Design and evaluation of the antimicrobial properties of ackee seed extract silver nanoparticle film formulations

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Polymers in Medicine, ISSN 0370-0747 (print), ISSN 2451-2699 (online) *Polim Med.* 2020;50(2):65–73

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Funding sources
None declared

Conflict of interest
None declared

Received on August 13,2020
Reviewed on October 25, 2020
Accepted on November 12, 2020

Abstract

Background. Plant-extract-reduced metal nanoparticles provide means of overcoming microbial resistance. Incorporating them into appropriate pharmaceutical formulations will enhance their portability and ease of administration.

Objectives. To synthesize silver nanoparticles using methanol extracts of the seeds of *Blighia sapida* as capping agents and formulating the products in antimicrobial films.

Material and methods. Phytochemical screening of the methanol extract of *Blighia sapida* K.D. Koenig (ackee) seeds was performed and its antioxidant properties were determined using DPPH (1,1-diphenyl--2-picrylhydrazyl) assay. The green synthesis of ackee seed extract silver nanoparticles (ASAgNPs) was accomplished with reacting 1 mM of aqueous silver nitrate ($AgNO_3$) and the methanol extract in a flask; the bioreduction was performed at 37°C for 72 h. The resulting nanoparticles were lyophilized and characterized using UV-visible spectrophotometry, Fourier-transform infrared spectroscopy (FTIR) and photomicrography. The nanoparticles were further formulated into films using starch and carboxymethyl cellulose using the solvent evaporation method. The extract, biosynthesized nanoparticles and film formulations were screened for antimicrobial activity against several pathogens using the agar well diffusion method.

Results. The methanol seed extracts of the ackee fruit contained saponins, tannins, flavonoids, terpenoids, and anthraquinones. The extract exhibited significant antioxidant properties. The nanoparticles and film formulations had a broader range of activity against microbes than the plant extract, exhibiting significant activity against *Escherichia coli* ATCC 700728, *Salmonella typhi* ATCC 14028, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853. Activity was also observed with *Candida krusei*, *C. albicans*, and *Penicillium* sp. It is noteworthy that this last organism showed resistance to fluconazole.

Conclusions. Ackee seed extract silver nanoparticles exhibited a synergistic antimicrobial activity against several pathogens. Film formulations of the nanoparticles retained this antimicrobial activity and allowed the product to be presented in a consumer-ready form.

Key words: silver nanoparticles, ackee seeds, antimicrobial films, *Blighia sapida*

Cite as
Odeniyi MA, Olusomoka E, Odeniyi OA, Adebayo-Tayo BC. Design and evaluation of the antimicrobial properties of ackee seed extract silver nanoparticle film formulations. *Polim Med*. 2020;50(2):65–73. doi:10.17219/pim/130388

DOI
10.17219/pim/130388

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Introduction

The need for new, potent and affordable drugs for the treatment of microbial infections in the developing world is one of the issues facing global health today. However, finding effective drugs for the treatment of these infections is hindered by factors ranging from microbial resistance to safety, compliance and cost. The use of medicinal plants for curative purposes is as old as mankind, but coupled with the latest developments in nanotechnology, they can be used to treat diseases. A synergistic formulation is expected to result from combining the antimicrobial properties of plant extracts with the metal nanoparticles in the form of film formulations for ease of use.

The green synthesis of metal nanoparticles from plant extracts is an attractive alternative to physical and chemical methods. This method is simple, the costs are low, the production time is short, and it is amenable to large-scale production, does not require extreme temperature or pressure, and eliminates the need for toxic reagents.[1–3] The synthesis of metal nanoparticle using plants has the additional advantage of stabilizing the nanoparticles, since plant biomolecules exert a twofold effect of reducing and capping the biosynthesized nanoparticles.[4–6]

Ackee (*Blighia sapida* K.D. Koenig; Family: Sapindaceae) is a herbaceous, biennial plant. It is native to West Africa and is also cultivated in India and the American tropics. It is well-distributed throughout Nigeria and is found in drier forests of the savannah region.[7] Ackee seeds contain bioactive substances such as saponins, flavonoids, tannins, terpenoids, alkaloids, steroids, and anthraquinones.[8–10] These bio-constituents contribute to its antioxidant, anti-inflammatory, anti-diarrheal, and antimicrobial activities. Ackee provides medicinal value for traditional healers in Nigeria and across Africa for the treatment of several ailments.[11] Ackee fruit is rich in essential fatty acids, vitamin A, zinc, and protein.[8,12]

While several studies have reported the antibacterial activity of silver nanoparticles (SNPs) synthesized from plant extracts, no research has been performed on the synthesis of SNPs from *B. sapida* and subsequent formulation into antimicrobial and antioxidant films for ease of application.

Material and methods

Collection of ackee seeds and preparation of plant material

Seeds of *Blighia sapida* were collected during the fruiting season from the Botanical Garden, University of Ibadan, Nigeria. The seeds were thoroughly washed, rinsed and oven-dried at a temperature of 40°C. The oven-dried seeds were then blended and extracted using methanol.

Method of extraction

Two kilograms of the dried seed sample was transferred into a glass container; 7.5 L of pure methanol was added, then stirred every 2 h with a glass rod and allowed to stand for 72 h. The solvent (now containing the extract) was collected using a muslin bag. The filtrate was further filtered using Wattman No. 1 filter paper. This process was repeated twice with another 5.0 L of pure methanol added each time to the chaff. The combined filtrate was then concentrated with the aid of a rotary evaporator (Heidolph Laborota 400; Heidolph Instruments, Kelheim, Germany) set at 40°C, after which the sample was further concentrated using a vacuum oven set at 40°C. The dried extract was weighed and the percentage yield was calculated.

Both qualitative and quantitative phytochemical screening of the plant extract were performed using standard procedures.

Antioxidant activity according to DPPH scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical thanks to the free electron that is delocalized around the molecule, thereby preventing the dimerization that other free radicals undergo. This delocalization gives the molecule its deep violet color which is characterized by an absorption band in ethanol solution at a wavelength of about 517 nm. The DPPH is reduced and the violet color is lost when it is placed in a substrate that can release a hydrogen atom. To determine the antioxidant potential of the test samples, the change in optical density of DPPH radicals was monitored. The sample extract (0.2 mL) was diluted with methanol and 2 mL of a DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm.[13] The percentage of DPPH radical scavenging was calculated using the equation:

$$\% \text{ inhibition of DPPH radical} = ([A_{br} - A_{ar}]/A_{br}) \times 100,$$

where A_{br} is the absorbance before the reaction and A_{ar} is the absorbance after the reaction had taken place.

Total antioxidant capacity according to phosphomolybdenum complex formation

The measurement of total antioxidant capacity employed a spectrophotometric principle based on the reduction of Mo (VI) to a green phosphate Mo (V) complex by the sample analyte at an acidic pH. In a test tube, 0.1 mL of the sample solution (100 µg) was combined with 1 mL of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tube was covered and kept in a boiling water bath for 90 min, then cooled to room temperature. The absor-

bance of the aqueous solution was measured at 695 nm against a blank using a UV spectrophotometer (CE7400 AQUARIUS, Cambridge, UK).[12,13]

Synthesis of the silver nanoparticles using the *Blighia sapida* seed methanol extract

The methanol extract of ackee seeds was used for the biosynthesis of silver nanoparticles. Mixtures of the methanol extract at concentrations of 1:4 and 1:9 were mixed with 1 mM of an aqueous solution of silver nitrate ($AgNO_3$) in a 250-milliliter Erlenmeyer flask containing 100 mL of 1 mM of the aqueous solution of $AgNO_3$. The resulting ackee seed silver nanoparticles (ASAgNSPs) were placed into an incubator for complete bio-reduction at a temperature of 37°C for 24–72 h and were visually observed for changes in color.[6]

Characterization of the plant extract silver nanoparticles

The biosynthesized ASAgNSPs were characterized using UV-visible spectroscopy. The reduction of $AgNO_3$ to Ag^+ by the plant extract was verified using an UV-visible spectrophotometer (CE7400 AQUARIUS, Cambridge, UK). The absorption spectra of the samples were recorded at intervals of 24–72 h.

Fourier-transform infrared analysis

The Fourier-transform infrared analysis (FTIR) analysis of the ASAgNSPs was performed using a potassium bromide (KBr) pellet (Perkin Elmer, Waltham, USA) in transmission mode. Transmission spectra were obtained using 64 scans at a resolution of 8 cm^{-1} in the spectral range of 4000–400 cm^{-1}.

Antimicrobial characteristics of the synthesized ASAgNSPs

The ackee seed methanol extract and biosynthesized ASAgNSPs were screened for antimicrobial activity using the agar well diffusion method to compare their effectiveness against different microorganisms.

Using the cup-plate method, a sterile nutrient agar was prepared and poured into sterile Petri dishes and allowed to solidify. Each plate was inoculated with 25 µL (containing about 10^8 CFU/mL) of either *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 35218, *Citrobacter freundii* ATCC 8090, *Salmonella typhi* ATCC 14028, *E. coli* ATCC 700728, or *E. coli* ATCC 11775. Four wells with a diameter of 8 mm were bored using a sterile cup borer. The respective wells were filled with the methanol extract of ackee seeds, the synthesized silver nanoparticles of the extract, the $AgNO_3$solution, and 25 µL of streptomycin (1 mg/mL) serving as a positive control. The plates were incubated at 37°C overnight. The antibacterial activity of each component was measured in terms of the mean diameter (in mm) of the zone of inhibition produced by each component at the end of the incubation period.

Antifungal properties of synthesized *Blighia sapida* nanoparticles

A potato dextrose agar medium was prepared and poured into sterile Petri dishes and allowed to solidify. Fungal pathogens (25 µL) were spread onto respective plates labelled *Aspergillus niger*, *Rhizopus* sp., *Candida albicans*, *C. krusei*, and *Penicillium* sp. Four wells 8 mm in diameter were bored using a sterile cup borer. The methanol extract, fluconazole (as a positive control), dimethyl sulfoxide (as a negative control), the $AgNO_3$ solution, and the silver nanoparticles were transferred into the respective wells and the plates were incubated at 28 ±2°C overnight. The antifungal activity of each component was expressed in terms of the mean diameter (in mm) of the zone of inhibition produced by each component against the fungi at the end of the incubation period.

Formulation of antimicrobial film

Three different kinds of film were formulated: 1 film without silver nanoparticles, serving as the blank or control, and 2 other films with different concentrations of the silver nanoparticles (1:4 and 1:9).

Ten grams of carboxymethyl cellulose (CMC) sodium salt was mixed with 1 L of distilled water in a large beaker using a magnetic stirrer. Corn starch (1.2 g) was gelatinized in 50 mL of distilled water at 80°C for 45 min. The gelatinized starch was added to the CMC solution and allowed to mix for 1 h. Then, 0.6 g of aluminum sulfate in 20 mL of distilled water was added to the beaker in order to investigate the optimal cross-linkage; the solution was allowed to mix for another 30 min. The Petri dishes were spread with 30 mL of the gelatinized starch-CMC solution and the silver nanoparticles were loaded onto them and dried at 70°C until a film formed.

Antimicrobial assay of formulated films

An antimicrobial assay was carried out using the films containing biosynthesized ASAgNSPs, and a control film against the clinical isolates on which the biosynthesized nanoparticles were effective, through the standard disk diffusion method. The 5-millimeter disk-shaped films were placed on sterile microbe-swabbed media in Petri dishes and incubated at 37°C (for bacteria) or 28 ±2°C (for fungi) for 24 h and 72 h, respectively. The diameter of the zone of inhibition was measured and recorded in millimeters.

Thickness and folding endurance of film

After being cut into 1 × 1-inch strips, the films – with or without nanoparticles – had their thickness measured with a micrometer screw gauge. The thickness of the film reflects how well the polymer is incorporated into the formulation. The folding endurance of the film describes the number of times the film can be bent over or folded at a particular point until it breaks.

Statistical analysis

Statistical analysis was carried out with one-way analysis of variance (ANOVA) and the t-test, using GraphPad Prism v. 7 software (GraphPad Software Inc., San Diego, USA). At a 95% confidence interval (95% CI), p-values less than or equal to 0.05 were considered significant.

Results and discussion

Phytochemical analysis of the methanol extract of ackee seeds revealed that they contained phytoconstituents such as saponins, tannins, flavonoids, terpenoids, steroids, alkaloids, and anthraquinones. Cardiac glycosides were not found, though saponin was present (Table 1). Saponins primarily modify the composition of the rumen microbial population, which results in a modification of rumen fermentation. According to Delmas et al.,[14] saponins are very toxic to fungi. The antifungal activity of saponins against *Trichoderma viride* was formerly used as a method of identifying them.

Tannins inhibit extracellular microbial enzymes, reduce bioavailable iron, and form hydrogen bonds, specific interactions with proteins such as enzymes or cell envelopes, and complex formulations with polysaccharides. Tannins have been found to have antimicrobial activity against fungi, bacteria and yeast.[15]

Flavonoids exhibit a wide range of activity, ranging from antimicrobial to anti-inflammatory, analgesic, antiallergic, and antioxidant effects. They help reduce the risk of cancer and prevent menopausal symptoms.[16] Their antibacterial effects are thought to come from their ability to form complexes with bacterial cell walls and extracellular and soluble proteins. Quercetin, a known flavonoid found in apples, has been shown to possess antioxidant properties. Both tannins and flavonoids have been found to propagate synergistic effects, which are responsible for high antioxidant activity.

The seeds of *Blighia sapida* have some alkaloidal content, and alkaloids are very useful defense systems for plants. They protect the plant against herbivores and pathogens. Hence, it can be said that *Blighia sapida* seeds have anti-inflammatory, antioxidative, anticarcinogenic, anti-allergic, immunomodulatory, antifungal, antibacterial, and protective functions. In addition, they are useful in the production of soap due to their high saponin content.

The functional groups identified by the FTIR analysis of ackee seeds were primary and aromatic alcohols, amine, amide, carbonyl, carboxylic, and alkyl halide groups (Fig. 1). These molecules have been indicated in the bio-reduction of silver ions.[6]

The DPPH assay is a fast, reliable, and reproducible parameter for analyzing the in vitro antioxidant activity of pure compounds and plant extracts.[17,18] The percentage of scavenging antioxidant activity is dependent on the concentration of extract used. A decrease in the absorbance value of the methanol extract with a corresponding increase in the concentration of the extract signifies a good radical scavenging activity of the extract (Table 2). The percentage of scavenging activity of the extract increases with the concentration of the extract; the highest percentage of scavenging activity in this study (62.1%) was found at a concentration of 1000 µg/mL. The standard ascorbic acid exhibited a higher percentage of scavenging activity than the extract at the same concentration because it contains more phenolic compounds than the extract. The total antioxidant capacity of the methanol extracts of ackee seeds showed an increase in absorbance values with a corresponding increase in the concentration of the extract, indicating that the extract possesses good antioxidant activity.

The method used for formulating the film dosage form was proposed by Suo et al.[19] and Weerawarna.[20] It in-

Table 1. Phytochemical constituents of *Blighia sapida* seeds

Test	*B. sapida*
Saponins	++
Tannins	+
Flavonoids	+
Cardiac glycoside	–
Terpenoids	+
Steroids	+
Alkaloids	+
Anthraquinones	+

Table 2. DPPH scavenging activity of methanol extracts of *Blighia sapida* seeds

Concentration	Ackee seed methanol extract [%]	Standard [%]
50 µg/mL	–	95.1
100 µg/mL	2.4	95.5
200 µg/mL	9.7	95.6
400 µg/mL	26.3	95.7
600 µg/mL	46.5	95.7
800 µg/mL	58.7	95.8
1000 µg/mL	62.1	96.9

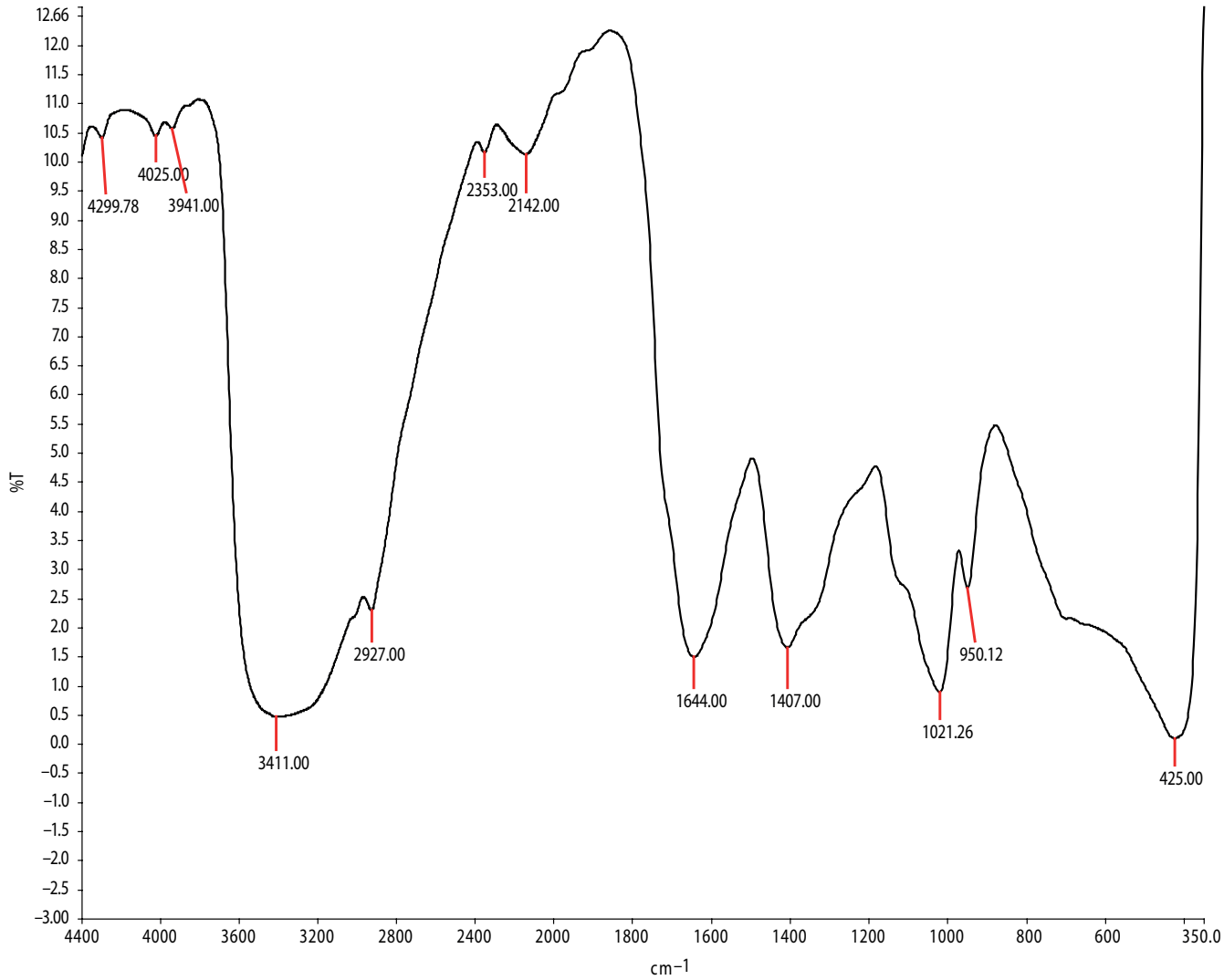

Fig. 1. FTIR spectroscopy of the biosynthesized silver nanoparticle (1:9) from ackee seed extracts

volves using a cross-linker, aluminum sulfate, which helps in holding the polymer chains together to form a film with the desired properties. The use of 2 or more polymers have been found to improve the physical properties of films, such as thickness and mechanical strength; therefore, a blend of sodium carboxymethyl cellulose and corn starch were used in the preparation. A small amount of a plasticizer – 1% glycerol – was also incorporated into the formulation to improve the flexibility of the film. The plasticizer and the polymers used in the preparation were compatible, as this is a crucial criterion that must be met.

The stability of the films was dependent largely on the level of cross-linkage across the polymer chains and the plasticizing effects of the plasticizers used. Using cross-linkers in the preparation improve the physical stability of the film by imparting thickness and mechanical strength and by preventing dissolution. However, ionic functional groups across the chain have been found to encourage water diffusion within the network.[21] It has also been discovered that the concentration of plasticizer in the preparation could result in either brittle or excessively smooth films. Hence, an appropriate amount of plasticizer is required to formulate a film with the desired characteristics (Fig. 2).

In the antimicrobial test carried out on the extract, the extract only demonstrated activity against 1 bacterium, *E. coli* ATCC 25930 (12 mm), and activity against 4 strains of fungi, *C. albicans* (14 mm), *Rhizopus* (22 mm), *C. krusei* (14 mm), and *A. niger* (14 mm). The extract showed the highest level of antifungal activity against *Rhizopus*, with a recorded zone of inhibition of 22 mm in diameter. Therefore, the extract had a more pronounced antifungal effect, encouraging its use in topical or dermatological preparations (Table 3). The biosynthesized nanoparticles (1:4 and 1:9) displayed good antimicrobial activity against the tested pathogenic organisms, as shown in Tables 3, 4.

The 1:9 ASAgNSPs demonstrated activity against 8 bacterial organisms, with most activity against *S. aureus* ATCC 29213 (a 11-mm zone of inhibition). The least activity was observed against *E. coli* ATCC 25930, with zones of inhibi-

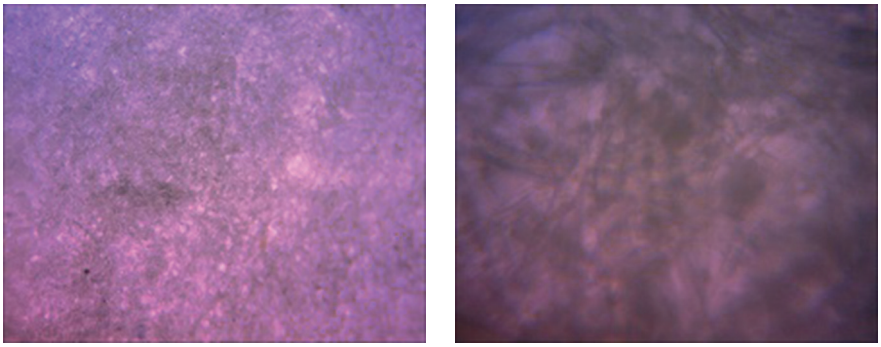

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Fig. 2. Photomicrograph of film strips without silver nanoparticles at different magnifications

Table 3. Antimicrobial activity of ackee seed extract silver nanoparticles (ASAgNPs) against various microorganisms, according to the diameter of the zone of inhibition (in mm)

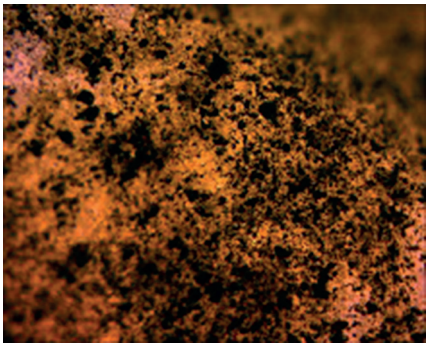
Test organism	*Blighia sapida* methanol extract	ASAgNPs		Streptomycin	Silver nitrate solution
		1:4 concentration	1:9 concentration		
Escherichia coli ATCC 25930	12.0	–	2.0	18.0	6.0
Citrobacter freundii ATCC 8090	–	4.0	9.0	18.0	3.0
Staphylococcus aureus ATCC 29213	–	12.0	11.0	16.0	7.0
Salmonella typhi ATCC 14028	–	9.0	6.0	18.0	12.0
Escherichia coli ATCC 700728	–	6.0	10.0	15.0	3.0
Pseudomonas aeruginosa ATCC 27853	–	12.0	10.0	22.0	9.0
Escherichia coli ATCC 11775	–	9.0	10.0	20.0	–
Escherichia coli ATCC 35218	–	6.0	6.0	20.0	4.0

Table 4. Inhibitory activity of ackee seed extract silver nanoparticles (ASAgNPs) against fungal organisms, according to the diameter of the zone of inhibition (in mm)

Test organism	*Blighia sapida* methanol extract	ASAgNPs		Fluconazole	Silver nitrate solution
		1:4 concentration	1:9 concentration		
Candida krusei	14.0	3.0	5.0	12.0	–
Candida albicans	14.0	2.0	6.0	14.0	2.0
Rhizopus	22.0	6.0	6.0	–	–
Penicillum sp.	–	2.0	4.0	–	2.0
Aspergillus niger	14.0	–	–	–	–

tion 2 mm in diameter. The 1:4 ASAgNSPs showed activity against 7 of the tested bacterial organisms; *E. coli* ATCC 25930 was resistant to it. The highest level of antibacterial activity was against *S. aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853, with zones of inhibition 12 mm in diameter. Of the tested fungal pathogens, only *Penicillum* sp. demonstrated resistance to the biosynthesized 1:4 nanoparticle, while the other ASAgNSP (1:9) showed good antifungal activity against 4 fungal pathogens (Fig. 3). The strongest antifungal activity of the biosynthesized 1:4 nanoparticle was against *Rhizopus* (6 mm), while the least antifungal activity was against *C. albicans* (2 mm) and *Penicillum* sp. (2 mm). The biosynthesized 1:9 nanoparticle recorded its highest antifungal activity against *C. albicans* (6 mm) and *Rhizopus* (6 mm), with the least antifungal activity being against *Penicillum* sp. (4 mm). However, *Rhizopus* and *Penicillum* sp. showed resistance to the control (fluconazole) (Fig. 4).

The ASAgNSP films (Tables 5, 6) also demonstrated good antimicrobial activity against both tested bacterial and fungal pathogens (Fig. 5). The highest antibacterial activity for the 1:9 ASAgNSP film was demonstrated against *E. coli* ATCC 700728, *Citrobacter freundii* ATCC 8090 and *P. aeruginosa* ATCC 27853 – each with zones of inhibition 15 mm in diameter. The least antibacterial activity was against *Salmonella typhi* ATCC 14028 (10 mm). However, *Klebsiella pneumoniae* showed resistance to the silver nanoparticle film. The 1:4 ASAgNSP film displayed the most antibacterial activity against *E. coli* ATCC 700728, with a 15-mm-diameter zone of inhibition. The least activity was again against *S. typhi* ATCC 14028 (10 mm), while *E. coli* ATCC 25930 and *K. pneumoniae* showed resistance



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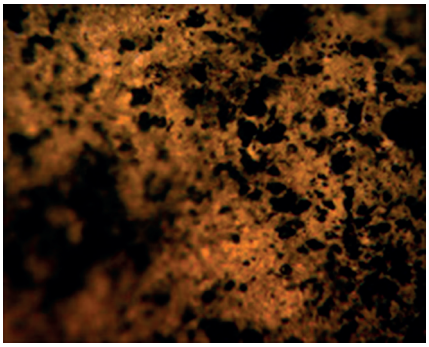

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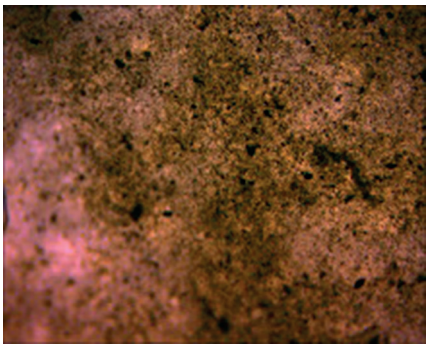
Fig. 3. Photomicrograph of ackee seed extract silver nanoparticles (1:4)



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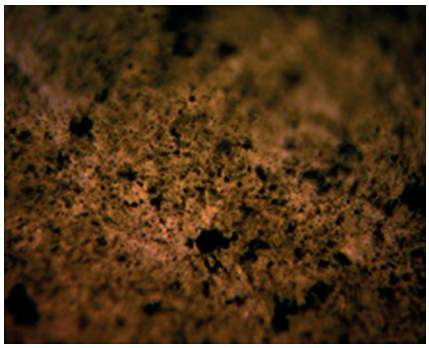

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Fig. 4. Photomicrograph of ackee seed extract silver nanoparticle film strips (1:9)

Table 5. Antimicrobial activity of ackee seed extract silver nanoparticle (ASAgNP) films against various microorganisms, according to the diameter of the zone of inhibition (in mm)

Test organism	Blank film	ASAgNP Film (1:4)	ASAgNP Film (1:9)
Escherichia coli ATCC 25930	–	–	11.0
Citrobacter freundii ATCC 8090	–	12.0	15.0
Staphylococcus aureus ATCC 29213	–	12.0	13.0
Salmonella typhi ATCC 14028	–	10.0	10.0
Escherichia coli ATCC 700728	–	15.0	15.0
Pseudomonas aeruginosa ATCC 27853	–	14.0	15.0
Escherichia coli ATCC 11775	–	12.0	12.0
Escherichia coli ATCC 35218	–	13.0	13.0
Klebsiella pneumoniae	–	–	–
Bacillus cereus	–	11.0	13.0
Proteus sp.	–	11.0	14.0
Aeromonas hydrophila	–	14.0	11.0

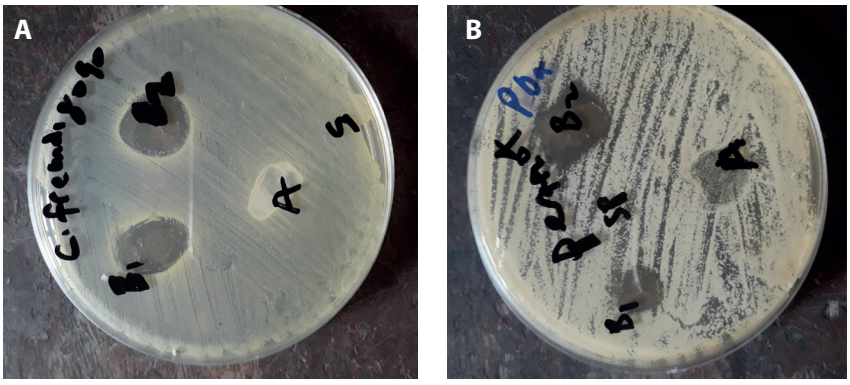

Fig. 5. Antimicrobial activity of ackee seed extract silver nanoparticle film strips against *Citrobacter freundi* (A) and *Penicillium* sp. (B)

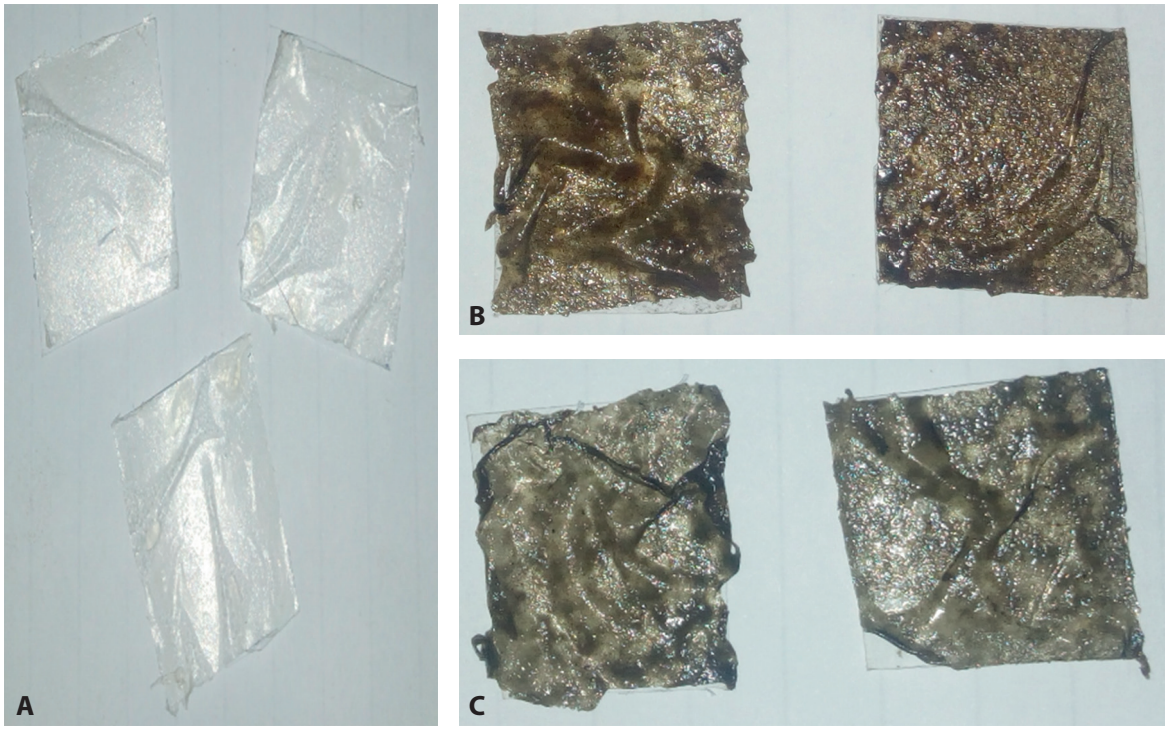

Fig. 6. Blank film strips without silver nanoparticles (A), film strips with ackee seed extract silver nanoparticles at a 1:4 concentration (B) and at a 1:9 concntration (C)

Table 6. Inhibitory activity of ackee seed extract silver nanoparticle (ASAgNP) films against fungal organisms, according to the diameter of the zone of inhibition (in mm)

Test organisms	Blank film	ASAgNP film (1:4)	ASAgNP film (1:9)
Candida krusei	–	7.0	11.0
Candida albicans	–	11.0	15.0
Rhizopus	–	8.0	8.0
Penicillum sp.	–	9.0	13.0
Aspergillus niger	–	–	–

to this silver nanoparticle film. The ASAgNSP film also demonstrated good antifungal activity against 4 out of the 5 tested fungal pathogens. The highest level of antifungal activity for the 1:9 film was seen against *C. albicans* (15 mm), with the least activity being against *Rhizopus* (8 mm); *A. niger* showed resistance to the 1:4 ASAgNSP film (Table 6). The highest level of antifungal activity for this film was also against *C. albicans*, with an 11-mm-diameter zone of inhibition; the least antifungal activity was against *C. krusei* (7 mm). No significant difference was observed in the activity of the 2 formulations. Furthermore, *A. niger* showed resistance to the film. The film without silver nanoparticles (blank) showed no antimicrobial activity. This demonstrates that the other components used in the formulation of the film had no antimicrobial properties themselves.

The biosynthesized silver nanoparticle served as a carrier for the extract, while the extract also served as a capping agent, both showing synergistic effects. The biosynthesized silver nanoparticle contained silver, which boosted the antimicrobial effects of the nanoparticle, as silver is found to have antimicrobial properties. The slightly higher values ($p > 0.05$) of the zones of inhibition of the silver nanoparticle film and the biosynthesized 1:9 nanoparticle in comparison to the 1:4 one suggests that more nanoparticles formed in the former (Table 5).

The thickness of the film is solely dependent on the amount and type of polymers used in the preparation. A film made from 2 or more polymers would yield a film of the desired thickness. The blank film was the thickest (0.21 mm), while the films containing silver nanoparticles were of relatively similar thickness (0.13 mm). The folding endurance is used to evaluate the mechanical properties of the film. It is determined by folding or bending the film at the same point to determine the number of times it can be folded until it breaks or cracks. The more folds, the higher the folding endurance and the higher the mechanical strength of the film. The film strips showed an appreciable level of folding endurance, as the fewest was 6 folds, recorded for the 1:9 ASAgNSP film. The most folds was for the film with no silver nanoparticles (blank), whereas the 1:4 ASAgNSP film was folded 10 times before it finally broke. The presence of pores within the film strips facilitated the diffusion of the materials incorporated into the film into the surrounding inoculated medium, thereby eliciting the antimicrobial activity (Fig. 6).

Conclusions

An environmental-friendly, non-toxic, cost-effective method has been devised for the biosynthesis of silver nanoparticles using *Blighia sapida* methanol extract as a capping agent. This method has been found to be a good alternative compared to the chemical synthesis of silver nanoparticles, and can be used in the commercial production of biosynthesized silver nanoparticles.

The biosynthesized ackee seed silver nanoparticle film displayed good antimicrobial activity against clinical pathogenic organisms, demonstrating a broad spectrum of activity against both gram-negative (*E. coli*), gram-positive (*S. aureus*) and fungal pathogens (*Penicillum* sp.); thus, they could be of great importance as microbial growth inhibitors, making them useful in antimicrobial control systems and medical devices.

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