Antioxidant properties of silver nanoparticles biosynthesized from methanolic leaf extract of *Blighia sapida*

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ABSTRACT

Synthesis of nanoparticles by biological methods using microorganisms, enzymes or plant extracts has been suggested as possible ecofriendly alternative to chemical and physical methods which involve the use of harmful reducing agents. In the current study, silver nanoparticles (AgNPs) were synthesized by green approach from methanolic leaf extract of *Blighia sapida*. The synthesized AgNPs were characterized by UV-visible (UV-vis) spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy and Scanning Electron Microscopy (SEM). The antioxidant activity was evaluated using DPPH radical scavenging assay, determination of total reductive potential, total phenolics content (TPC) and total flavonoids content (TFC) of the synthesized AgNPs. SEM analysis revealed that the size of the synthesized silver nanoparticles ranged from 50-70 nm with maximum UV-vis absorbance at 413 nm. DPPH radical scavenging activity, reducing power, total phenolic and total flavonoid contents of the synthesized AgNPs increased in a dose dependent manner as compared to ascorbic acid the standard reference used. This result confirmed that *Blighia sapida* is a potential biomaterial for synthesizing AgNPs which can be exploited for its antioxidant activity.

Keywords: *Blighia sapida*, antioxidant activity, silver nanoparticles, DPPH radical scavenging activity, free radicals

1. Introduction

Free radicals play a key role in oxygen dependent (aerobic) living systems. They are part of cell respiration and other important cellular processes but are also involved in aging and disease development [1, 2]. Free radicals are unstable molecules with free unpaired electrons.
They are highly reactive because the free electrons always attempt to bond with other electrons and form covalent pairs. In this process, the free radicals remove the electrons from other molecules. Beyond affecting regulation of cells, it can also damage molecules such as carbohydrates, fats, proteins and nucleic acids [3, 4]. There are different sources of free radicals within the cells and its environment. In aerobic organisms, free radicals are produced during normal metabolic processes [5]. The major sources include electron transfer in the plasma membrane and cell respiration in the mitochondria membrane. Mitochondria are the main source of the oxidative damage because free radicals such as superoxide can escape from the electron transport chain [6, 7]. In order to prevent intracellular damage by free radicals, cells have built up intracellular antioxidant system. This process converts free electrons into a non-reactive form by proteins.

Antioxidants control oxidative reactions by inhibiting, delaying or hindering the oxidation of the biomolecules [8, 9]. The key antioxidant enzymes possess certain elements that shield and protect proteins [10, 11]. Non enzymatic antioxidants can also neutralize radicals for example water soluble substances such as Vitamin C, glutathione or fat-soluble substances such as Vitamin E, β-carotene [8, 12, 13]. Synthetic antioxidants such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) have newly been reported to be harmful for human health [14, 15]. Thus, the search for effective, non-toxic, natural compounds with antioxidative activity has been increased in recent years. Recently nanomaterials have started playing a fundamental role in human life and health owing to their substantial benefits of biomedical applications [16] like medical imaging, drug delivery, diseases diagnosis [17], cancer treatment [18, 19], treatment of infectious diseases [20, 21], treatment of neurodegenerative disorder including Parkinson disease [22] and so on.

Moreover, the strong antioxidant property exhibited by some nanomaterials is opening exciting potential to develop new regimens with enhanced and targeted actions. For example, gold, silver and selenium nanoparticles have been shown to possess ability to reduce oxidative stress due to their efficient redox-active radical-scavenging properties [23, 24, 25, 26]. *Blighia sapida* is a soap berry perennial herbaceous plant that is prominently found in Western Tropical Africa (Figure 1). It belongs to the family Sapindaceae. In Nigeria, it is commonly known as Ackee (English), Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo) [27]. It is an evergreen tree which grows to a height of between 7 and 25m. It occurs naturally from Senegal, Cameroon and Equatorial Guinea. The tree is often cultivated for its edible fruit in many areas of tropics and subtropics. When ripe, Ackee fruit splits to expose a fleshy cream colour pulp aril attached to a shiny black oblong seed [28]. The fleshy fruit can also be
curried, used in soups and stews as a meat substitute [29]. The fruit must be allowed to open fully before it is detached from the tree because unripe or overripe arils as well as the seed contain hypoglycin and its derivatives which are strongly toxic [30]. *Blighia sapida* is useful in Africa traditional medicine. In Cote d’ Ivore, it is commonly used for the treatment of yellow fever, epilepsy, oedema and as a laxative and diuretic [31]. Sap from the terminal bud is instilled in the eyes to treat ophthamia and conjunctivitis [32]. The pulp of grand up leafy twig is rubbed on the forehead to treat migraine/headache [31, 33]. The root, bark, leaves, capsules and seeds were identified for the cure of 22 diseases in Benin [34]. The plant was reported to have antioxidant activity [35, 36, 37]. Therefore the aim of this study was to synthesize silver nanoparticles from the leaves of *Blighia sapida* and to investigate its possible antioxidant potentials.

![Figure 1. Blighia sapida plant (a), Blighia sapida fruit (b), Blighia sapida ripe fruit showing a fleshy cream colour pulp aril and shiny black oblong seed (c)](image)

### 2 Materials and Methods

#### 2.1 Reagents

All reagents were of analytical grade and obtained from Sigma Aldrich Chemical, Germany.

#### 2.2 Plant materials

Fresh leaves of *Blighia sapida* were collected from the Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso. The plant was authenticated at the herbarium unit of the Department of Pure and Applied Biology, Ladoke Akintola University of Technology Ogbomoso (Voucher number : LHO - 538). Healthy leaves with no sign of damage were air dried under shade at room temperature for four weeks and pulverized into powdered form.
2.3 Preparation of plant extract
Methanolic leaf extract of *Blighia sapida* was prepared by soaking 2 kg of the pulverized plant material in 6 l of methanol for 4 days. The liquid portion was then separated from the leaf shaft with the aid of Whatman (No. 1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath set at 40 °C to obtain a dry residue which is the crude methanolic extract (yield weight 150 g) which was stored in a desiccator until further use.

2.4 Phytochemical screening
The methanolic extract was screened for the presence or absence of alkaloids, flavonoids, tannins, phenols and steroids using standard methods [38, 39].

2.5 Synthesis of silver nanoparticles
The extract was centrifuged at 4,000 rpm for 20 min. The supernatant obtained was used to synthesize silver nanoparticles as described by Lateef *et al.* [40]. Exactly 1 ml of the supernatant was added to the reaction vessel containing 40 ml of 1 mM silver nitrate (AgNO₃) solution for the reduction of silver ion. The reaction was carried out at room temperature (30±2 °C) for 2 h. The formation of silver nanoparticles was monitored through visual observation of the change in colour.

2.6 Characterization of silver nanoparticles
The formation of silver nanoparticles from *Blighia sapida* methanolic leaf extract was also determined by measurement of the absorbance spectrum of the reaction mixture using UV-visible spectrophotometer (UV-2450 Shimadzu Model, USA). The spectrum data recorded was then plotted.

The silver nanoparticles solution was centrifuged at 10,000 rpm for 20 min. The solid residue was then dried at room temperature and the powder obtained was used for FTIR measurement using KBr pellet with an LF-45 fluorescence spectrophotometer FTIR instrument (Shimadzu Model IR, USA) in the wavelength 4000-500 cm⁻¹ [40, 41].

Scanning electron microscopy was done using a Hitachi scanning electron microscope (Model, S-2600N-Tokyo Japan) operating in the high vacuum anode with an acceleration voltage of 20 KV. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed.
using a blotting paper and the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

2.7 Antioxidant assay

2.7.1 DPPH radical scavenging assay

The 1,1, diphenyl-1-2 picrylhydrazyl (DPPH) scavenging potential of the silver nanoparticles from Blighia sapida methanolic leaf extract was determined using the method of Mensor et al. [42]. Different concentrations (50, 75, 100, 125 and 150 µg/mL) of the silver nanoparticles and the standard (ascorbic acid) were taken in different test tubes. Thereafter, 1 ml of freshly prepared DPPH (0.3 mM) was added to each test tube and vortexed thoroughly. Finally, the solution was incubated in dark place for 30 min. For control, 2 ml of methanol was added instead of silver nanoparticles and run simultaneously with the test. The percentage DPPH radical scavenging activity of the silver nanoparticles was calculated using the formula.

\[
\% \text{ DPPH Radical Scavenging Activity} = \frac{A_c - A_s}{A_c} \times 100
\]

\(A_c\) is the control absorbance of DPPH radical + methanol; \(A_s\) is the sample absorbance of DPPH radical + sample AgNPs / Ascorbic acid.

2.7.2 Reducing power assay

The reducing power of the silver nanoparticles was determined by the method of Linn et al. [43] with slight modification. Different concentrations of AgNPs (50, 75, 100, 125 and 150 µg/ml) were prepared and separately mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. Further, 2.5 ml of tricholoroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, the supernatant solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml FeCl₃ (0.01%) and absorbance were measured at 700 nm in UV-visible spectrophotometer.

2.7.3 Determination of total phenolics content

The total phenolics content (TPC) of the silver nanoparticles synthesized from methanolic leaf extract of Blighia sapida was determined using the Folin Cioclateau assay [44]. About 1 ml of different concentrations (50, 75, 100, 125 and 150 µg/mL) of the AgNPs was taken into a 25 ml volumetric flask and 10 ml of distilled water and 1.5 ml of Folin Cioclateau reagent were added to it. The mixture was kept for 5 min and then 4 ml of 20% sodium carbonate was added and made up to 25 ml with distilled water and allowed to incubate for 30 min at room
temperature. The total phenolic content was determined using spectrophotometry at 765 nm and was expressed as gallic acid (GA) equivalent (µg/g).

2.7.4 Determination of total flavonoids content

Total flavonoids content (TFC) of the silver nanoparticles from methanolic leaf extract of *Blighia sapida* was estimated using the aluminum chloride colorimetric method of Chang *et al.* [45]. About 0.1 ml of different concentrations of the silver nanoparticles (50, 75, 100, 125 and 150 µg/ml) were separately mixed with 0.1 ml of 10% AlCl₃.6H₂O, 0.1 ml of 1M sodium acetate and 2.8 ml of distilled water and incubated at room temperature for 30 min. Total flavonoid content was determined using spectrophotometry at 415 nm and was expressed as µg/g quercetin equivalent.

3. Results and Discussion

Qualitative phytochemical screening revealed the presence of phenolics, alkaloids, flavonoids, tannins, saponins and steroids. Preliminary identification of nanoparticles formation was determined by observing the colour change. The AgNPs were characterized with colour formation produced from the reduction of silver ion by the plant extract. The intensity of the colour increases as the bio-reduction of silver ion progresses and become stable when the reduction is completed. The colour of the solution changed from light yellow to dark brown (Figure 2). Varying colours including yellow, brown and dark brown has been reported by several authors [24, 40, 46].

![Figure 2. Colour change in the reaction mixture (silver nitrate + *Blighia sapida* extract)](image)

(a) Colour of the mixture after mixing the extract with silver nitrate solution (b) Colour change of the mixture after 2 h
The reduction of Ag\(^+\) to AgNPs was further confirmed using UV-Vis spectroscopy. UV-vis spectroscopy is a valuable technique used to detect the characteristics surface plasmon resonance (SPR) pattern of the metal nanoparticles. Metal nanoparticles exhibited SPR phenomena when metal electrons in the conduction band collectively oscillate in resonance with certain wavelength of incident light. Figure 3 depicts the UV-visible spectrum of the biosynthesized AgNPs from methanolic leaf extract of *Blighia sapida* with absorption spectrum at 413 nm which is a characteristic band for silver. No other peak was observed in the spectrum indicating the formation of AgNPs. These values are within the range reported for AgNPs [40, 47].

**Figure 3.** UV-vis spectrum of silver nanoparticles synthesized from methanolic leaf extract of *Blighia sapida*

FTIR spectroscopy was used to identify the possible biomolecules present in *Blighia sapida* leaf extract which are responsible for reducing the Ag\(^+\) to Ag\(^0\), capping and stabilizing the
silver nanoparticles. FTIR spectra recorded for the biosynthesized AgNPs is presented in Figure 4. The FTIR spectrum of the nanoparticles produced showed peaks at 3325, 2902, 2382, 2117, 1635, 1288, 1145, 505, 457 and 422 cm⁻¹. The vibrational peak around 3325 cm⁻¹ is assigned to O-H that could possibly emanate from carbohydrate or phenolics. The peak at 2902 cm⁻¹ is ascribed to C-H of the alkyl group, the peak around 2117 cm⁻¹ is due to C=C from alkene or aromatic amine, 2382 cm⁻¹ corresponds with C=C from alkyne, the peak at 1635 cm⁻¹ suggest C=O from carboxyl group or esters and N-H binding vibration of proteins, the peak at 1145 cm⁻¹ is likely due to C-O from phenols while the corresponding peaks at 505, 457 and 422 cm⁻¹ can be ascribed to O-H from phenols or conjugated chromophore. The appearance of these peaks suggested the presence of protein, carbohydrates, hydrocarbons and phytochemicals such as phenolics in the AgNPs which may be responsible for stabilizing and capping effects on the nanoparticles. The biomolecules may have interacted with the silver ions through their oxygen donor atoms and are adsorbed on the surface of the metal ions. The tentative assignment of the peak is supported by other literature reports [48].

Figure 4. FTIR spectrum of silver nanoparticles synthesized from methanolic leaf extract of Blighia sapida
The size, shape and morphology of the synthesized AgNPs were determined by scanning electron microscopy. The synthesized AgNPs were spherical in shape with diameter range of 50-70 nm (Figure 5).

Figure 5. SEM image of silver nanoparticles synthesized from methanolic leaf extract of *Blighia sapida*

*In vitro* antioxidant activity of the synthesized AgNPs was studied by analyzing four antioxidant capacities which are indicative of the antioxidant potential of the synthesized AgNPs. The studied parameters were DPPH radial scavenging activity, reductive potential, total phenolics and total flavonoids contents of the synthesized AgNPs at different concentrations from 50-150 µg/ml. DPPH is a stable organic free radical that has been used for investigating the free radical activities and thus antioxidant activity of various natural products [49, 50, 51]. Figure 6 shows the dose response for the DPPH scavenging activity of the synthesized AgNPs. The AgNPs synthesized from the methanolic extract of *Blighia sapida* are potential free radicals scavengers with effective inhibition activity in a dose dependent manner. The varying concentration of the AgNPs (50, 75, 100, 125 and 150 µg/ml) significantly scavenged DPPH by 58.10, 59.26, 62.33, 71.24 and 75.42% respectively. However, these activities are less than that of ascorbic acid, the standard reference used. The reducing power of a compound is related to its electron transfer ability and therefore may serve as a significant indicator of its potential antioxidant activity [52]. The reductive capabilities of the biosynthesized AgNPs is shown in Figure 7. The reducing power of the AgNPs increased with increasing amount of sample. The biosynthesized AgNPs exhibited a maximum reducing capability of 53.52% at 150 µg/ml which is lower than that of ascorbic acid.
acid (70.19%). Figures 8 and 9 depict the total phenolics and total flavonoids contents of the biosynthesized AgNPs. The AgNPs contained phenols and flavonoids in a concentration dependent manner. At a concentration of 150 µg/ml, the AgNPs has total phenolic content of 36.52 µg/g and total flavonoid content of 10.14 µg/g respectively. However, these values are less than that of phenolic and flavonoid contents of ascorbic acid, the standard antioxidant.

![Figure 6. DPPH radical scavenging activity of AgNPs and ascorbic acid](image1)

![Figure 7. The reducing power assay of the AgNPs and ascorbic acid](image2)
Phytochemicals like flavonoids, phenolics, anthocyanins have been of great interest as the sources of natural antioxidants. Plant materials such as leafy vegetables, fruits, seeds, cereals
have been reported to contain natural antioxidants [53, 54]. Flavonoids are a class of secondary plant phenols with powerful antioxidant properties [55]. Phenols are regarded as the most important oxidative components of plants hence, correlation between the concentration of total phenolics and antioxidant capabilities have been reported [56]. The antioxidant activity of phenol is mainly due to their redox properties which play an important role in scavenging and neutralizing free radicals. In the current study, the antioxidant activity of the AgNPs may be attributed to the high phenolics and flavonoids content in the plant. These plant phenolics are strong antioxidants with high reducing capacity [57]. The ability of *Blighia sapida* to reduce silver ions or to form silver nanoparticles by reducing silver ions is likely due to the presence of phenolic compounds which are electron donors.

4 Conclusion
This study reported the green synthesis of silver nanoparticles by simple and ecofriendly manner using the methanolic leaf extract of *Blighia sapida*. The leaf extract contains protein, carbohydrates and phenolic compounds which might be responsible for the bio-reduction of silver ion and capping/stabilization of the silver nanoparticles as was confirmed by FTIR studies. The biosynthesized silver nanoparticles from leaf extract of *Blighia sapida* showed antioxidant activity through its DPPH radical scavenging activity, its reductive potential, high phenolics and flavonoids contents. This study provides an insight into the usage of *Blighia sapida* leaf as a good source of naturally occurring antioxidant and could have great importance as therapeutic agent in preventing or ameliorating oxidative stress related diseases.

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