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Antifungal, Anticoagulant, Thrombolytic and Antioxidant Activities of Gold Nanoparticles Phytosynthesized from *Blighia sapida* Husk Extract

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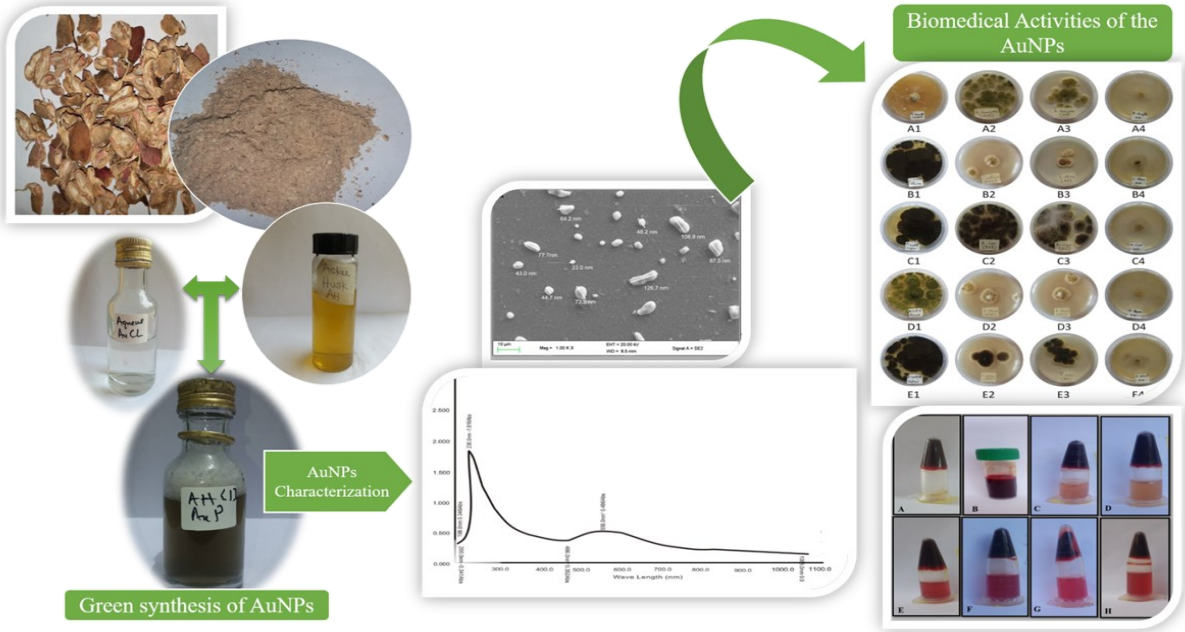
Highlights

- AuNPs inhibits the mycelia growth of five toxigenic/pathogenic fungi
- AuNPs prevents the coagulation of fresh human blood
- AuNPs rapidly dissolves pre-formed healthy donor human blood clots within 30 min
- AuNPs displays DPPH scavenging activity at concentration dependent rate

Graphical Abstract



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Abstract

The green synthesis of gold nanoparticles (AuNPs) using crude aqueous extract of Ackee (*Blighia sapida*) husk as reducing/capping agent was investigated for its antifungal, anticoagulant and thrombolytic activities in this work. The synthesis of AuNPs was monitored through the colour change for 15 min at photoactivation which turned out to be dark brown colouration and further characterized using UV-Vis spectroscopy, Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The UV-visible spectrum of the AuNPs displayed clear peak at 556.0 nm which was not observed in the spectra of the aqueous gold chloride and the crude aqueous extract of the plant. The prominent peaks of AuNPs in FTIR spectrum were 3792.9, 3346.2, 2971.4 and 1704.9 cm^{-1} which lent credence to the fact that proteins and phenolic compounds were involved in the biofabrication and capping of the AuNPs. Energy dispersive X-ray (EDX) analysis showed that gold (Au) was the prominent metal present; the AuNPs were fairly spherical in shape with sizes ranging from 23 to 126.7 nm. The biosynthesized AuNPs showed significant effect against the growth of *Candida albicans* (38.0%), *Aspergillus niger* (50%), *Aspergillus flavus* (63.4%), *Aspergilus fumigatus* (61.4%) and *Fusarium solani* (58.9%). The free radical scavenging properties of the AuNPs was very potent against the stable organic free radical DPPH, the varying concentration of the AuNPs (10, 20, 40 and 60 µg/ml) scavenged DPPH by 80.75, 81.11, 87.91 and 89.34%. Furthermore, the AuNPs showed anticoagulant activities of 48.70, 49.13, 50.00 and 54.74% at 250, 500, 750 and 1000 µg/ml and thrombolytic activities of 54.31, 56.46, 75.00 and 96.12% at 250, 500, 750 and 1000 µg/ml. The biomedical and antifungal properties of AuNPs against toxigenic/pathogenic fungi established in this research work would grant lasting solution to many medical, agricultural and biotechnological challenges in this dispensation.

1. Introduction

Nanotechnology is becoming a household name in virtually all fields in science and technology. Since its publicity in 1974 [1] till present, a plethora of milestone have been accomplished and many unattainable heights of manipulating matter at atomic level had been surmounted with feat of discoveries and innovations [2-5]. Nanotechnology, as one major unprecedented breakthrough in this dispensation of science [6], have proven the versatility of plants, plant wastes [7] and plant materials through many researches [8-11].

The importance of plants to man can never be over emphasized, as they play great roles in the survival of the ecosystem. Plants are rich in organic compounds in forms of phytochemicals or macromolecules [12] that are highly essential for cell metabolism or nourishments of the body, chelating of heavy metals [13], and bio-reduction [14,15] to mention a few. Moreover, the importance of plants to mankind is beyond their medicinal and edible properties. Plants are also one of the main parent raw materials for agro-based and biochemical industries. On the evidence of these facts, the roles of plants in science and technology advancement cannot be decried as useless. Researchers throughout the dispensation of innovations in science and technology have exploited many avenues in which plants could be useful; plants are still useful virtually in all areas in science and technology. The role of plant materials in the eco-friendly synthesis of nanoparticles are significant due to great deal of macromolecules they constitute [16,17]. Plants are very good bioreducing agents which made them highly necessary in the green synthesis of nanoparticles/nanomaterials [15].

Ackee (*Blighia sapida*), is a tree crop on which ackee fruit grows. It is an evergreen, tree and the fruit itself resembles a pear. It is

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green in its unripe stage, and then reddish fruit that is very recognizable when ripen. The fruit eventually splits to reveal three large black seeds surrounded by spongy flesh called the aril, which is the edible part of the fruit [18]. Some of the most important health benefits of ackee include its ability to lower blood pressure, boost energy levels, support healing and growth of worn out tissues, aid digestion, protect against diabetes, lower cholesterol, build strong bones, improve the immune system, and increase blood circulation [19].

Considering the health importance of *Blighia sapida*, this work was aimed at phytosynthesis of gold nanoparticles from aqueous husk extract of *Blighia sapida* and evaluation of its antifungal, anticoagulant, thrombolytic and antioxidant properties.

2 Materials and Methods

2.1 Sample collection and Preparation

The Ackee fruits were collected from 1500 LT Area, LAUTECH, Ogbomoso and Abaa area, Ogbomoso, Oyo state by plucking. The fruits were examined closely to ensure ripening and opening without any blemish or signs of infections. The fruits were conveyed to the laboratory in sterile polythene bags. The sample (whole fruit) was separated into husk, seed and the edible part after which the husks were washed with sterile distilled water, drained aseptically, air dried and powdered aseptically after two weeks of drying. Then, 0.1 g of the powder was weighed and suspended in 10 ml of distilled water, and heated in water bath at 60 ^{o}C for 1 h. The extract was filtered using Whatman No. 1 filter paper and then centrifuged at 4000 rpm for 15 min after which the supernatant was collected in screw cap sterile bottle.

2.2 Biofabrication and Characterization of Gold Nanoparticles

One ml (1 ml) of the husk extract was reacted with 40 ml of the gold chloride (1 mM) solution [15] and then photo-activated as it enhances the formation of the gold nanoparticles. The formation of AuNPs was visually observed by checking the color change. As the experimental control, a solution of the aqueous solution ($HAuCl_3$) alone was also used without colour change during photoactivation. A change in color of the reaction mixture treated with extract of *B. sapida* was visually observed, followed by the measurement of its absorbance spectrum using UV–visible spectrophotometer (CECIL CE model 7200 USA) operated at the range of 200–800 nm. FTIR spectroscopy analysis was also carried out on the synthesized AuNPs, using IRAffinity-1S Spectrometer (SHIMADSU: IR Affinity-IS-USA) to identify the functional groups of the various biomolecules that took part in the green synthesis of the nanoparticles and its stability. The morphology of the gold nanoparticles was determined using Scanning Electron Microscopy (SEM) and the elemental composition of the biosynthesized gold nanoparticles was determined through Energy dispersive X-ray (EDX) analysis.

2.3 Biomedical Applications of the Biofabricated Gold Nanoparticles

2.3.1 Antifungal Assay

The antifungal activities of the AuNPs were determined using mycelial growth inhibition method [20]. AuNPs were prepared and incorporated into potato dextrose agar (PDA) at concentration of 200 µg/ml. Agar plug (7 mm) from 48h-old fungal cultures of *Aspergillus niger, Aspergillus fumigatus, Fusarium solani, Candida albicans* and *Aspergillus flavus* were used to inoculate the PDA plates at the center. All the plates were incubated at 28 ± 2 ^{o}C for 72 h. The fungal growths were measured after 72 h and the percentage growth inhibitions were calculated using the formula:

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$$\frac{D(control) - D(test)}{D(control)} X100\%$$

Where D is the diameter of fungal growth on the plates.

2.3.2 Anticoagulant and Thrombolytic Activities of Biofabricated Gold Nanoparticles

The anticoagulant activity of the AuNPs was investigated as previously described by Lateef *et al.* [21]. Exactly 100 µl of the AuNPs (100 µg/ml) was added to 0.5 ml of blood freely provided by a healthy donor, while blood collected into EDTA served as positive control and blood collected in clean-tube served as negative control. In addition, *Blighia sapida* husk extract and aqueous gold solution were also used to treat blood samples, these were held at room temperature (30 ± 2 °C) for 30 min, and thereafter examined for anticoagulation.

The thrombolytic activity of the biofabricated gold nanoparticles was also determined using the methods of Azeez *et al*. [22]. Tubes containing 0.5 ml of blood were held at 37 °C for 30 min for clotting to occur and then examined. The weight of clean tube (W1) was subtracted from the weight of tube and blood clot (W2) to obtain the weight of blood clot (W3). Thereafter, 100 µl of the gold nanoparticles, gold chloride solution, and *Blighia sapida*husk extract were added to each blood clot tube. Then, incubation was done at 37 °C for 90 min, the tubes were later inverted to confirm clot lysis. The lysed clot was drained, and the weight of the tube with remaining clot was taken (W4) to obtain the weight of clot that was not lysed (W5). The percentage thrombolytic activity was obtained through the formula below:

$$\frac{W3 - W5}{W3} X100\%$$

2.3.3 Antioxidant Activities of Biosynthesized Gold Nanoparticles

The 1,1, dipheny 1-2 picrylhydrazyl (DPPH) free radical scavenging potential of the biosynthesized gold nanoparticles was investigated employing the method of Mensor *et al*. [23]. Different concentrations (10, 20, 40 and 60 µg/mL) of the gold nanoparticles and the standard (ascorbic acid) were prepared. Then, 1 ml of the prepared DPPH (0.3 mM) was added to each concentration containing the gold nanoparticles. The standard and other solutions were then incubated in dark place for 1 h. For the control, 2 ml of methanol was added to the prepared DPPH and was also incubated in the dark. The optical densities of the incubated solutions were taken at 517 nm after an hour of the radical scavenging activities and the result were calculated in percentage using the formula:

$$\text{DPPH scavenging (\%)} = \frac{Abscontrol - Abssample}{Abscontrol} x100$$

3. Results and Discussion

3.1 Biogenic formation of the AuNPs

The biofabricated gold nanoparticles stabilized within 15 min at photoactivation, producing dark brown coloration (Figure 1). AuNPs solutions exhibiting color ranging from yellowish brown through cherry red, green brown, red brown, pale yellow to dark green have been reported by several authors [24, 25]. This change in coloration established the role of the plant materials properties used i.e. the presence of different macromolecules such as proteins in the extracts that played catalytic and stabilization roles in the formation of the nanoparticles [9].

3.2 Characterization of the Biofabricated Gold Nanoparticles

The UV-Vis spectrum of the gold nanoparticles displayed maximum absorbance at a wavelength of 536 nm as shown in Figure 2c and this is within the surface plasmon reson-

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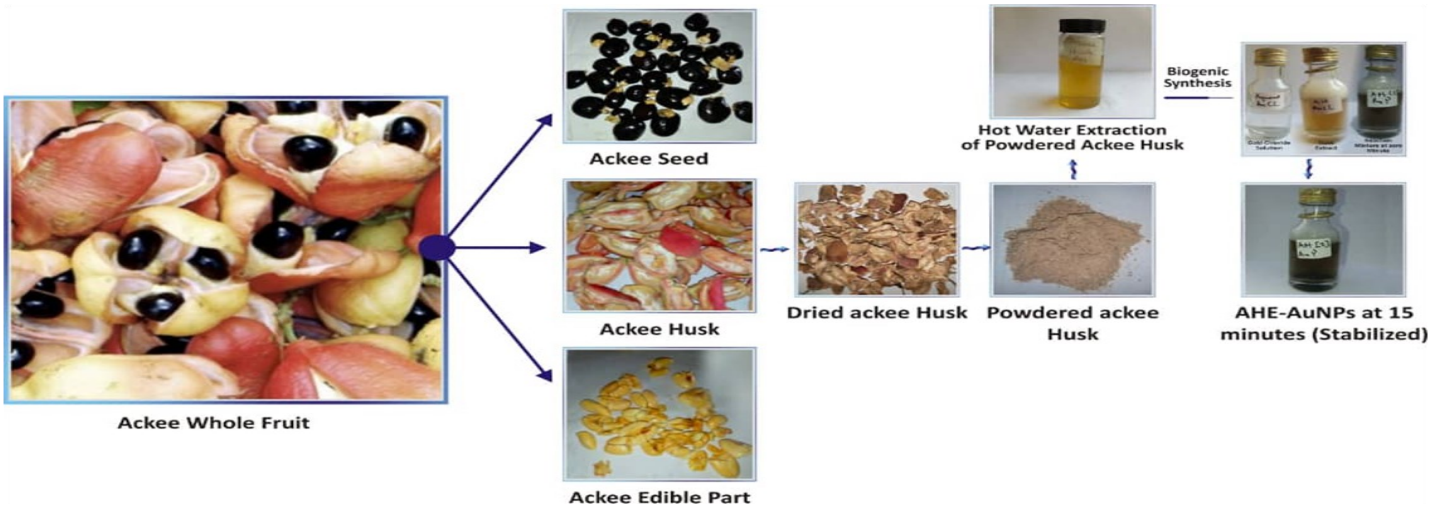

Fig. 1: Green synthesis stages of AuNPs from the *Blighia sapida* husk extract

ance range of 510-614 reported for AuNPs [8-10, 25-28]. Figure 2a shows the spectra of the precursor (aqueous gold chloride) while Figure 2b shows the spectra of the reducing/capping agents (aqueous crude extract of the *Blighia sapida* husk). Figures 2a and 2b were also presented to establish the occurrence of new reaction in Figure 2c at the mixture of $HAuCl_3$ (the precursor) and aqueous crude extract of the *Blighia sapida* husk (the reducing/capping agent) which resulted in the reduction of Au^{3+} to Au^0.

Fourier Transform Infra-red spectroscopy spectrum for photosynthesized AuNPs (Figure 3) manifested strong peaks at 3792.9, 3346.2, 2971.4 and 1704.9 cm^{-1}, implicating proteins and phenolic compounds as the capping and stabilization molecules involved in the biotransformation process that produced the AuNPs. The band 3294 cm^{-1} is typical of N–H bond of amines, while that of 1635 cm^{-1} is indicative of C=C stretch of alkenes or C=O stretch of amides [15, 29]. The phytochemicals/macromolecules present in the crude plant extract evidently make a coating that shields the gold nanoparticles. Respectively plant secondary metabolites engineered the biofabrication and capping of the AuNPs [10].

According to CFNI's "Food Composition Tables for the English-speaking Caribbean" [30], the contents of a 100 g serving of "Ackee, canned, drained" are as follows: Water (76.7 g), Energy (625 kJ or 151 kcal), Protein (2.9 g), Fat (15.2 g), Saturated fat (0 g) Cholesterol (0 mg), Total carbohydrate (0.8 g), Dietary fibre (2.7 g), Calcium (35 mg), Iron (0.7 mg), Potassium (270 mg), Sodium (240 mg), Zinc (1 mg), Vit A, thiamin (0.03 mg), riboflavin (0.07 mg), niacin (1.1 mg), total folacin (41 microgram), and Vit C (30 mg)[31]. All the macromolecules present in Ackee as reported above lent credence to the strong peaks observed in the FTIR spectrum of the AuNPs.

The AuNPs were fairly spherical in shape with sizes ranging from 23 to 126.7 nm (Figure 4a), which is in agreement with those earlier reported. Huang *et al.* [16] reported size range from 21.5 to 80 nm; Narayanam and Sakthivel [32] reported size range from 6.7 to 57.9 nm; Islam *et al.* [33] reported size range from 50 to 80 nm on the gold nanoparticles synthesized from plant system [34]. The EDX patterns (Figure 4b) showed the intense presence of gold in the AuNPs colloidal suspension.

3.3 Antifungal Activities of AHE-AuNPs

The biosynthesized AuNPs showed significant effect against the growth of *Candida albicans* (58.9%), *Aspergillus niger* (50%), *Aspergillus flavus* (63.4%), *Aspergillus fumigatus* (61.4%) and *Fusarium solani* (38.0%) (Figure 5) as against the abundant growth on the control plates. According to Yazar and Jutta [35], fungi like molds and some yeast are known to be toxigenic because of their

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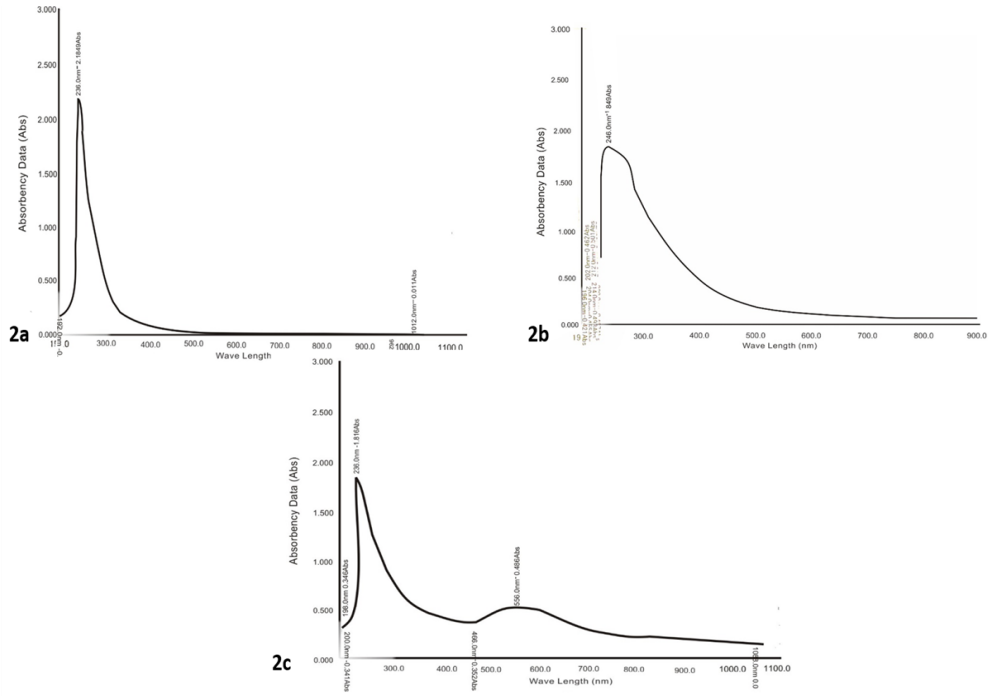

Fig. 2**:** (2a), the UV-vis absorption spectrum of the aqueous gold chloride; (2b), the UV-vis absorption spectrum of the *Blighia sapida* husk extract; (2c), the UV-vis absorption spectrum of the photosynthesized AuNPs

ability to produce mycotoxin as their secondary metabolites [36, 37]. Mycotoxins are poisonous chemical compounds [38], and toxic to man if consumed. Furthermore, toxigenic fungi are associated with deterioration, spoilage and contamination of stored farm produce likes seeds [39] leading to shortage of food and great loss to farmers. In developing countries like Nigeria, this has been a nightmare for local farmers and large scale farmers causing their net gains to be a fractions of the initial values [38, 40, 41].

A lot of chemical methods have been employed in inhibiting the infestation of toxi-

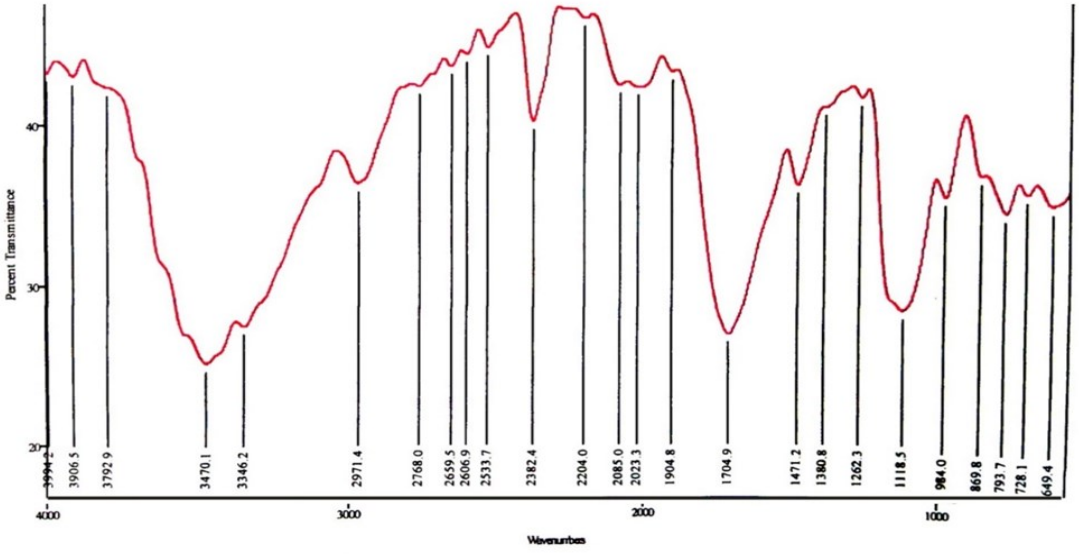

Fig. 3: The FTIR spectrum of the biofabricated AuNPs

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genic fungi but all these approaches come with problems associated with pollution, food poisoning and even mortality [42]. On the evidence of these challenges, a need for safe and ecofriendly approach is needed, and this antifungal study of the green synthesized gold nanoparticles have proven to be ecofriendly and safe in the inhibition of mycelia growth of some toxigenic fungi. To the best of our knowledge, no work has been reported on the antifungal activities of gold nanoparticles synthesized from *Blighia sapida* husk extract.

3.4 Thrombolytic Activities of the AuNPs

The thrombolytic properties of the bio fabricated gold nanoparticles is shown in Figure 6. The biofabricated AuNPs rapidly dissolved pre-formed healthy donor human blood clots in the Eppendorf tube within a period of 30 min. The control experiment performed with the gold chloride solution and the husk extract showed lesser dissolution of

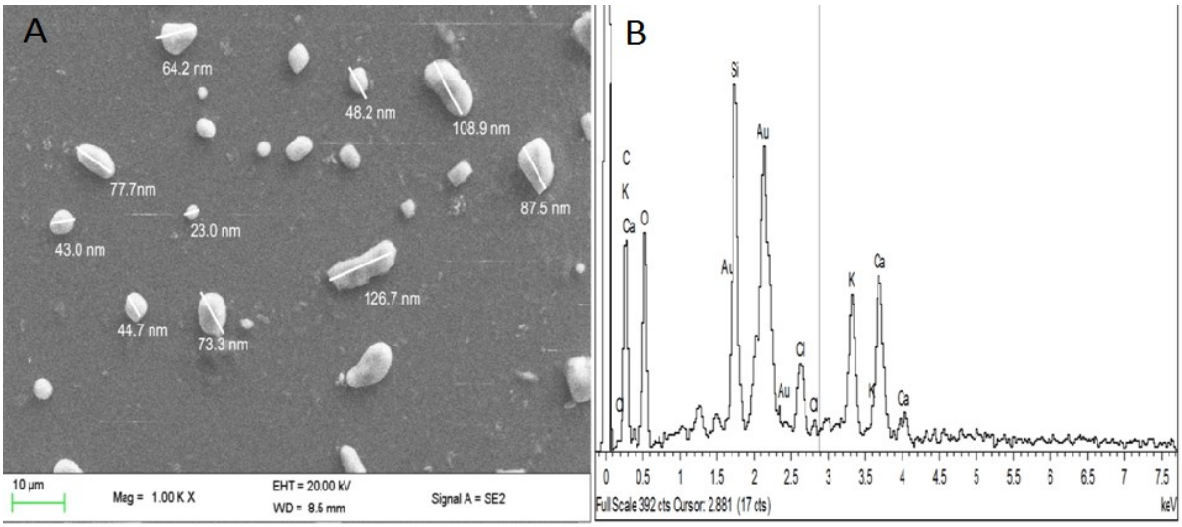

Fig. 4: A, Scanning Electron Microscope micrograph of the bio fabricated AuNPs; B, Energy dispersive X-ray signal of the biofabricated AuNPs

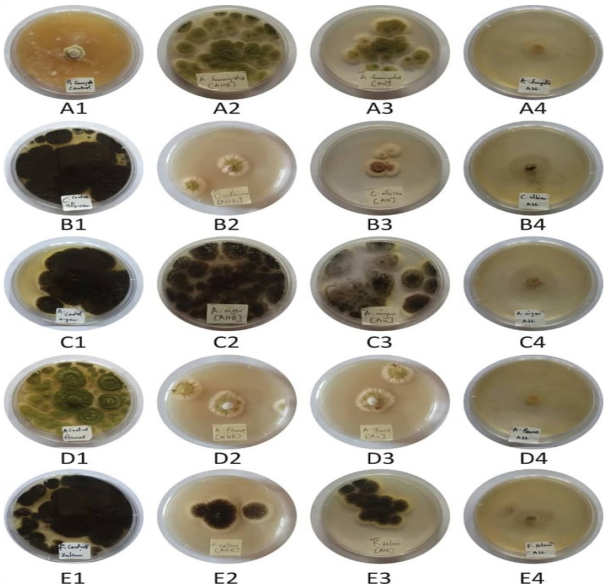

Fig. 5: Antifungal activities of biosynthesized AuNPs, Ackee husk extract and aqueous gold chloride; A: *Aspergillus fumigatus*, B: *Candida albicans*, C: *Aspergillus niger*, D: *Aspergillus flavus*, E: *Fusarium solani*. 1: PDA only, 2: PDA + *Blighia sapida* husk extract, 3: PDA + $HAuCl_3$, 4: PDA + biosynthesized AuNPs.

of blood clot (Figure 6). The gold nanoparticles showed thrombolytic activities of 48.70, 49.13, 50.00 and 54.74% at 250, 500, 750 and 1000 µg/ml (Table 1). These thrombolytic activities observed were consistent with those obtained using AuNPs on similar studies [8, 28, 43]. The treatment of thrombus through nanoparticles is highly necessary due to the properties they possessed, and this research has provided more solution that may aid more breakthrough in the treatments of thrombus/embolism.

3.5 Anticoagulant Activities of AuNPs

The anticoagulation activities of the biofabricated gold nanoparticles is shown in Figure 7. The AuNPs prevented coagulation of fresh human blood in a similar way as obtained in EDTA bottle. In the control samples, blood clots were formed in fresh

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collected in ordinary clean bottle and those treated with plant extracts alone. The synthesized gold nanoparticles showed anticoagulant activities of 54.31, 56.46, 75.00 and 96.12% at 250, 500, 750 and 1000 µg/ml (Table 2). The results obtained are in conformity with anticoagulant potentials of gold nanoparticles synthesized from diverse biomolecules as previously reported [8, 22, 28, 43, 44].

The blood coagulation system is important to ensure steady blood flow, prevents bleeding, and also assists in the innate immune system to prevent spread of infectious agents. However, blood clot resulting from infections can damage tissues and cause organ failure, which are frequently associated with the incidences of cardiovascular disorders, autoimmune reactions, allergic responses, injuries and emergence of cancer. In medicine, the use of heparin are faced with the challenges associated with drug instability, complications resulting from excessive bleeding, and high cost of treatment. These challenges can be mitigated through nanobiotechnology. The prevention of aggregation of platelets by non-toxic bio fabricated AuNPs to inhibit the formation of blood clots should be encouraged.

Table 1: Thrombolytic activities of the biosynthesized gold nanoparticles

Samples	W_3 (g)	W_5 (g)	$\frac{W_3 - W_5}{W_3} \times 100$
BSHE	2.32	1.23	46.00
$AuCl_3$	2.32	1.25	46.12
AuNPs (µg/ml)			
250	2.32	1.19	48.70
500	2.32	1.18	49.13
750	2.32	1.16	50.00
1000	2.32	1.05	54.74

BSHE, *Blighia sapida* husk extract

Table 2: Anticoagulant activities of the biosynthesized gold nanoparticles

Stages	W_3 (g)	W_5 (g)	$\frac{W_3 - W_5}{W_3} \times 100$
BSHE	2.32	1.26	45.68
$AuCl_3$	2.32	1.02	56.03
AuNPs (µg/ml)			
250	2.32	1.06	54.31
500	2.32	1.01	56.46
750	2.32	0.58	75.00
1000	2.32	0.09	96.12

BSHE, *Blighia sapida* husk extract

3.6 Antioxidant Activity of the AuNPs

The *in vitro* antioxidant activities of the AuNPs were investigated employing DPPH free

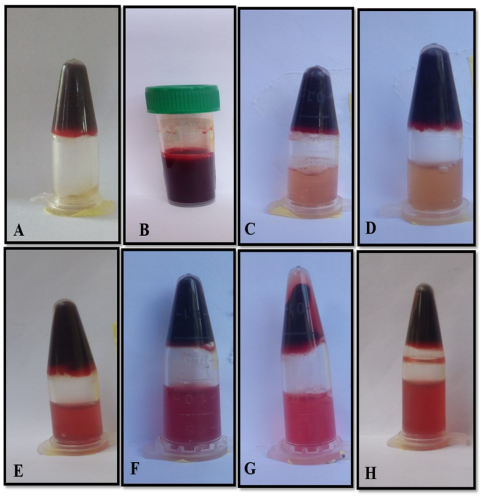

Fig. 6: Thrombolytic properties of the biofabricated gold nanoparticles (A, Clot blood; B, Blood + EDTA; C, Blood + $AuCl_3$; D, Blood + *Blighia sapida* husk extract; E, Blood + AuNPs (250 µg/ml); F, Blood + AuNPs (500 µg/ml); G, Blood + AuNPs (750 µg/ml); H, Blood + AuNPs (1000 µg/ml)

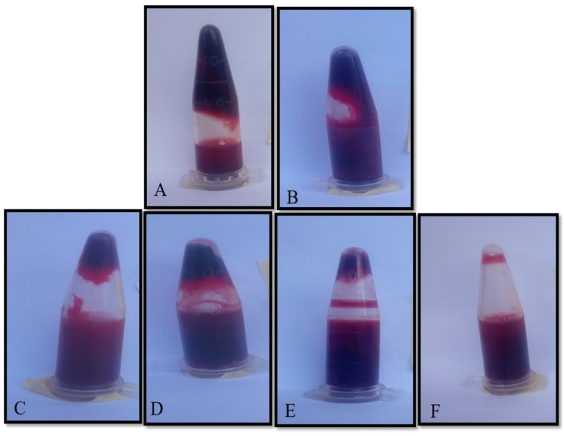

Fig. 7: Anticoagulant properties of the biofabricated gold nanoparticles (A, Blood + $AuCl_3$; B, Blood+ *Blighia sapida* extract; C, Blood + AuNPs (250 µg/ml); D, Blood + AuNPs (500 µg/ml); E, Blood + AuNPs (750 µg/ml); F, Blood + AuNPs (1000 µg/ml)

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Table 3: Antioxidant activities of the biosynthesized gold nanoparticles

Stages	Free radical scavenging (%)
Control	
Ascorbic acid	74.22
AuNPs (µg/ml)	
10	80.75
20	81.11
40	87.91
60	89.34
Blighia sapida husk extract	62.31

radical scavenging assay at different concentrations of 10, 20, 40 and 60 µg/ml. DPPH has been known to be used in investigating free radical scavenging properties of biosynthesized nanoparticles [7, 15, 43] as it is a stable organic free radical [45]. Table 3 shows the dose (concentration) dependent increase for the DPPH scavenging activity of the AuNPs. The varying concentration of the bio synthesized AuNPs (10, 20, 40 and 60 µg/ml) scavenged DPPH by 80.75, 81.11, 87.91 and 89.34% respectively. However, the free radical scavenging activities of the biosynthesized nanoparticles are greater than that of the crude extract of Ackee husk and ascorbic acid used as the control. This lent credence to the reducing power of organic compounds responsible for the bio-fabrication of the AuNPs and the larger surface area, a feat characterized with nanoparticles.

The generation of free radical in the body is almost inevitable due too heavy exposure to fumes, CO from incomplete combustions [46, 47] and poor nutrition producing reactive oxygen species (ROS) in the body leading to chains of reaction later resulting to oxidative stress and when left unchecked could cause cardiovascular disease [48], cancer [49] and ageing to mention a few. The effect and damage caused by free radicals in the body or any medium are fatal. The free radical scavenging potentials of the green synthesized gold nanoparticles from ackee husk in this study evidently proffer a solution to the problem.

4. Conclusion

This research work establishes the fact that the extract obtained from the husk of *Blighia sapida* fruit can be used for the green synthesis of gold nanoparticles. The *Blighia sapida* husk extract served both as the reducing and capping agents against the gold chloride which was the precursor, leading to the formation of agglomerated, spherical-shaped gold nanoparticles with sizes ranging from 23 to 126.7 nm. The gold nanoparticles synthesized showed inhibitory properties at 200 µg/ml. against the mycelia of five toxigenic fungi. Its biomedical application also proved its relevance in modern medicine. This research work has lent credence to the relevance of an agrowaste (*Blighia sapida* fruit husk) in the green synthesis of gold nanoparticles, and also established the effective use of *Blighia sapida* husk as capping/reducing agent in the green synthesis of gold nanoparticles and the demonstration of its antifungal, antioxidant, anticoagulant and thrombolytic activities.

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