DRUG DISCOVERY

15(36), 2021

Evaluation of Flavonoid and Phenol content and Antioxidant Properties of Silver Nanoparticles of Unripe Pawpaw and Banana peel

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To Cite:
Adesipe TI, Iweala EJ. Evaluation of Flavonoid and Phenol content and Antioxidant Properties of Silver Nanoparticles of Unripe Pawpaw and Banana peel. *Drug Discovery*, 2021, 15(36), 122-130

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Peer-Review History
Received: 18 March 2021
Reviewed & Revised: 19/March/2021 to 14/May/2021
Accepted: 15 May 2021
Published: May 2021

Peer-review
External peer-review was done through double-blind method.

DISCOVERY SCIENTIFIC SOCIETY

ABSTRACT

The present study is aimed at determining the total flavonoid and phenol contents and antioxidant properties of Silver nanoparticles (AgNPs) biosynthesized using unripe pawpaw peel (UPPAE) and banana peel (UBPAE) aqueous extracts. AgNPs of UPPAE and UBPAE were synthesized separately by reducing AgNO3 with UPPAE and UBPAE respectively. Primary characterization was done on the synthesized AgNPs with a UV-visible spectrophotometer, and their antioxidant properties were evaluated using DDPH, ABTS, FRAP assays. The reaction mixtures of AgNO3 solution and the agro-waste extracts turned dark brown for AgNO3 + UPPAE and light brown for AgNO3 + UBPAE and also displayed a UV-visible spectrum of 450 nm and 421 nm respectively which is characteristic of silver nanoparticles. The result of the total flavonoid contents revealed that AgNPs-UPPAE had the highest amount of flavonoid content (499.89 ± 1.96 mgQE/g) and phenol content (85.30 ± 0.57 mgGAE/g). However, the antioxidant result revealed that AgNPs-UBPAE displayed the highest DPPH scavenging activity with an IC50 value of 52.98 µg/ml when compared to AgNPs-UPPAE (52.98 µg/ml) and Ascorbic acid (59.93 µg/ml). AgNPs-UPPAE and AgNPs-UBPAE showed maximum ABTs scavenging activities with IC50 values of 52.20 µg/ml and 52.45 µg/ml respectively which is comparable to Ascorbic acid (50.95 µg/ml). The result of FRAP revealed that AgNPs-UPPAE and AgNPs-UBPAE had the highest FRAP value at a concentration of 25µg/ml unlike Ascorbic acid whose highest FRAP value was at 100 µg/ml. This result reveals the potential use of AgNPs-UPPAE and AgNPs-UBPAE as alternative natural antioxidants for the management of oxidative stress-induced ailments.

Keywords; Silver nanoparticles, Agro-waste, Total phenol and flavonoid contents, Antioxidant properties

ISSN 2278–540X EISSN 2278–5396 I OPEN ACCESS

1. INTRODUCTION

Oxidative stress caused by the formation of highly reactive oxygen species (ROS) has been linked to the development of certain conditions such as aging, cellular injury, cancer, and renal hepatic, neurodegenerative, and cardiovascular disorders (Sushant *et al.*, 2019; Losada-Barreiro *et al.*, 2017). Because the formation of reactive oxygen species is inevitable in the body as they are by-products of metabolic activities in the body (Gabriele *et al.*, 2017; Navarro-Yepes *et al.*, 2014), endogenous antioxidant enzymes, such as catalase, glutathione peroxidase, deactivates these free radicals in order to prevent oxidative stress (Kurutas, 2016). However, these endogenous antioxidants may not suffice in the presence of elevated reactive oxygen species level. Therefore exogeneous antioxidants especially those of natural origin are required (Jaouad and Torsten, 2010; Rahman, 2007) since the prolonged usage of common commercially available synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA) and tert-butylhydroquinone (TBHQ) produces adverse effect (Kumar et al., 2014; Wichi,1989).Herbal materials including plants, whole fruits and peels has been reported to contain phenolic compounds which are excellent electron donors, as their hydroxyl groups contribute to antioxidant activity of their parent material (Sushant *et al.*, 2019; Bendary *et al.*, 2013).In order to ameliorate the pharmacokinetics of medicinal plant, herbal biomolecules can be encapsulated with suitable nano materials since the delivery of herbal therapeutic molecules as drugs is challenging (Martínez-Ballesta *et al.*, 2018; Gloria *et al.*, 2017). Nanoparticles have gained immense scientific interest as they are in effect an aqueduct between massive materials and submicroscopic structures. Silver nanopaticles have been recognized all over the world, admist several metal nanoparticles because they are very effective, less toxic and most suitable for medicinal applications (Ratan *et al.*, 2020; Annu *et al.*, 2018; Patra *et al.*, 2018; Patil and Kumbhar, 2017; Kanav *et al.*, 2016; Rai *et al.*, 2009). Several authors has proposed the use of biological method of AgNPs synthesis which uses plant extract over physicochemical methods and even other biological methods of synthesis that uses enzymes or microorganism because the use of plant extracts is ecofriendly, does not require elaborate processes, has greater yield and does not require the use of toxic chemicals (Retan *et al.*, 2020; Ana-Alexandra *et al.*, 2016; Mital et al., 2013). However, in the recent years, attempts are being made to substitute plant parts with agro industrial wastes in order to develop greener and more workable processes (Margarita and Victoria, 2019; Poadang *et al.*, 2017; Borase *et al.*, 2014). Agricultural by-products from industries processing have been reported to be an essential source of natural antioxidants (Deng *et al.*, 2012) since they contain compound such as phenols and flavonoids that are powerful antioxidant agents (Biljana and Djendji, 2019). Unripe pawpaw and banana peels are often thrown away during the preparation of their fruits causing unsightly pollution (Mordi *et al.*, 2016; Maisarah *et al.*, 2013).However reports has shown that these fruit peels contain flavonoid and phenol compounds (Felix *et al.*, 2016, Anuj *et al.*, 2016, Aquino *et al.*, 2016) which are not just antioxidant agents but are also among the named biomolecules that serves as a reductant and also as capping agents for silver nanoparticles synthesis (Singh *et al.*, 2020; Anupam *et al.*, 2019).Biogenic silver nanoparticles exhibits enhanced therapeutic activities due to biomolecules attached on the surface of the nanoparticles (Anupam *et al.*, 2019).

Therefore in this study we have attempted to synthesize AgNPs using UPPAE and UBPAE, compare their (AgNPs-UPPAE and AgNPs-UBPAE) total phenol and flavonoid contents and their antioxidant activity using (2,2-diphenyl-1-picrylhydrazy l, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), Ferric reducing antioxidant power assays.

2. MATERIALS AND METHOD

The samples (Unripe pawpaw and banana) employed for this study were obtained from the market. Reagents and chemicals used were of analytical grade.

Sample Preparation

The entire samples were rinsed under the running tap and peels of individual samples were removed using table knife, rinsed again with distilled water and diced into tiny pieces.

Extract Preparation

The aqueous extract of unripe pawpaw peels (UPPAE) and unripe banana peels (UBPAE) were prepared following the method described by Abhay and Rupa (2016). About 25g of UPPAE and UBPAE were kept separately inside two beaker containing 100ml distilled water each and then heated in a water bath for 30 min at 60^{o}C .The aqueous extracts were filtered separately with Whatmann No. 1 filter paper before centrifuging for 10 min at 1000 rpm.

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Synthesis of Silver nanoparticles

The nanoparticles were biosynthesized at room temperature. 10 ml each of UPPAE and UBPAE were added separately into two flask containing 90ml of aqueous 1 mM AgNO3 (Nooshin *et al.,* 2017).

Characterization of biosynthesized AgNPs

Formation of the reduced silver nanoparticles in colloidal solution was monitored by using a UV–vis Spectrophotometer. The absorption spectra of the supernatants were recorded in the range of 300 and 600 nm wavelength.

Determination of Total Phenol content

Folin Ciocalteu reagent was used to quantitatively determine the total phenol content of the biosynthesized nanoparticles and Gallic acid was used as the standard. The phenolic contents were estimated as Gallic acid equivalents GAE/g of samples using the standard curve of Gallic acid. All determinations were done in triplicate (Chandra *et al.,* 2014, Singleton and Rossi, 1965).

Determination of Total Flavonoid content

The total flavonoid content of the biosynthesized AgNPs were determined using the Aluminum chloride colorimetric method and Quercetin was used to make the standard calibration curve (Sushant *et al.,* 2019, Chandra *et al.,* 2014).

Antioxidant assay

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

DPPH radical scavenging activity

The DPPH free radical scavenging activity of the nanoparticles was evaluated by adding different concentrations (25 μg/ml-100 μg/ml) of either AgNPs-UPPAE, AgNPs-UBPAE or Ascorbic acid (standard) to 200 μM freshly prepared methanolic solutions of DPPH at room temperature in the dark. Absorbance was taken for each reaction mixture at 517 nm after 30 min (Kumar *et al.,* 2014, Blois, 1954).

Radical scavenging activity was calculated by the following formula;

DPPH scavenging activity (%) = [(ABScontrol – ABSsample)/(ABScontrol)] × 100

where, ABS control is the absorbance of DPPH + methanol and ABSsample is the absorbance of DPPH + sample (Nanoparticles/standards).

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

Stock solution of ABTS radical cation was made by dissolving ABTS (7 mM) with potassium persulfate ($K_2S_2O_8$, 2.4 mM). The mixture was left to stand in the dark at room temperature for 12 h. The working solution was then prepared by mixing the two stock solutions in equal proportions (1:1 v/v). The working solution of ABTS was diluted in 60 ml of methanol to obtain the absorbance of 0.708± 0.001 units at 734 nm using the spectrophotometer. 100 μl of the nanoparticles or standards(Ascorbic acid) prepared in methanol at different concentration (25 μg/ml-100 μg/ml) were mixed with 100 μl working solution .The reaction mixture was then allowed to stand at 30°C for 7 min, then the absorbance was measured by using a UV-visible spectrophotometer at 734 nm.

ABTs Scavenging Activity (%) = [1−(Abs Sample)/(Abs Control)] × 100

Where, Abs control is the absorbance of ABTS radical + methanol and Abs sample is the absorbance of ABTS radical + sample (Nanoparticles/standard).

Ferric Ion Reducing Antioxidant Potential (FRAP) Assay

The stock solutions prepared were 300 mM acetate buffer (3.1 g CH_3COONa and 16 ml CH_3COOH), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-striazine) solution in 40 mM HCl, and 20 mM FeCl3 solution. The temperature of the fresh working solution prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL FeCl3 solution was raised to 37°C before using. Different concentrations (25 μg/ml-100 μg/ml) of 100 μL of either AgNPs-UPPAE, AgNPs-UBPAE or Ascorbic acid (standard) were allowed to react with 2900 μL of FRAP solution in the dark for 30 min. Absorbance were recorded at 593 nm for the coloured product (ferrous

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tripyridyltriazine complex) The standard curve was linear between 200 to 1000 μM FeSO4. Results are expressed in μM Fe (II)/ g extract.

3. RESULT

Addition of the 90 ml of 1 mM $AgNO_3$ aqueou solution to two different flasks containing 10ml of UPPAE and UBPAE each, resulted in color changes of the reaction media; dark brown for $AgNO_3$ + UPPAE and light brown for $AgNO_3$ + UBPAE. The formation of brown colour indicates that both extracts successfully reduced Ag^+ to Ag^O.

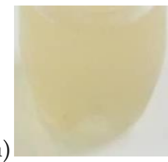
a)  b) 

Figure 1- aqueous extracts of a) Unripe pawpaw peel extract, b) unripe banana peel

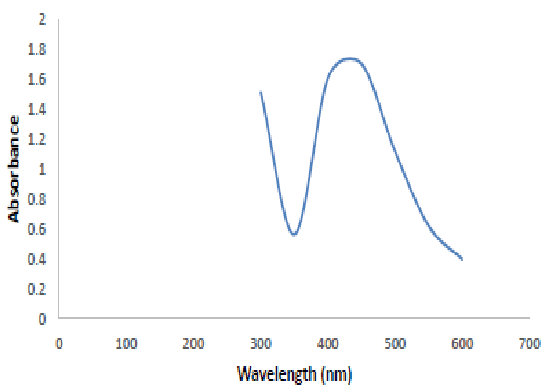
a)  b) 

Figure 2-a) *$AgNO_3$ + UPPAE=AgNPS-UPPAE b) $AgNO_3$ + UBPAE =AgNPS-UBPAE after incubation for 24hrs*

Characterization of Silver Nanoparticles

The ultraviolet-visible (UV-Vis) spectrum of the reaction media at 24h interval was observed to be 450nm for AgNPs-UPPAE (Figure 3a) and 421nm for AgNPs-UBPAE (Figure 3b). These absorption spectra observed are characteristic of silver nanoparticles and further confirms the synthesis of silver nanoparticles of UPPAE and silver nanoparticles of UBPAE.

a)



b)

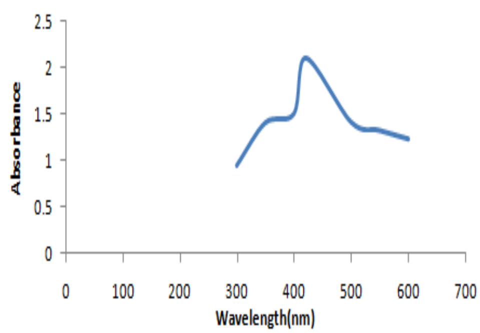


Figure 3 -*UV Visible spectrum of a)AgNPs-UPPAE and b)AgNPs-UBPAE*

Table 1: Total phenolic content of AgNPs-UPPAE and AgNPs-UBPAE

Samples	Phenolic contents (mgGAE/g)	Flavonoid content (mg QE/g)
AgNPs-UPPAE	85.30 ± 0.57	499.89±1.96
AgNPs-UBPAE	70.64 ±0.27	464.34±4.27

Results are expressed as mean ± SD (n = 3)

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Determination Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of the biosynthesized AgNPS were expressed as mg of Gallic acid (GAE)/ g and mg Quercetin acid equivalent QE/g dry wt respectively as shown in table 1.

ANTIOXIDANT ACTIVITY OF BIOSYNTHESIZED AgNPs

DPPH Radical Scavenging Activity

The DPPH radical scavenging activities of biosynthesized AgNPs and Ascorbic acid are presented in Figure 4. All the samples showed concentration-dependent increases in radical scavenging capacity. The greatest DPPH radical scavenging potency with a minimum IC_{50} value was recorded for AgNPs-UBPAE (52.98 μg/ml) followed by AgNPs-UPPAE (54.78 μg/ml) and Ascorbic acid (59.93μg/ml).

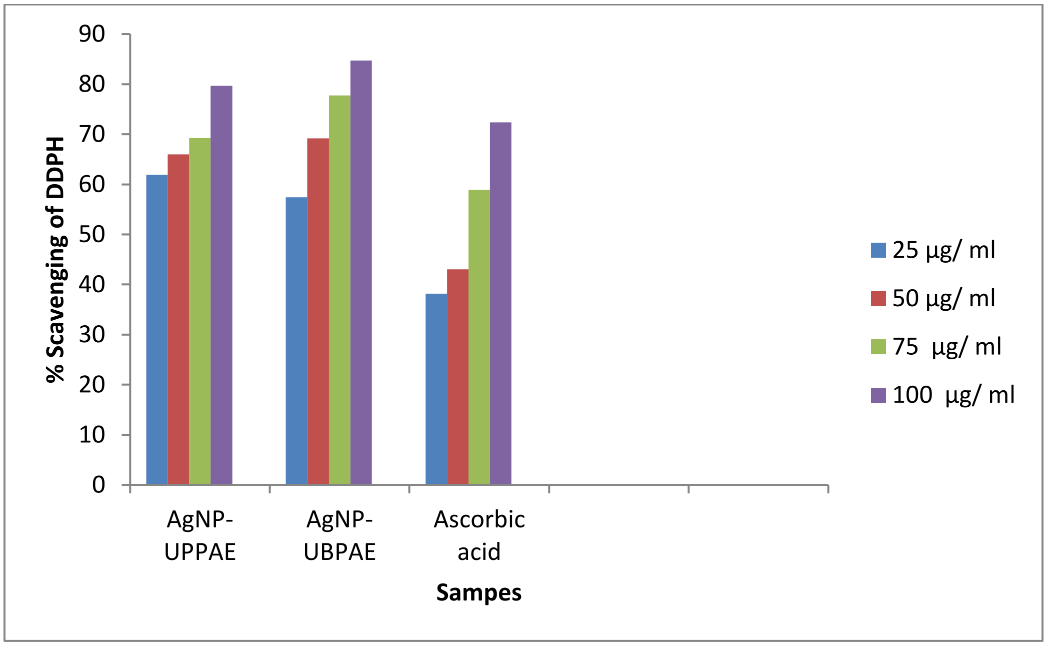

Figure 4 - Comparison of DPPH• scavenging activity of AgNPs-UPPAE, AgNPs-UBPAE and ascorbic acid. Results expressed as the mean ± standard deviation (n = 3) at concentrations of 25, 50, 75 and 100 μg/mL

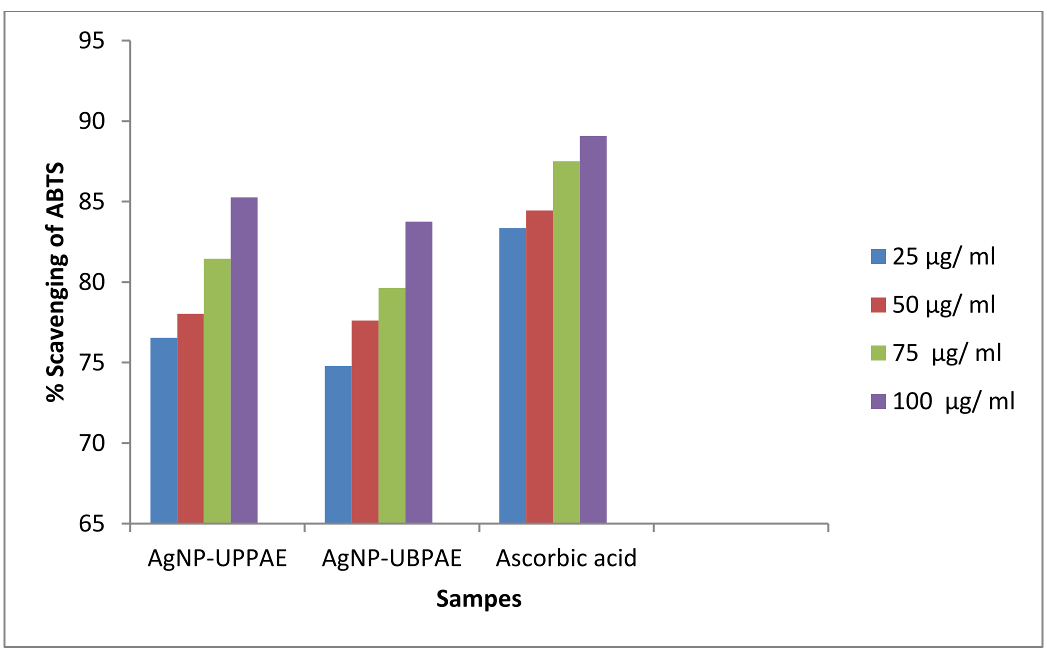

Figure 5 - Comparison of ABTS• scavenging activity of AgNPs-UPPAE, AgNPs-UBPAE and ascorbic acid. Results expressed as the mean ± standard deviation (n = 3) at concentrations of 25, 50, 75 and 100 μg/mL

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ABTS Scavenging Activity

The ABTS scavenging activities of biosynthesized AgNPs and Ascorbic acid are presented in Figure 5. All the samples showed concentration-dependent increases in radical scavenging capacity. The greatest ABTS radical scavenging potency with a minimum IC_{50} value was recorded for Ascorbic acid (50.95 μg/ml) followed by AgNPs-UPPAE (52.20 μg/ml) and AgNPs-UBPAE (52.45 μg/ml).

Ferric ion reducing antioxidant potential (FRAP) Assay

The result of FRAP radical scavenging is presented in Figure 6 below. The result revealed that AgNPs of UPPAE and AgNPs of UBPAE had the highest FRAP value at concentration of 25μg/ml (0.64± 0.006 and 0.80± 0.003 μM Fe (II)/g respectively) unlike Ascorbic acid whose highest FRAP value was at 100μg/ml (0.60± 0.002 μM Fe (II)/g).

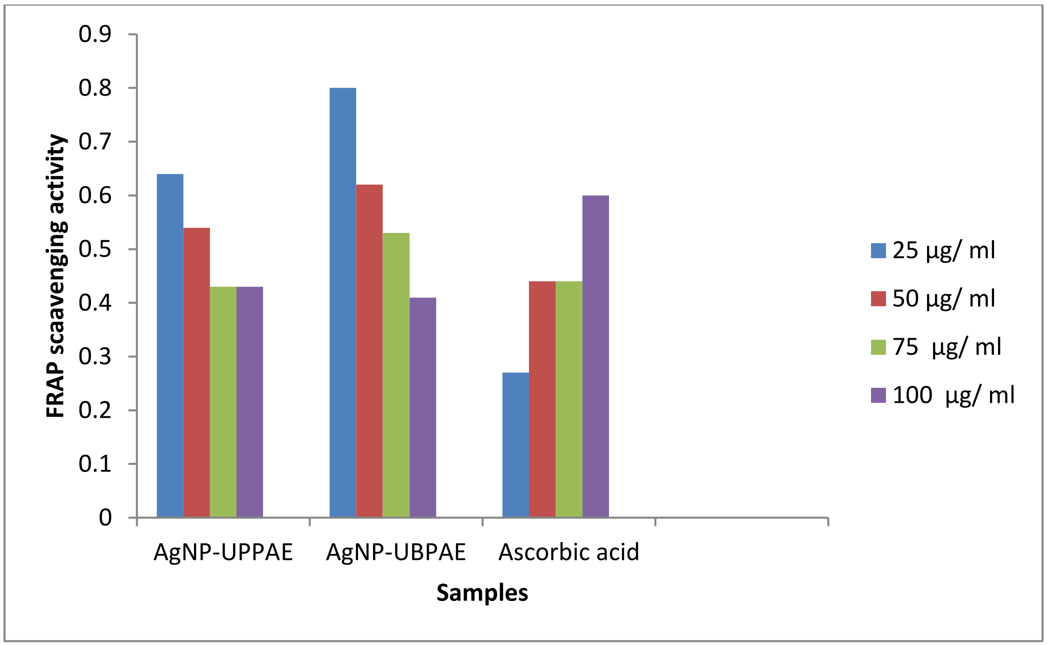

Figure 6 - Comparison of FRAP• scavenging activity of AgNPs-UPPAE, AgNPs-UBPAE and ascorbic acid. Results expressed as the mean ± standard deviation (n = 3) at concentrations of 25, 50, 75 and 100 μg/mL

4. DISCUSSION

Since rules controlling organic solid waste management and ecological worries has been expanding (Das *et al.*, 2019; Omran *et al.*, 2018; Reena and Menon, 2017) and report has shown that agrowaste contains useful bioactive compounds (Rafik *et al.*, 2018; Rehan *et al.*,2018), fruit wastes such as peels could be utilized in a productive way for nanotechnology-based applications. In this study, aqueous extracts of unripe pawpaw and banana peels which are by products of the food industry were used to synthesize silver nanoparticles (Fig 1). The biosynthesis of AgNPs-UPPAE and AgNPs-UBPAE was initially confirmed by the colour change in the reaction mixture to dark brown for $AgNO_3$ + UPPAE and light brown for $AgNO_3$ + UBPAE (Fig 2). Similar colours has been reported by other researchers as AgNPs usually looks brownish in aqueous medium due to surface Plasmon vibrations (Dada *et al.*, 2019; Olugbemi 2019; He *et al.*, 2018; He *et al.*, 2017; Hyllested *et al.*, 2015; Krithiga et al., 2015; Banerjee *et al.*, 2014). After the bio-synthesis of AgNPs-UPPAE and AgNPs-UBPAE, the formation of AgNPs was monitored using UV–VIS absorption spectroscopy in the wavelength range of 300–600 nm. Normally, AgNPs displays a surface plasmon resonance (SPR) band between 450–550 nm because of the excitation of free electrons (Das *et al.*, 2019; Mousavi *et al.*, 2018; Gloria et *al.*, 2017). In the present study, the SPR value of AgNPs-UPPAE and AgNPs-UBPAE was detected at 450nm and 421nm respectively (Fig 3). This SPR values has been reported for several biosynthesized silver nanoparticles (Reham *et al.*, 2020; Dada *et al.*, 2019; Hina *et al.*, 2018; Składanowski *et al.*, 2016). After the primary characterization of the biosynthesized AgNPs, the total phenol and flavonoid contents and antioxidant activities were determined. The antioxidant activities of AgNPs-UPPAE and AgNPs-UBPAE were determined because there is an increasing concern that prolonged usage of common commercially available synthetic antioxidants, such as butylated

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hydroxytoluene (BHT), butylated hydroxyanisol (BHA) and tert-butylhydroquinone (TBHQ) produces adverse effect (Kumar *et al.*, 2014; Wichi,1989) and since the formation of reactive oxygen species which could result to oxidative stress at elevated concentration is inevitable in the body (Gabriele *et al.*,2017; Navarro-Yepes *et al.*, 2014), there is a need for exogeneous antioxidant agents especially those of natural origin (Jaouad and Torsten, 2010; Rahman, 2007). The positive results of the ferric ion antioxidant power and scavenging activities of AgNPs of UPPAE and AgNPs of UBPAE against DDPH and ABTS in this study are presented in (Fig 4-6); these positive results can be attributed to the smaller size and elevated levels of the phenol and flavonoid contents of the biosynthesized AgNPs when compare to those of their parent materials; unripe pawpaw and banana peel reported in the literature. The total Phenol and Flavonoid contents of AgNPs-UPPAE was found to be 85.30 ±0.57mg GAE/g and 499.89±1.96mg QE/g respectively while AgNPs-UBPAE had a total Phenol and Flavonoid contents of 70.64 ±0.27mg GAE/g and 464.34±4.27mg QE/g. This result of the total phenol and flavonoid contents of AgNPS-UPPAE is higher than the total phenol and flavonoid contents of aqueous extract of unripe pawpaw peel 126.75±0.20 mg GAE/100 g and 166.11±0.01 mg QE/100 g as reported by Dada et al., (2016). 685.57 mg GAE/100 g and the aqueous extract of banana peel with total and phenol contents of 9.89 ± 0.16 mg GAE/g and 8.56 d ± 0.22 mg GAE/100 g respectively as reported by (Ahmed et al., 2019).

5. CONCLUSION

The results proved that silver nanoparticles were successfully synthesized using the peel extracts of unripe pawpaw and banana at room temperature. The synthesis of the nanoparticles was confirmed by the color change of both extracts to dark brown and light brown respectively after the addition of $AgNO_3$ solution. The AgNPs were further characterized primarily by UV–analysis. The use of peel extracts for synthesizing metallic nanoparticles is not expensive, can be easily scaled-up, is environmentally friendly and also allows for the availability of a product that is void of toxic contaminants, as necessary in therapeutic applications (Ratan *et al.*, 2020) and in order to develop greener and more sustainable processes (Margarita and Victoria, 2019). Furthermore, the synthesized AgNPs showed good antioxidant activities proving its pertinence in medicines.

Funding:

This study has not received any external funding.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for experimentation.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Data and materials availability:

All data associated with this study are present in the paper.

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