GREEN SYNTHESIS OF SILVER NANOPARTICLES FROM AQUEOUS EXTRACT OF UNRIPE PAWPAW PEEL AND ITS ANTIMICROBIAL AND ANTIOXIDANT PROPERTY

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ABSTRACT

Green synthesis is considered an alternative approach for synthesizing silver nanoparticles (AgNPs) as it is eco-friendly and cost effective.The present work investigated the synthesis of silver nanoparticles using unripe pawpaw peel extract (UPPE) and assayed for its antimicrobial and antioxidant properties.
Method:AgNps were synthesized from 1 mM $AgNO_3$ solution through the aqueous extract of unripe pawpaw peel. Primary characterization of synthesized silver nanoparticles was carried out using UV-visible spectroscopy. The antimicrobial and antioxidant activities of the synthesized AgNps was investigated using standard methods of analysis. The colourless reaction mixture of $AgNO_3$ solution and aqueous extract of unripe pawpaw peel turned brown and displayed UV-visible spectra of 450nm which is characteristic of silver nanoparticles . The present study revealed that silver nanoparticles prepared from unripe pawpaw peel extract showed greater antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Aspergillus niger* which are common food related pathogens than Gentamycin. The antioxidant assay also revealed that maximum scavenging activity against DPPH was exhibited by the synthensized nanoparticles which showed a concentration-dependent scavenging activity with IC_{50} of 54.78 µg/ml when compared to the standard with IC_{50}: 59.93 µg/ml. Ascorbic acid exhibited the highest scavenging activity against ABTS with (IC_{50}: 50.95 µg/ml), followed by the synthensized AgNps (IC_{50}: 52.20 µg/ml).optimization of this green synthesis would support the production of agnps with great therapeutic potentials.

Abbreviations:ABTS-2: 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); DPPH: 1-diphenyl-2-picrylhydrazyl**.**

Keywords: Antimicrobial, Antioxidant, Uv visible, Silver nanoparticles, Unripe pawpaw peel

INTRODUCTION

Any microscopic particle with at least one dimension less than 100nm is known as nanoparticle. Nanoparticle research is inevitable because of its application and synthesis (Gopinath *et al*., 2012). They are applied in catalysis bio-sensing, imaging, drug delivery, nano-device fabrication and in medicine of due to their catalytic, optical, magnetic, as well as biological properties (Jain, Huang, El-Sayed, & El-Sayed, 2008). The use of environmentally friendly substances that does not require the utilization of toxic chemical materials for the synthesis of nanoparticles is beneficial for pharmaceutical and biomedical applications (Jain *et al.,* 2009: Reenaa & Aathira, 2017). The use of microorganisms and enzymes have been suggested as possible natural alternatives for nanoparticles synthesis (Mohanpuria, Rana, & Yadav, 2008), however they involve elaborated process of culturing and maintaining of the cell (Saxena, Tripathi, Zafar, & Singh, 2012). Reports has shown that green synthesis which uses plants extract as reducing and capping agents are more advantageous because it involves a single-step method for the nanoparticle biosynthesis process and it is human friendly (Ana-Alexandra, Alexandrina, Rodica-mariana, & Ioana-Raluca, 2016; Valli and Vaseeharan, 2012 ; Kumar & Yadav, 2009). Several works has been carried out on the biological synthesis of nanoparticles by using plant extracts (Prathibha, Packiyam, Bhat, Jayadev & Shetty, 2015: Kirubha & Alagumuthu 2015), but very few studies are available on the biosynthesis of silver nanoparticles from peel extracts (Kokila, Ramesh, & Geetha, 2015: Reenaa & Aathira, 2017) especially from unripe peels. Silver nanopaticles has been recognized all over the world because it is considered nontoxic to human when compared to other metal nanoparticles (Rai, Yadav, & Gade, 2009). Silver nanoparticles (AgNPs) because of its large number of applications such as in optics (Sivanesan et al., 2011), selective coatings for solar energy absorption (Liu , Qu , Zhang, Tan, & Wang , 2013), biolabeling catalysts (Zhang, Shao, & Zhang , 2011), antimicrobial and antioxidant agents (Otunola,

Afolayan, Ajayi, &Odeyemi, 2017) owing to their unique properties have been receiving broad interest. This study describes the biofabrication of Silver nanoparticles from fruit waste materials especially unripe pawpaw fruit peel extracts. Characterization of Silver nanoparticles was done by using UV- visible spectroscopy, which gives a preliminary confirmation of Silver nanoparticles. An attempt has been made to compare the antibacterial and antioxidant activity of the synthesized Silver nanoparticles prepared to standard drugs.

MATERIAL AND METHODS

Materials

Fresh unripe pawpaw was obtained from (Federal Polytechnic Ilaro Farm). All chemicals used were of analytical grade. The standard cultures of microorganisms used was obtained from Molecular Biology laboratory, Covenant University.

Preparation of Unripe Pawpaw Peel Extract (Uppe)

Pawpaw peels were cut into small pieces (< 5 mm), washed three times with tap water and three times with distilled water to remove external dirt impurities present in it. The peels were dried on paper toweling. About 25 g of peel was kept in a 100 ml beaker containing 100 ml distilled water and boiled at 60 ^{0}C for 30 min. The extract was filtered through Whatman no. 1 filter paper and centrifuged at 1000 rpm for 5 minutes to remove insoluble fractions and macromolecules. It was stored at 4°c for further use.

Green Synthesis

The method employed by Olugbemi, 2019 was utilized. In brief, 90 ml of 1mM $AgNO_3$ solution was added to 10ml of extract and it was kept under dark condition for 24 hours at room temperature. The formation of silver nanoparticles was confirmed by color change.

Characterisation of Silver Nanoparticles

The synthesized AgNPs were monitored using UV--vis spectrophotometer between the range of 300nm and 600nm.

Antimicrobial Activity

The antimicrobial potency of biosynthesized AgNps was tested against *Escherichia coli* (gram negative bacteria*), Staphylococcus aureus* (gram positive bacteria), *Aspergillus niger (fungus)*using Agar well diffusion method as described by Abhay and Rupa (2016) with appropriate modifications. Muller Hinton agar was poured into petri dishes. After solidification of nutrient agar plates, 0.1ml of standardized inoculum 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 24hrs and was inspected for zone of inhibition. Gentamycin was used as positive control in this experiment.

Antioxidant Assay

The antioxidant activities of the biosynthesized AgNPs was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzthiazoli ne-6-sulfonic acid) (ABTS) assays.

2, 2-Azino-Bis (3-Ethylbenzthiazoline-6-Sulfonic Acid) (Abts) Assay

ABTS scavenging activity was determined according to the method described by Gloria, Anthony,Emmanuel, &Samuel, (2017).. The stock solutions consisting of 7 mm ABTS solution and 2.4 mm Potassium persulfate solution were prepared. the working solution was prepared by mixing the two stock solutions in equal proportions (1:1 v/v) and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS + solution with 60 ml of methanol to obtain an absorbance of 0.708 ± 0.001 units at 734 nm using the spectrophotometer. A volume of 100 µl of methanol was added into all the wells with the exception of second (b) and third (c) rows. Exactly 200 µl of the nanoparticles (0.5 mg/ml) or

standards (Ascorbic acid) prepared in methanol were added in triplicates to the third row (c). A 2-fold serial dilution was done by mixing the contents in each well of the third row (starting from the first column) and transferring 100 µl into the second well of the same column, and the procedure was repeated up to the 7th well of the same column, and the last 100 µl from the 7th well was discarded. a 2-fold dilution yielding concentrations of the plant extracts and standards ranging from 0.01 to 0.5 µg/ml was thus prepared in the wells. The AgNPs (100 µl) and the control were allowed to react with 100 µl of the ABTS + solution, and the absorbance was measured at 734 nm after 7 min using the spectrophotometer. The ABTS of the AgNPs was then compared with that of the standard, and the percentage inhibition was calculated as follows:

$$\text{ABTS SCAVENGING ACTIVITY (\%)} = [1-(ABS_{SAMPLE})/(ABS_{CONTROL})] \times 100$$

Where, $ABS_{control}$ is the absorbance of ABTS radical + methanol and ABS_{sample} is the absorbance of ABTS radical + sample(Nanoparticles/standard).

1,1-Diphenyl-2-Picryl Hydrazylradical Scavenging Assay

For the DPPH radical scavenging ability a stock solution of 0.135 mm DPPH radical was prepared in methanol. A volume of 100 µl of methanol was added into all the wells with the exception of second (b) and third (c) rows. Exactly 200 µl of the nanoparticles (0.5 mg/ml) or standards (Ascorbic acid) prepared in methanol were added in triplicates to the third row (c). A 2-fold serial dilution was done by mixing the contents in each well of the third row (starting from the first column) and transferring 100 µl into the second well of the same column, and the procedure was repeated up to the 7th well of the same column, and the last 100 µl from the 7th well was discarded. A 2-fold dilution yielding concentrations of nanoparticles and standards ranging from 0.01 to 0.5 mg/ml was thus prepared in the wells. The reaction mixture was then vortexed thoroughly and left in dark at room temperature for 30 min, after which the absorbance was measured in a spectrophotometer at 517 nm (Gloria et al., 2017). The DPPH radical scavenging ability of the nanoparticles was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(ABS_{control} - ABS_{sample})/(ABS_{control})] \times 100$$

where, $ABS_{control}$ is the absorbance of DPPH + methanol and ABS_{sample} is the absorbance of DPPH + sample (Nanoparticles/standards).

RESULTS AND DISCUSSION

Results

Synthesis and Characterization of Silver Nanoparticles

Addition of the spice extracts to aqueous $AgNO_3$ solution resulted in changes of color of the mixtures from faint yellow to colloidal brown indicating AgNP formation.

Figure 1: Unripe pawpaw peel extract before and after reaction with silver nitrate

The ultraviolet-visible (UV-Vis) spectrum of AgNPs (Figure 2) was recorded from the reaction medium at 24h interval. The AgNPs from the unripe pawpaw peel extracts gave absorbance peak of 450nm. The peak due to silver ion at 300 nm was found missing.

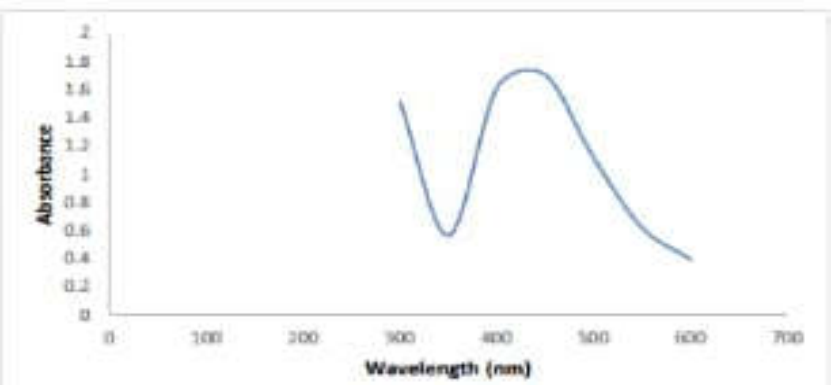

Figure 2: Ultraviolet-visible spectra of silver nanoparticles after 24hours of incubation

The UV-Vis spectra also revealed that the AgNPs formed rapidly and remained stable even after 24h.

Antimicrobial Activities of Silver Nanoparticles

The antibacterial and antifungal activities of the AgNPs was evaluated by Agar dilution method using Mueller–Hinton agar. Agar dilution is one of the most commonly used techniques for determining antimicrobial activities.

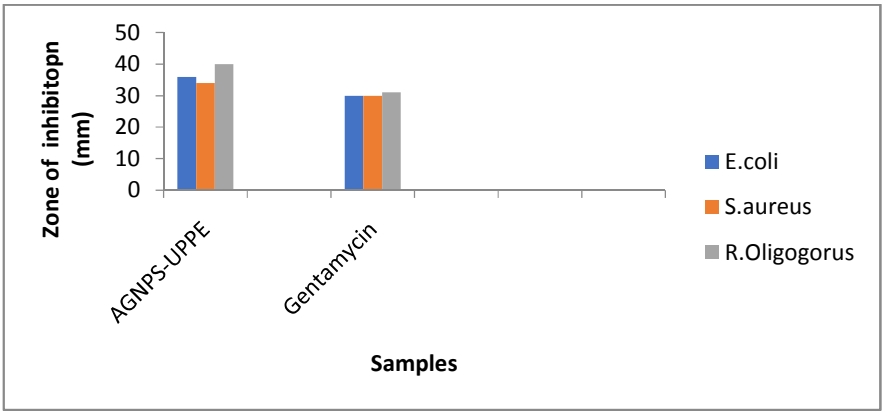

Figure 3: Antibacterial activity of silver nanoparticles against two bacterial isolates and one fungi isolate.

Antioxidant Activity of Silver Nanoparticles

The antioxidant activity of synthesized AgNps was evaluated by DPPH and ABTS radical scavenging assay, and Ascorbic acid was used as a positive control. The AgNps exhibited potential free radical scavenging activity against both radicals as shown in Figure 4 & 5. The maximum scavenging activity against DPPH was exhibited by the synthensized nanoparticles which showed a concentration-dependent scavenging activity with IC_{50} of 54.78 µg/mL when compared to the standard with IC_{50}: 59.93 µg/mL. Ascorbic acid exhibited the highest scavenging activity against ABTS with (IC_{50}: 50.95 µg/mL) while the synthesized AgNps (IC_{50}: 52.20 µg/mL).

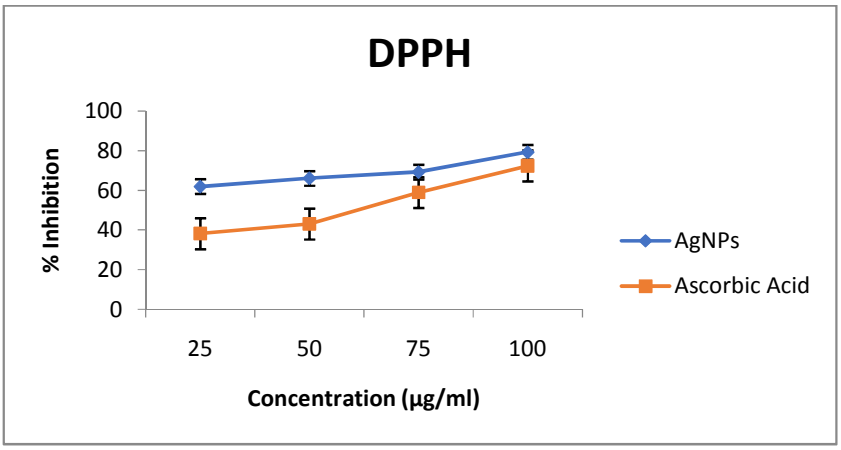

Figure 4- Chart of DPPH free radical scavenging of Synthesized AgNPs and Ascorbic Acid(control).

The data represent percentage inhibition DPPH free radical scavenging activity. Each point represents the values obtained from the experiment performed in triplicate (mean± SEM)

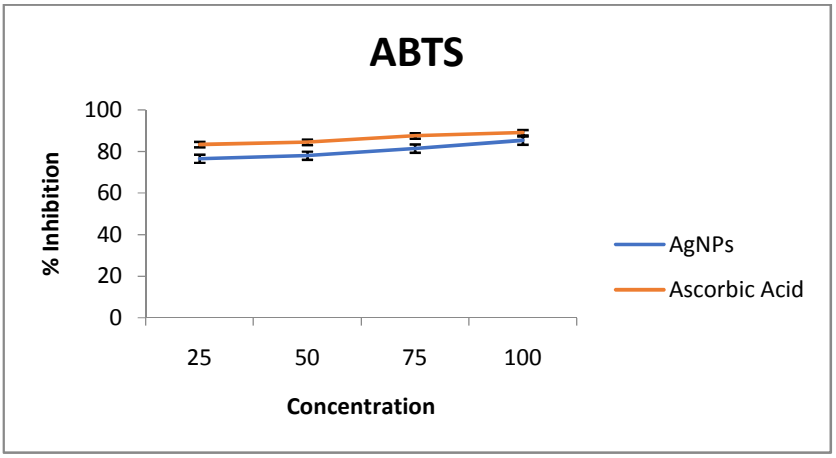

Figure 5- ABTS radical scavenging activity of Synthesized AgNPs from Unripe pawpaw peel and Ascorbic Acid (control).

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

DISCUSSION

Green synthesis of nanoparticles which uses plants extract as reducing and capping agents are the most advantageous of biogenic synthesis because it involves a single-step method and it is human friendly. An important factor for AgNps synthesis is colour change (Lalitha , Subbaiya & Ponmurugan ,2013) . According to Hyllested , Espina-Palanco , Hagen , Mogensen and Neipp (2015), AgNPs appear brown in aqueous medium as a result of surface Plasmon vibrations. The formation of silver nanoparticles (AgNPs) from aqueous extract of unripe pawpaw peel was primarily observed by the colour change from faint yellow to colloidal brown Similar color changes as been reported by other researches ranging from light yellow, yellowish brown, to dark brown confirming the formation of AgNPs (Krithiga , Rajalakshmi & Jayachitra, 2015; Banerjee , Satapathy & Mukhopahayay, 2014. The nanoparticles were primarily characterized by UV-Vis spectroscopy, which has

proved to be a very useful technique for analysing nanoparticles. An absorption peak of 450nm was observed, which is characteristic of the Ag, arising from the excitation of longitudinal plasmon vibrations of AgNPs in the solution (Gloria et al., 2017). The AgNPs showed strong antimicrobial activity against all the tested organism. This result is in correlation with the report of Abdul-Rehman et al., (2016) that most plant extract AgNPs exhibit enhanced broad-spectrum antimicrobial activities. This suggests that Ag nanoparticles from unripe pawpaw peels could be projected as future generation antimicrobial agents. Radical scavenging activity of compounds are widely tested using DPPH and ABTS since they provide easy and rapid evaluation. This present study reveals that the synthesized AgNps exhibit potential free radical scavenging activity against both radicals. The antioxidant activities of AgNps from plant sources such as Spices (Gloria et al., 2017)and *Sonneratia apetala* leaves (Peddinti & Vanga, 2017) have been reported.

CONCLUSION

AgNPs was successfully synthesized and characterized primarily from $AgNO_3$ and aqueous extracts of unripe pawpaw peel. The synthesized nanoparticles exhibited potent antibacterial and antioxidant activities. Further optimization of the current green-synthesis could be effective in the treatment of several diseases and for food preservation.

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