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MEETING THE NUTRITIONAL NEEDS OF SUB-SAHARAN AFRICA BY EXPLORING THE POTENTIALS OF ACKEE SEED ARIL - (*Blighia sapida* K.D. KOENIG. - SAPINDACEAE) IN SOUTHWEST, NIGERIA

Omotayo, O. E.[1,*], and Faniyi, O. B.[2]

[1]Pure and Applied Biology Programme, College of Agriculture, Engineering and Science, Bowen University, P.M.B 284, Iwo, Osun State, Nigeria.

[2]Microbiology Programme, College of Agriculture, Engineering and Science, Bowen University, P.M.B 284, Iwo, Osun State, Nigeria.

[*]Corresponding Author's Email: olumide.omotayo@bowen.edu.ng

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ABSTRACT

Blighia sapida commonly known as ackee, is a relatively well-known, yet mostly underutilized fruit tree crop of West Africa. The main aim of this study was to ascertain the nutritive components of locally cultivated ackee fruit arils with a view to establishing their nutritive potential. Ackee fruit arils were collected from the tree stands in various field locations within Southwest Nigeria and subjected to phytochemical, Fourier Transform-Infrared, and proximate analyses. Results show the presence of phenols, glycosides, alkaloids, flavonoids, saponins, and terpenoids in varying degrees in the phytochemical screening. FT-IR spectroscopy of the aqueous and methanol extracts indicated the presence of amines, phenols, alcohols, alkanes, esters, and aldehydes. The proximate analysis result revealed the presence of high moisture content, ash content, crude protein and fats, and low crude fibre in the ackee fruit aril. The confirmed presence of these active functional groups and nutritive constituents in the fruit underscores the antioxidant properties and nutritive benefits of the plant. Further work needs to be done to determine how to best elucidate active principles from the ackee aril in order to explore their potential as food additives to solve the nagging dietary imbalance issues ravaging the world.

Keywords: Fruit utilization, phytochemicals, food additives, ackee.

INTRODUCTION

Fruits are generally established as nutritive storehouses of nature and are deemed quite indispensable in the array of natural plant products derived from any given natural environment which may be exploited for the benefit of humankind. Plants themselves are generally known to be valuable sources of a wide range of secondary metabolites which are used as pharmaceuticals, agrochemicals, flavours, fragrances, colours, biopesticides, and food additives (Pande and Gupta, 2013). The occurrence of natural antioxidants in plants has led to the production of functional food and drugs with health-promoting properties from plants due to their lower toxic effects and higher efficiencies, especially in disease conditions that are often drug-resistant (Samsonowicz *et al.,* 2019). Phytochemicals (which are also abundantly present in fruits) are chemical compounds that occur naturally in plants and have health benefits and antimicrobial activities against pathogenic bacteria (Tagoe *et al*., 2011). The presence of antioxidants and phytochemicals in many local fruits grown in sub-Saharan Africa have been proven to be of great benefit to the general economic and social well-being of the local populace inhabiting this sub-region.

Food security issues are currently re-emerging as a growing public safety concern in sub-Saharan Africa particularly in West Africa (Aworh, 2021). West and Central Africa have been recognized to be facing an unprecedented food security crisis for the past two consecutive years due to the compounded consequences of the 2021 cereal deficit, deteriorating security situation, socioeconomic impacts of the COVID-19 pandemic and disruptions to the food and energy supply due to the Ukraine conflict (Diouf *et al.,* 2023). Key factors affecting this phenomenon include climate change, natural disasters such as flooding and famines which occur more frequently across many countries, the aftershocks of the COVID-19 pandemic as well as increasing armed human conflict situations leading to less production of food by traditional agriculturists and farmers (Ouko and Odiwuor, 2023). As a result of these factors, less food is being produced coupled with increasing inflation trends as well as

hike in transportation fares of food products out of rural areas into heavily populated urban areas, a near crisis situation is fast developing in the food and agricultural production industry (Wudil *et al.,* 2022). In the face of these mounting challenges, consumption of fruits remains a stable, natural recourse to meet up nutritional needs of the teeming populace in this region of sub-Saharan Africa.

Blighia sapida (commonly known as ackee fruit) is a woody perennial multipurpose fruit tree species belonging to the Sapindaceae family native to the Guinean forests of West Africa (Wray *et al.,* 2020). Ackee is an inherent tree crop of West Africa, prevalent in tropical and subtropical environments (Aloko *et al.,* 2019) having various parts of it employed in traditional medicine for the treatment of common illnesses such as fever, malaria, internal haemorrhage, dysentery, yellow fever, and diabetes (Lawal *et al.,* 2018). Bioactive molecules such as total phenol, ascorbic acid, hypoglycin A, squalene, etc. have also been identified from the plant (Grande-toval *et al*., 2019). The fleshy arils of the ripened fruits are edible. Seeds and capsules of the fruits are used for soap-making and fishing, and some parts of the plant have medicinal properties, useful in African traditional medicine (Olawale *et al.,* 2016). Thus, significant scientific knowledge on the health benefits of these phytochemicals present and their antioxidant potentials could ensure the development of more efficient ways to translate plant extracts into useful products with improved commercial value. The aqueous, methanol, and petroleum ether extracts of the fruits of *Blighia sapida* have been screened for antimicrobial activity (Ekué *et al*., 2010). The ripe ackee seeds have been reported to contain higher levels of phenols and flavonoids than any other parts of the plant; with phenols being regarded as antimicrobial agents with biostatic properties (Okwu, 2004). Though readily consumed in in southwest Nigeria, ackee has been reported as a scantily researched and underutilized indigenous fruit tree of African origin (Awodoyin *et al.* 2015; Ngwa and Nnam, 2018). This study therefore seeks to establish the bioactive principles contained in this fruit that could be helpful for meeting the nutritional needs of consumers.

MATERIALS AND METHODS

Sample Collection

Fresh ackee seed arils from ripened fruit pods were collected from a tree stand each within a residential location in Ogbomosho city, Oyo State, and the campus grounds of Bowen University (BU), Iwo, Osun State, Nigeria (N 07.622494º; E 004. 201091º; Elevation 269 m). Bowen University (BU), Iwo is located at latitude 7 47′ N and longitude 4^{O} 33'W with an elevation that varies between 250 and 300 m above sea level (Ipeaiyeda and Dawodu, 2014). These were placed aseptically inside clean polythene bags and conveyed to the Central Laboratory Facility of the College of Agriculture, Engineering and Science, BU, for further analysis to be carried out. The seed arils were removed from the fruit pod and the arils were duly separated from the seed and kept under refrigerated conditions.

Extraction Procedure

The seed arils were blended and soaked in 2500 ml of each solvent (methanol and distilled water) for 24 hours, and were then filtered using Whatmann No. 1 filter paper and the resultant extract in the solvents were evaporated to dryness in a Soxhlet apparatus and used as crude extract. Proximate analysis of the fresh ackee seed arils was conducted and the aril extracts were subjected to qualitative tests for the identification of various phytochemical constituents using standard procedures.

Proximate analysis of Ackee aril *(Blighia sapida)*

Determination of moisture content

A clean empty dish was dried in the oven at 100 ^{0}C for 5 minutes; which was then transferred into a desiccator to cool. The dry empty dish was then weighed and recorded. Three grams (3 g) of the fresh ackee aril (W3) were evenly spread within the dry dish and then weighed and recorded (W1). The dish and contents were then heated in the oven at 105 ^{0}C for 3 hours, after which it was removed from the oven and then transferred into the desiccator. The final weight of the dish and the dried aril sample was then taken and recorded (W2). The determination of moisture content was carried out in triplicate.

$$\%\text{Moisture content} = \frac{W1 - W2}{W3} \times 100$$

W_1 = Fresh weight of sample + weight of empty dish
W_2 = Final weight of sample + dish
W_3 = Initial weight of sample

Determination of Ash content
A crucible was washed and dried in the oven at 100 ^{0}C for 5 minutes. It was then transferred into the desiccator to dry and cool. The weight of the dry crucible was first taken; after which 3 g of the sample was measured into the dry crucible. The weight of the dry crucible and its contents were then recorded. The sample was first pre-ashed on a hot plate for 15 minutes at 100 ^{0}C in a fume cupboard before the crucible and ashed sample were transferred into the muffle furnace and set at 600 ^{0}C for 5 hours. It was removed after five hours (5 h) and transferred into the desiccator to cool before final weight was taken and recorded.

$$\% \text{ ash} = \frac{weight\ after\ ashing - tare\ weight\ of\ crucible}{Initial\ weight\ of\ sample}$$

Determination of Crude protein and Nitrogen (Using the Kjeldahl method)
Two grams (2 g) of the pulverized sample was weighed and transferred into a Kjeldahl flask. Eight (8) grams of the catalyst (mixture of 96% anhydrous Na_2SO_4, 3.5% $CuSO_4$, and 0.5% SeO_2) and 20 ml of H_2SO_4 was added. The flask was then heated in an inclined position in order to prevent sample loss and ensure efficient digestion of the sample. By the time the initial frothing ended, a loose pear stopper was fit at the top of the flask and heated strongly. The flask was shaken from time to time and the heating continued for 1 hour after the liquid became clear. When digestion was complete, the flask retaining the digest was cooled and then 400 ml of ammonium-free water and a large piece of granulated zinc was added. Fifty millilitres (50 ml) of 2% boric acid solution and screening methyl red indicator was then added to the solution in the flask. The diluted digest was made alkaline by adding 80 ml of 50% NaOH solution. The distillate was finally titrated with standard H_2SO_4 (0.2N). The nitrogen values obtained was converted into percentage of crude protein by multiplying with a factor of 6.25 assuming that protein contains 16% as described by the procedure of Olawale *et al., (*2016).
Crude Protein (%) = Nitrogen x 6.25

Determination of crude fat
Two grams (2 g) of the pulverized samples was weighed into the thimble with porosity which permitted rapid passage of the solvent. The thimble was covered with deflated cotton wool, 25 ml of petroleum ether was then poured into the flask and the Soxhlet extractor was fixed. The whole set up was put on electro-mantle and it was heated at 50 ^{0}C. The sample was extracted for five hours (5 h). The solvent was evaporated till no odour of solvent was perceived. The procedure was carried out in duplicate fashion.

$$\% \text{ Crude fat} = \frac{Average\ weight\ of\ oil}{2.0} \times \frac{100}{1}$$

Determination of crude fibre
Two grams (2 g) of a fat-free sample was weighed and then transferred into a 600 ml beaker and 200 ml of pre-heated 1.25% H_2SO_4 was added and the solution was gently boiled for 30 minutes, the volume of acid was maintained by the addition of hot water. The Buckner flask funnel fitted with the Whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was then filtered hot through the funnel under sufficient suction to remove the acid. The residue was then washed several times with boiling water until it became neutral to litmus paper and was then transferred back into the beaker. Two hundred millilitres (200 ml) of pre-heated H_2SO_4 were added and the solution was gently boiled for 30 minutes. It was then filtered and washed thoroughly with hot water once and twice with ethanol. The residue was dried at 65 ^{0}C for 24 hours and weighed. The residue was then transferred into a crucible and placed in a muffle furnace (550 ^{0}C) and ashed for 4 hours. It was transferred into a desiccator and finally weighed. The crude fibre percentage weight of the sample was determined by the following formula:
Calculation:

$$\% \text{ Crude fibre} = \frac{Average\ weight\ of\ fibre}{2.0} \times \frac{100}{1}$$

Fourier Transform-Infrared analysis ackee aril extract
Blighia sapida seed aril extracts were loaded into the FT-IR spectroscope and the spectroscopy results were recorded on an Agilent Technology FT-IR spectrometer (Cary 630) a scan range between 600 cm^{-1} to 4,000 cm^{-1}.

Phytochemical screening of ackee arils extracts

The following phytochemicals including tannins, phenols, flavonoids, alkaloids, anthraquinones, saponins, steroids, phenols, and glycosides **w**ere extracted based on standard biochemical procedures outlined in the Bergey's manual of systematic bacteriology (Brenner *et al.,* 2005)

RESULTS

Proximate analyses of each of ackee aril extracts as shown in Table 1 indicates the presence of crude proteins, nitrogen, fat and fibre. The results show that the fresh ackee arils generally displayed high values for moisture and ash contents. Methanolic and aqueous extracts of *B. sapida* for crude nitrogen, protein and crude fat contents recorded similar values for both extracts, while the crude fat content obtained with aqueous extract of *B. sapida* showed much higher values compared to the methanolic extract. Plate 1 shows de-podded mature ackee arils attached to their seeds.

Table 2 displays the findings of the FT-IR analysis of the aqueous and methanol extracts of the ackee aril (*Blighia sapida*). The spectrum of *B. sapida* generally fell between the wavelength regions 600 and 4000 cm^{-1}. Out of fourteen (14) functional compounds found in the general ackee aril extract profile, twelve compounds were observed as peaks for the aqueous solvent, while eleven compounds occurred as peaks for the methanol solvent. The results of FT-IR analysis confirmed the presence of functional groups such as O-H, C=C, C-H, C-O, and C-Cl (Table 2). The highest peaks appear at the wavelength of 3341.6 cm^{-1} and 3375.1 cm^{-1} for aqueous and methanolic extracts respectively which indicates the presence of O-H stretching and H-bonded of alcohols and phenols.

Plate 1: Ackee arils with attached seed

Table 1: Proximate analysis of the ackee aril extract from Ogbomosho collection stand

S/N	Parameter	Methanolic extract (%)	Aqueous extract (%)	Fresh ackee (%)
1	Moisture content	-	-	67.3
2	Ash content	-	-	41.1
3	Crude protein and Nitrogen	10	8	-
4	Crude fat	3	29.9	-
5	Crude fibre	2.4	2.1	-

*Values are means of duplicate determination.

Table 2: FT-IR analysis of the aqueous and methanol extracts of the ackee aril from Bowen University campus (*Blighia sapida*).

Table 2: FT-IR analysis of the aqueous and methanol extracts of the ackee aril from Bowen University campus (*Blighia sapida*).

S/No	Wavelength (cm^{-1})		Functional Group assignment	Expected Phyto compounds identified
	Aqueous Extract	Methanol Extract		
1	3341.6	3375.1	O-H bond	Alcohols and phenols
2	2916.6	2916.6	C-H bonds	Aliphatic hydrocarbons.
3	2955.8	2849.5	C-H bonds	Aliphatic hydrocarbons
4	2851.4	-	C-H bonds	Aliphatic hydrocarbons.
5	1735.1	1701.5	carbonyl (C=O) groups	Esters, aldehydes, and ketones
6	1638.2	1617.7	C=O bond	Aromatic compounds
7	1386.6	1515.2	C-H bond	Alkanes and methyl groups.
8	1248.7	1375.4	C-H bond	Alkenes and aromatic compounds
9	1215.1	1151.7	C-O bond	Ethers and esters
10	1177.8	-	C-O bond	Ethers and esters
11	-	1064.2	C-O bond	Alcohols
12	1108.9	-	C-N bond	Primary amines.
13	-	1034.3	C-O	Ethers
14	661.6	717.5	C-Cl bond	Alkyl chlorides

Table 3: Qualitative phytochemical screening of *Blighia sapida* aril extracts from Bowen University campus

	Phenols	Glycosides	Alkaloids	Flavonoids	Saponins	Tannins	Steroids	Anthraquinones	Terpenoids
Methanolic extract	++ +	+	++	++	+	-	-	-	-
Aqueous extract	+	+	+++	+	+	-	-	-	+++

Key:
+ = presence of phytochemicals
- = absence of phytochemicals
++ = moderate concentration
+++ = high concentration

Figure 1 (Supplementary file 1) shows the FT-IR spectra of *B. sapida* aqueous extract. An OH stretching vibrating mode with hydrogen bonding is displayed at wavelength 3341 cm^{-1} in the spectra of *B. sapida* aqueous extract. The occurrence of a narrow band of wavelength peaks at 2916.6, 2955.8, and 2851.4 cm^{-1} showed the presence of alkane molecules (C-H stretch). The wavelength peak at 1735.1 cm^{-1} provides evidence of the presence of a carboxylic acid group (C=O stretch) in the extract compound. A peak at 1108.9 cm^{-1} confirms the presence of aromatic compounds (C-N stretch). The peaks of 1215.1 and 1177.8 cm^{-1} also indicate the presence of ethers and esters, while the lowest wavelength peak of 661.6. cm^{-1} indicated the presence of alkyl chlorides.

Figure 2 (Supplementary file 2) shows the FT-IR spectra of *B. sapida* methanol extract. The wavelength peaks at 3375.1, 2916.6, 1701.5, 1619.5, 1515.2, 1151.7, and 1064.2 cm^{-1} respectively showed the presence of alcohols and phenols, (O-H stretch, H-bonded), carbonyls (C=O stretch), esters, aldehydes, and ketones (C=O, C-H stretch), aromatic compounds (C=O bond), and alkyl chlorides (C-Cl bond).

The results of the qualitative phytochemical screening of the ackee arils extracts are presented in Table 3, showing the occurrence of phenols, glycosides, alkaloids, flavonoids and saponins at varying degrees derived with methanolic solvent while the aqueous solvent successfully extracted phenols, glycosides, alkaloids, flavonoids, saponins and terpenoids at varying degrees.

DISCUSSION

Extensive literature search reveals the rich phytochemical profile and nutritional contents of the ackee fruit (Aloko *et al.,* 2019), which has also been confirmed by this study. Ackee fruit remains one of the outstanding local African fruits with a high diversity profile of phytochemicals including flavonoids, terpenoids, glycosides and phenols. The proximate analysis of the fresh ackee arils in this study showed high moisture content, indicating their potential for short-term storage and thus the need to preserve them in low temperature conditions to prevent spoilage by microorganisms. High-fat content was observed for the ackee aril, which was similarly observed by Dossou *et al.,* (2014). However, the low crude protein and fibre content of ackee implies the need for complementing with other protein-rich foods in the diet. Ackee aril has been identified to contain an appreciable source of natural antioxidants like citric acid, squalene and oleic acid (Grande-Tovar *et al.,* 2019), as well as providing a rich source of omega class of fatty acids; including omega-3, omega-6 and omega-9 fatty acids (Machel, 2017).

Interestingly, no two bioactive chemicals will have the same FT-IR spectrum (Easmin *et al.,* 2017; Wongsa *et al.,* 2022). This study's findings attest to the functional groups' presence in the ackee plant aril extracts (Konappa *et al.,* 2020). According to the FT-IR spectroscopy results for aqueous and methanol extracts in this study, the absorption spectra fell between 600 and 4000 cm^{-1}. The existence of multiple functional groups may be seen in both samples as indicated by multiple peaks, indicating that both extracts contained various functional metabolite groups (Bhat *et al.,* 2018). There are a few strong bands at different frequencies that indicate the existence of N-H, C-H, OH, and C = O skeletal vibrations that indicate the presence of different metabolites in the extracts, including amines, phenolics, alkanes, esters, and aldehydes (Table 2). In a similar study by Omotayo *et al.,* (2022), FT-IR analysis confirmed the presence of hydroxyl groups, phenols, alkanes, aromatic amines, carboxylic acids, esters, and ether compounds in mango leaf and bark extracts. The confirmed presence of these active functional groups in the plant compounds is highly implicated in the antimicrobial effects of such plant extracts. Ackee arils applied in this study were also seen to possess similar functional compounds found in a potent tropical medicinal spice (ginger) according a recent study by Wong *et al.,* (2023), using FT-IR analysis, to contain functional groups including phenolic compounds, aromatic compounds, carbonyl compounds and hydroxyl groups, according to the developed FT-IR spectrum graph.

CONCLUSION

The confirmation of the presence of phytochemicals such as phenols, flavonoids and glycosides in the ackee aril extracts indicates the potential of the ackee aril as a functional food with antioxidant properties that may improve health status of its consumers in the region. Also, the high fat content of the ackee aril may have great implications in meeting the nutritional needs of the human communities in this region of the country. Therefore, it is recommended that further scientific exploration of its potentials as food additives and supplements will surely contribute to reducing nutrition related problems for citizens in the country.

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CONFLICT OF INTEREST

The authors declare that no competing conflict of interest exists.

AUTHORS' CONTRIBUTIONS

OEO: Conceptualization, Visualization, Project Administration, Supervision, Writing – Review and Editing, Resources**;** FOB: Investigation, Formal Analysis, Writing – Original Draft, Resources.

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