Saponin, Antioxidant and Free Radical Scavenging Properties of Blighia *sapida* Pods

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ABSTRACT

Objective: To evaluate the saponin, antioxidant and free radical scavenging properties of *Blighia sapida* (ackee) pods. Saponins exhibit a wide range of medicinal properties which includes anticancer and anti-inflammatory activities.

Methods: Pods from the *Blighia sapida* fruit were evaluated for the presence of saponins by Fourier Transform Infra-Red Spectroscopy (FTIR). Methanolic extracts of the pods were also analyzed for total phenolics, antioxidant and free radical scavenging activities. Antioxidant activity was determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay and total phenolics by the Folin-Ciocalteu assay.

Results: The IR spectrum exhibited peaks which are consistent with the presence of triterpenoidal saponins. The peaks observed indicated the presence of hydroxyl (3317 cm^{-1}), alkene (1633 cm^{-1}) and ester (1030, 1242, 1367 cm^{-1}) functionalities. The pods of the fruit contain high levels of antioxidant (1.81 ± 0.11 mg/g) and free radical scavenging activities (69.70 ± 1.32 %).

Conclusion: *Blighia sapida* pods may be an untapped source of medicinally active compounds containing antioxidant, anticancer and free radical scavenging properties.

Keywords: Blighia *sapida*, ackee, pods, triterpenoid, saponins, antioxidant activity

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INTRODUCTION

Ackee (*Blighia sapida*) is native to West Africa but has been naturalized in Jamaica. The tree can be found interspersed throughout the island or in groves. The fruit is initially green in colour changing to red as the fruit matures. Upon full maturity, the pods open revealing three to four yellow arilli with black seeds. Only the arilli of fully mature fruits should be eaten. This is due to the presence of a toxic non proteinogenic amino acid, hypoglycin A, which is present in the immature fruit (1-3). As the fruit matures, the levels of hypoglycin A decreases from 711 mg/100 to concentrations below the detection limit of 1.2 mg/100 g in the edible ripe arilli (4). Ackee is a commercially significant local and export crop. The arilli of the mature fruit may be blanched, seasoned and cooked with codfish, (Jamaica's national dish) or processed in brine and canned for exported to the United States of America, Canada and the United Kingdom. In Benin the fruit is dried and traded in local markets (5). After processing the pods and seeds of the fruit are discarded.

Ways of utilizing byproducts of the ackee industry are being investigated. In Africa, the seeds and pods have been used in soap making (5) and the pods used for the laundering of clothes. Saponins are of emerging commercial significance with applications in the food, cosmetics and pharmaceutical industries (6). They display a wide array of medicinal properties such as anti-cholesterolemic (7), anti-inflammatory (8, 9), and anticancer (10) activities, and are precursor to therapeutic drugs such as cortisone, and contraceptive estrogens. They also exhibit surfactant properties. Structurally saponins are glycosides of triterpenes, steroids or alkaloids and are classified based on the nature of the aglycone (sapogenin). Oligosaccharides may be linked to the sapogenin via an ether or ester linkage at one or two glycosylation sites producing a mono-desmosidic or bidesmosodoc saponin, respectively (11).

FTIR spectroscopy was utilized to characterize the saponins present in the pods of the ackee fruit. The total phenolics, antioxidant and free radical scavenging activities of extracts of the pods of the fruit were also evaluated.

MATERIALS AND METHODS

Chemicals

All chemicals were analytical grade. Folin-Ciocalteu reagent and 1,1-diphenyl-2-picrylhydrazyl were purchased from Sigma Aldrich (USA).

Plant material

Mature ackee fruits were harvested from trees located on the campus of the University of the West Indies, Kingston, Jamaica. Fruits were separated into their individual components. Pods were dried to constant weight (55°C for 6 days, Gallenkamp Laboratory Oven OV-330, England) and milled using an Ika-Werke M20 Analytical Mill, Staufen, Germany (30 sec at 25°C).

Phytochemical screening

The saponin test was carried out on aqueous extracts of the milled ackee pods (12). Milled ackee pods (1 g) were extracted with distilled water (100 mL). The extract was filtered and vigorously shaken.

Fourier transform infrared spectroscopy

A Bruker Vector 22 Fourier Transform-Infra Red (FTIR) Spectrometer was utilized to record the infrared spectra of dried, milled samples of ackee pods. OPUS software was used to acquire and manipulate spectral data.

Total phenolic content

Total phenolics were determined using Folin-Ciocalteu reagent with slight modifications (13). Samples (200 mg) were extracted with methanol (2 mL, 80 %) containing 1 % hydrochloric acid at room temperature on an orbital shaker (Gallenkamp, England) set at 200 rpm. Extracts were then centrifuged (3200 rpm, 10 min). The resulting supernatant (100 µL) was reacted with Folin-Ciocalteu reagent (10%. 750 µL) and mixed for 5 min followed by addition of Na_2HCO_3 solution (7.5 %, 750 µL). The solution was incubated at 22 ^oC in (1.5 h) and the absorbance measured at 760 nm using a spectrophotometer (Helios Omega, Thermo Fisher Scientific). A standard calibration curve of gallic acid (0 – 200 mg/L) was generated and the results expressed as mg gallic acid/g.

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was performed according to the method of Brand-Williams et al. (1995) (14). Samples were dissolved in methanol (1:3) and reacted (1 mL) with DPPH (0.004%, 1 mL, 3 min). The absorbance was measured at 517 nm using a spectrophotometer (Helios Omega, Thermo Fisher Scientific). A standard calibration curve was generated and the results expressed as mg/g gallic acid equivalents.

Free radical scavenging activity

The antioxidant activity was also calculated as free radical scavenging activity

$\% = (1 - A_1/A_o)*100$

Where

A_1 = Absorbance of sample at 517 nm

A_0 = Absorbance of control at 517 nm

Data analysis

Samples were analysed in triplicate. Means and standard deviations of the data were presented.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening of aqueous extracts of the ackee pod confirmed the presence of saponins. Persistent foaming was observed which constitutes a positive result.

Fourier transform infrared spectroscopy

Traditional methods of identifying and isolating saponins have oftentimes been tedious and time consuming. They frequently involve the utilization of solvent extractions. The use of rapid and greener technologies, for example, FTIR, is being investigated. Dried, milled samples of the ackee pod were evaluated using FTIR. Good correlation was observed between the IR spectrum of the ackee pod and that of other medicinal plants containing high levels of saponins (11). The FTIR spectrum of ackee pods showed characteristic triterpenoid saponin absorptions. A

hydroxyl functionality (-OH) was observed at 3317 cm^{-1}, aliphatic C-H stretching at 2924 cm^{-1} and an alkene functionality (C=C) at 1633 cm^{-1} (Figure 1). The geometry of the alkene functionality is *cis* which is inferred by a peak at 780 cm^{-1}. Oligosaccharide linkage absorption to sapogenins, (C-O-C), was evident at 1030 cm^{-1} (11). Saponins detected in ackee pods may be predominantly monodesmosidic as ester linkages were not detected. Oleanane-type triterpenoid saponins are characterized by a carbonyl functionality (C=O, 1740 cm^{-1}) due to oleanolic acid/ester which was absent from the IR spectra of ackee pods.

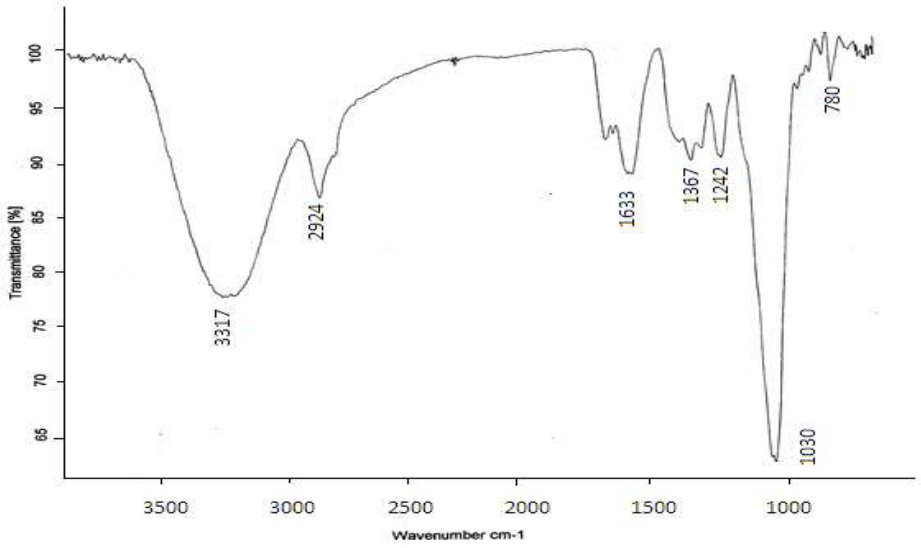

Fig 1. FTIR spectrum of dried, milled ackee pod

The structure of 3 complex saponins from ackee pods has been elucidated utilizing Nuclear Magnetic Resonance Spectroscopy (15). The saponins identified were blighoside A (-O-(α-l-arabinopyranosyl-(1→4)-3-O-acetyl-β-d-glucopyranosyl-(1→3)-α-l-rhamnopyranosyl-(1→2)-α-l-arabinopyranosyl-(1→3)) hederagenin), blighoside B (3-O-(α-l-arabinopyranosyl-(1→4)-3-O-acetyl-β-d-glucopyranosyl-(1→3)-α-l-rhamno-pyranosyl-(1→2)-α-l-arabinopyranosyl-(1→3)) oleanolic acid), and blighoside C (3-O-(4,6-di-O-acetyl-β-d-glucopyranosyl-(1→3)-α-l-

rhamnopyranosyl-(1→4)-3,6-di-O-acetyl-β-d-glucopyranosyl-(1→3)-α-l-rhamnopyranosyl-(1→2)-β-d-xylopyranosyl-(1→3)-β-d-xylopyranosyl-(1→3)) oleanolic acid. These saponins contained 4 to 6 monosaccharides attached to a triterpene aglycone. Blighoside A was the most abundant and is a tetrasaccharide containing rhamnose, glucose and two arabinose sugar units. These findings further validate the FTIR results which predicted the structure of the saponins to be triterpenoidal.

Antioxidant activity, free radical scavenging activity and total phenolics

The antioxidant activity of methanolic extracts of ackee pods was determined by the DPPH free radical scavenging assay and total phenolics by the Folin-Ciocalteu assay. In the DPPH assay, antioxidants are able to reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydrazine. Ackee pod extracts contained high levels of phenolics, antioxidant and free radical scavenging activities (Table 1).

Table 1. Antioxidant activity, total phenolic content and free radical scavenging activity of ackee pod methanolic extracts

Parameters	Pod
Antioxidant activity (mg/g)	1.81 ± 0.11
Total phenolics (mg/g)	3.00 ± 0.13
Free radical scavenging activity (%)	69.70 ± 1.32

Six known polyphenols, namely, gallic acid, chrysanthemin, methyl gallate, protocatechuic acid, isoquercitrin and ellagic acid, have been isolated from the pods of the fruit (16). There is growing interest in identifying natural sources of antioxidants due to the potential carcinogenicity of synthetic antioxidants. Saponins have been reported as having strong superoxide radical scavenging activity. α-Hederin, Hederasaponin-C, Hederacolchiside-E and Hederacolchiside-F, isolated from ivy, exhibited strong total antioxidant activity (17). At concentrations of 75 µg/mL, these saponins showed 94, 86, 88, and 75 % inhibition on lipid peroxidation of linoleic emulsion respectively (17). Saponins extracted from tea root extract (*Camellia sinensis*) also exhibited anti-inflammatory activity (18). The triterpenoid saponins, blighosides A, B and C were also found to be cytotoxic against human breast cancer cells (15). Ackee pods may be an untapped source of natural compounds with medicinal properties.

CONCLUSION

Ackee pods are a rich source of antioxidants and possess free radical scavenging properties. FTIR offers a direct, facile, nondestructive method of detecting saponins in the pods of the ackee fruit. The saponins present within the pod are triterpenoidal and may be of medicinal value.

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