phenolic content

The total phenolic contents of the unripe pawpaw peel extract (UPPA) and biosynthesized AgNPs (b-AgNPs) were expressed as mg of Gallic acid (GAE)/ g of extract as shown in table 1 below;

The result of total phenolic content (TPC) of peel extract alone or AgNPs showed that TPC was higher in the biosynthesized AgNPs (85.30 ± 0.57mgGAE/g) compared to the aqueous peel extract alone (23.95 ± 0.57mgGAE/g). Similar to this result, Sepideh*et al.*,[54] Abdel-Aziz *et al*.,[55] and Sultana *et al*.,[56] reported a higher total phenol content in synthesized AgNPs compared to the *Prosopis farcta* fruit extract, Chenopodiummurale and Houttuyniacordata leaf extracts respectively.

Antimicrobial Activities of Samples against E.coli

The antimicrobial activity of UPPA, b-AgNPs and standard antimicrobial drug, Ampicillin against pathogenic *E.coli* strain is shown in Figure 3 below;

The antimicrobial results revealed that AgNPs synthesized using aqueous extract of unripe pawpaw peel had a greater antibacterial activity with zone of inhibition of 36 mm against *E.coli* when compared with the peel extract alone with zone of inhibition of 27 mm. However, the standard antibacterial drug (Ampicillin) showed no zone of inhibition against the test pathogen. The result presented in this study validates that AgNPs have a broad antibacterial effect on antibiotic-resistant bacteria strains.[57] Contrary to this result, Muhammad *et al.*,[58]in his study reported that Callus extract of *F. indica* showed no antibacterial activity against *E.coli*, even though the Callus extract mediated AgNPs showed significant antibacterial activity against *E.coli* when compared to Ciprofloxacin. However, in this present work, the greater antibacterial activity exhibited by AgNPs synthesized using unripe pawpaw peel extract against *E.coli* could be as a result of capped phenolic compounds. Phenolic group has been implicated to enhance the conversion of silver nitrate to AgNPs due to its electron donating ability .[54,59]

Table 1: Total phenolic content of extracts of unripe pawpaw peel and biosynthesized AgNPs

Samples	**Phenolic contents (mgGAE/g)**
b-AgNPs	85.30 ± 0.57
UPPA	23.95 ± 0.57

Values are expressed as mean ± SD, n = 3

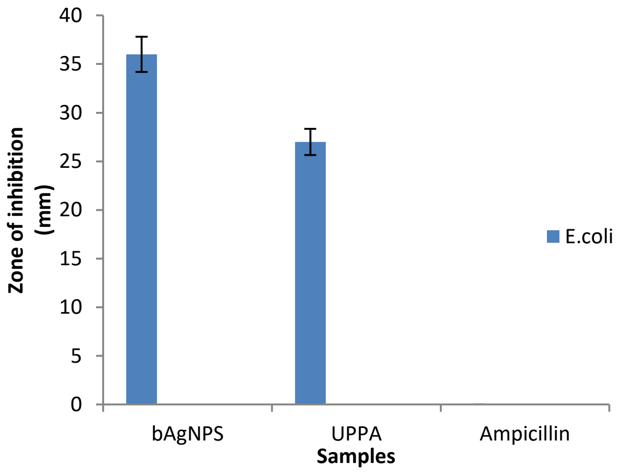

Figure 3: Antibacterial activity of b-AgNPs, UPPA and Ampicillin against *E.coli*

Conclusion

This study has revealed that silver nanoparticles can be synthesized in a simple and cost effective method using aqueous extract of unripe pawpaw peel. The total phenolic compound was higher in b-AgNPs compared to the pawpaw peel extract alone. Ampicillin showed no antibacterial activity against *E. coli* whereas, b-AgNPs showed a higher antibacterial activity against *E. coli* compared to the peel extract alone, indicating that the antimicrobial performance of aqueous extract of unripe pawpaw peels can be improved to a great extent if incorporated for the synthesis of AgNPs.

The results outlined in this study are novel, thus unripe pawpaw peel mediated AgNPs could emerge as an alternative to combat extraintestinal pathogenic *E. coli* resistant strains after proper pharmacologic evaluations.

Conflict of interest

The authors declare no conflict of interest.

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 them.

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