International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 3 Issue 9 ǁ September 2014 ǁ PP.31-40

Phytochemical Constituents of Seeds of Ripe and Unripe *Blighia Sapida* (K. Koenig) and Physicochemical Properties of the Seed Oil

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ABSTRACT : *The phytochemical properties and fatty acid composition of ripe and unripe Blighia sapida (ackee) seed as well as the physicochemical properties of the seed oil were quantified using standard analytical techniques. The common bioactive constituents; alkaloids, saponins, flavonoids, tannins and phenols were present in both seed samples. The ripe and unripe ackee seeds contained (mg/100g); tannins 4662.83±15.4, phenols 317.20±0.89 and flavonoids 5.17±0.09 were more in the ripe ackee seeds while alkaloids 0.48±0.02 and saponins 4208.33±17.61 were higher in the unripe ackee seeds. The ripe and unripe ackee seeds had an average oil yield of 15.61±0.01% and 14.05±0.02% respectively. The specific gravity of the ripe and unripe ackee seeds oil were 0.91±0.01and 0.90±0.00 mgcm^{-3}, while the saponification value of 96.77±0.03mg/g, acid value 66.09±0.11 mg/g, ester value 33.45±0.23 mg/g, iodine value 2.26±0.01 mg/g and peroxide value 2.05±1.15 mg/g were higher in the unripe ackee apple seeds. Gas chromatography of the ackee seed oil showed erucic acid, n-hexadecanoic acid, 9-octadecenoic acid, octadecanoic acid, 9-octadecanoic acid, 11-eicosenoic acid, eicosanoic acid and octadec-9-enoic acid as the major fatty acids present in the oil. This study shows that ackee seeds may find use in the production of therapeutic agents and industrial oil.*

KEY WORDS*: Ackee seeds, fatty acids, phytochemical constituents, physicochemical Properties, Seed Oil*

I. INTRODUCTION

The use of plant parts for ethno-medicinal purposes is well recognized in rural areas of many developing countries [1]. Higher plants have potency as sources of new antimicrobial agents [2]. Chemicals present universally in all plants can be classified as primary metabolites which include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls amongst others and secondary metabolites as alkaloids, terpenoids and acetogenins to different phenols. The qualitative and quantitative distribution of these metabolites differs from plant to plant and plant parts. Alkaloids found in low concentrations relative to the phenolic compounds are offset by their high biological potency in vegetative tissues. Besides this, alkaloids are found in higher concentration in storage tissues (roots, fruits and seeds) as compared to the green leaves [3]. Many of these alkaloids and glycosides are poisonous but still many are harmless and possess medicinal properties if used in little amount.

The amount of poisonous substances varies considerably from species to species and even from plant to plant, depending on the age and various ecological and climatic factors [4]. Oil seeds are energy dense foods containing protein and carbohydrate, the food energy they provide is mostly as fat (9 kcal or 37 kJ/g). Oil seeds vary widely in their fatty acid composition but are rich in monounsaturated fatty acids, an example is Peanuts oil or polyunsaturated fatty acids as found in sunflower seeds. Some seed oils contain substantial amounts of alpha linolenic acid; an n-3 fatty acid, and linoleic acid; an n-6 fatty acid both of which are essential fatty acids. From these two fatty acids, the body is able to synthesize all other fatty acids it requires. Arachidonic acid is synthesized from linoleic acid while from alpha linolenic acid, the long chain n-3 eicosapentaenoic and docosahaexanoic acids which are abundant in fish oils [5] can be synthesized. The levels of macronutrients, minerals and vitamins found in vegetable oils are not same as found in whole oil seeds. However, vegetable oils contain a number of phytochemicals and are the major source of natural plant sterols in the diet. Plant sterols have similar structure with cholesterol; hence reduce cholesterol absorption, and the levels of total and low density lipoprotein cholesterol (LDL-C) circulating in the blood. Refining vegetable oils decreases the sterol level (10 to 70%) depending on the oil and processing conditions employed, thereby decreasing their potential in lowering serum cholesterol [6]. The major sources of edible oils in Nigeria are peanut (*Arachis hypogoea*) and oil palm (*Elvesis guineensis*). These oils are utilized basically as cooking oils, for the manufacturing of soap, margarine and cosmetics [7].

With increasing demand which has led to importation of cooking oils, there is need to source for local oil- bearing fruit apart from vegetables which can be exploited for production of oils, both for consumption and industrial applications. *Blighia sapida*, commonly known as *Ackee* (English), *Gwanja Kusa* (Hausa; Northern Nigeria), *Isin* (Yoruba; Western Nigeria), *Okpu* (Igbo; Eastern Nigeria) and *Yila* (Nupe; North Central Nigeria) is a soap berry plant of the family *Sapindaceae.* It is a perennial herbaceous plant that is prominently found in Western Tropical Africa and was imported to Jamaica in the 16th century mainly as food for residents. It gained scientific recognition in 1793 when Captain William Bligh in honour of whom it was named '*Blighia sapida*' introduced it to England [8]. It is an evergreen tree, which grows to a height of between 7 and 25 m. *Ackee* tree grows well in Jamaica with little cultural attention and is cultivated mainly in the Parishes of Clarendon and St. Elizabeth. In folk medicine, extracts of *Blighia sapida* are commonly employed particularly in developing countries to treat a wide range of disease conditions. In Ivory Coast, the bark pulp serves as liniment for oedema intercostals pains. The juice extracts from the leaves and pulp serve as eye drop in ophthalmic and conjunctivitis [9] The roots are used in conjunction with *Xylopia aethiopica* as abortifacient [10]. Repeated small doses of an aqueous extract of the seed are employed in Brazil as parasites expellant, while the bark and leaves are considered stomachic in Colombia [11]. Although, literature shows some chemical and biological studies on *ackee* [12, 13, 14, 15], there is insufficient information on its potential application as industrial or pharmaceutical base. Therefore, the aim of this study was to assess the phytochemical and lipid characterization of the ripe and unripe *Ackee* (*Blighia sapida)* seeds that may be useful in its application as an industrial and pharmaceutical base.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals: All chemicals used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England unless otherwise stated.

2.1.2 Collection of Samples: *Blighia sapida (ackee)* seeds used for this study were obtained from Garatu village Niger State, Nigeria and were identified in the Department of Crop Science, Federal University of Technology Minna Niger State, Nigeria. Seeds were screened to remove bad ones, washed, sundried to constant weight, pulverized using pestle and mortar, sieved, put in an air-tight container and stored until further analysis.

2.2 Methods

2.2.1 Qualitative Phytochemical screening of *Ackee* Seed

Both qualitative and quantitative tests were carried out on the aqueous extract and powdered sample of the ripe and unripe seed sample using standard procedures described by (Sofowora, 1993), (Trease and Evans 1989), (Harborne, 1973), (Oloyed, 2005), (Chang, 2002), (AOAC,1984), (Obadoni et al, 2001).

2.2.1.1 Steroids

A 0.5 g portion of the ethanolic extract fraction of each plant was mixed with 2 ml of acetic anhydride followed by 2 ml of sulphuric acid. The colour changed from violet to blue or green in some samples indicated the presence of steroids **(**Sofowora, 1993**)**.

2.2.1.2 Terpenoids (Salkowski test)

A 5 ml portion of each plant extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml of concentrated H_2SO_4. A layer of reddish brown colouration was formed at the interface thus indicating a positive result for the presence of Terpenoids (Trease and Evans, 1989).

2.2.1.3 Flavonoids

A portion of powdered plant in each case was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration was observed that indicated the presence of Flavonoids (Harborne, 1973; Sofowora, 1993**).**

2.2.1.4 Tannins

A 0.5 g portion of the dried powdered sample was boiled in 20 ml of distilled water in a test tube and filtered. 0.1% ferric chloride ($FeCl_3$) solution was added to the filtrate. The appearance of brownish green or a blue-black colouration indicates the presence of tannins in the test samples (Harborne, 1973).

2.2.1.5 Phlobatannins

Aqueous fraction of the extract of each sample was boiled with 1% aqueous hydrochloric acid; the formation of red precipitate thus indicated the presence of Phlobatannins (Harborne, 1973; Sofowara, 1993**)**.

2.2.1.6 Cardiac glycosides (Keller-Killani test) : A 5 ml portion of each plant extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution, followed by the addition of 1 ml of concentrated sulphuric acid. Brown ring formed at the interface indicates a deoxysugar characteristic of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer, a greenish ring may also form just gradually throughout the thin layer (Harborne, 1973).

2.2.1.7 Saponins : A 2.0 g portion of the powdered sample was boiled in 20 ml of distilled water in a test tube in boiling water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously for the formation of emulsion characteristic of saponins (Obadoni et al, 2001).

2.2.1.8 Anthraquinones (Borntrager's test) : A 0.5 g portion of the plant extract was shaken with 5 ml of chloroform. The chloroform layer was filtered and 5.0 cm^3 of 10 % ammonia solution was added to the filtrate. The mixture was shaken thoroughly and the formation of a pink/violet or red, yellow colour in the ammoniacal phase indicates the presence of Anthraquinones (Harborne, 1973).

2.2.1.9 Reducing Sugar (Benedict test) : A 0.5 g portion of the extract was mixed thoroughly with 3cm^3 of distilled water and filtered. 3 drops of the filtrate was added to 3cm^3 of Benedict reagents and placed in a boiling water bath for 5minutes. The formation of a brick red precipitate indicates reducing sugar (Harborne, 1973).

2.2.1.10 Alkaloids : A 0.5g portion of the extract was stirred with 5cm^3 of 1% aqueous HCl on a steam bath. Few drops of picric acid solution was added to 2cm^3 of the extract. The formation of a reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids (Trease and Evans, 1989; Harborne, 1976).

2.2.2 Quantitative Phytochemical constituents of *Ackee* seed

2.2.2.1 Phenols : A 2g portion of the sample was defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours. The fat free sample was boiled with 50 ml of petroleum ether for the extraction of the phenolic component for 15 minutes. 5 ml of the extract was pipetted into a 50 ml flask, and then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to marked level and left to react for 30 minutes for colour development. This was measured at 505nm. Tannic acid was used to establish the calibration curve (Edeoga et al, 2005).

2.2.2.2 Flavonoids : Total flavonoid was determined using aluminium chloride colorimetric method (Chang et al, 2002). Quercetin was used as standard to generate the calibration curve. Exactly 0.5 ml of the diluted sample was added into test tube containing 1.5 ml of methanol. 0.1 ml of 10 % $AlCl_3$ solution and 0.1 ml sodium acetate ($NaCH_3COO^-$) were added, followed by 2.8 ml of distilled water. After incubation at room temperature for 30minutes, the absorbance of the reaction mixture was read at 415 nm. The amount of 10 % $AlCl_3$ was substituted by the same amount of distilled water in blank.

2.2.2.3 Alkaloids : A 0.5 g portion of the sample was dissolved in 96% ethanol: 20% H_2SO_4 (1:1). 1 ml of the filtrate was added to 5 ml of 60 % tetraoxosulphate (VI), and left undisturbed for 5 minutes. Then, 5 ml of 0.5 % formaldehyde was added and left to stand for 3 hours. The absorbance was read at 565 nm. The extinction coefficient (E_{296}, ethanol {ETOH} =15136M^{-1} cm^{-1}) of vincristine was used as reference alkaloid (Harborne, 1976).

2.2.2.4 Saponins : A 0.5 g portion of the sample was added to 20 ml of 1NHCl and was boiled for 4 hours. After cooling it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5 ml of acetone ethanol was added to the residue. 0.4 ml of each was placed in 3 different test tubes. 6 ml of ferrous sulphate reagent was added followed by 2 ml of concentrated H_2SO_4. It was thoroughly mixed after 10 minutes and the absorbance was read at 490 nm. Standard saponin was used to plot the calibration curve (Oloyed, 2005).

2.2.2.5 Tannins : A 0.2 g portion of the sample was measured into a 50 ml beaker. 20 ml of 50 % methanol was added and covered with para film and placed in a water bath at 77-80^oC for 1hour. It was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered using a double layered Whatman No. 41 filter paper into a 100 ml volumetric flask, 20 ml of water was added, 2.5 ml Folin-Denis reagent and 10 ml of 17 % Na_2CO_3 were added and mixed properly. The mixture was made up to the marked level with distilled water mixed well and left undisturbed for 20minutes for the development of a bluish-green colour. The absorbances of

the tannic acid standard solutions as well as the samples were read after colour development on a UV-Vis spectrophotometer model 752 at a wavelength of 760 nm (AOAC, 1984).

2.2.3Physicochemical properties of *Blighia sapida (ackee)* seed oil : Oil extracted from the ripe and unripe *ackee (Blighia sapida*) seed was characterized by the classical titrimetric and gravimetric analyses following [The British Pharmacopoeia (TBP) procedures, 2007].

2.2.3.1 Specific gravity : A 25 ml pycnometer was cleaned and dried, filled with water and weighed at room temperature (~27°C). The pycnometer was emptied, dried, cooled and weighed again on an electronic balance (Ohaus, USA). The apparatus was filled with *Ackee* oil and weighed. The specific gravity at 27°C was calculated using the Equation (1):

$$\text{S. G.} = \frac{\text{Mass of } ackee \text{ oil}}{\text{Mass of water}}$$

The values obtained were adjusted to 25°C using the formula:

$$G = G' + 0.00064\ (t - 25^0\text{C})$$

Where G is the corrected specific gravity, G′, calculated specific gravity (27°C), and t is the temperature (Cunniff, 1999). The determination was done in triplicates along with arachis oil as reference standard.

2.2.3.2 Acid value : A 12.5 ml of both ethyl ether and absolute alcohol were measured into a conical flask and 5 g of *Ackee* oil was added. The mixture was shaken and 0.5 ml of phenolphthalein was added and agitated vigorously. The mixture was then titrated with 0.1 M potassium hydroxide until a pink colour that persisted for 15 s was observed. The test was repeated with arachis oil (TBP) following random assignment. The acid value was calculated using the expression:

$$a = 5.610\ v/w$$

Where, a, is the acid value, v is the difference in volume of potassium hydroxide consumed in the titration and w, the mass of the sample used (TBP, 2007).

2.2.3.3 Ester value : One gram of *Ackee* oil was weighed into a 200 ml flask and 5 ml of ethanol (95%) added. To the mixture was added 5 drops of phenolphthalein indicator, and titrated with 0.1 M ethanolic potassium hydroxide until the colour turned pink. Twenty millilitres of 0.5 M potassium hydroxide was added along with 2 glass beads. A reflux condenser was turned on and the content was boiled for one hour. The mixture was removed from the heat source and 25 ml of distilled water was added along with 0.2 ml of phenolphthalein. The mixture was titrated to neutrality with 0.5 M hydrochloric acid. The ester value was determined by the expression (TBP, 2007):

$$E = 28.05\ v/w$$

where E, is the ester value, v is the difference in volume of hydrochloric acid consumed by the blank compared to that of the sample titrations and w is the weight of the sample.

2.2.3.4 Saponification value : Two grams of oil was weighed into a round-bottom flask, 25 ml of 0.5 M ethanolic potassium hydroxide was added followed by two dispersion beads and the mixture was boiled for 30 minutes in reflux condenser. The mixture was removed from the heat source and 1ml of phenolphthalein was added. The mixture was then titrated with 0.5 M hydrochloric acid until a pink colour persisting for more than 15 seconds was observed. Blank titrations excluding the oil were performed. The saponification values were calculated using the expression (TBP, 2007):

$$S = 28.05\ v/w$$

Where S, is the saponification value, v is the difference in volume of hydrochloric acid consumed by the blank compared to that of the sample titrations.

2.2.3.5 Peroxide value : The method specified by the [international standard organisation (ISO) 3960, 1975] was employed. 2 g of the sample was weighed into 500 ml conical flask, 10 ml of chloroform was added to dissolve the sample quickly by stirring, then 15 ml of acetic acid was added and 1ml of freshly prepared saturated potassium iodide solution was added. The flask was then closed immediately, stirred for 1minute and kept for exactly 5minutes away from light at room temperature. 75 ml of water was added to the flask and then shaken vigorously. Few drops of starch solution were added as indicator. The liberated iodine was titrated against 0.01N sodium thiosulphate solution. The same procedure was carried out for other samples and blank test was done by the same procedure but omitting test sample. The peroxide value (PI) expressed in milliequivalent of active oxygen per kilogram of sample is equal to:

$$PI = \frac{(V1 - V0) \times T \times 1000}{M}$$

Where V_0 is the volume of the sodium thiosulphate solution used for blank, V_1 is the volume of the thiosulphate used for determination of sample, T is the normality of the sodium thiosulphate used, and M is the mass of the test sample in gram.

2.2.3.6 Iodine Value : The method specified by the [international standard organisation (ISO) 3961, 1989] was used. 1g of sample was weighed into conical flask. 10 ml of chloroform and 12.5 ml of Dam's reagent was added to the flask. Stopper was then inserted and the content of the flask vigorously swirled. The flask was then placed in the dark for 1hr 30minutes. At the end of the time, 10 ml of potassium iodide solution and 75 ml of water were added. The content of each flask was titrated with 0.1mol/L sodium thiosulphate solution until the yellow colour due to the iodine almost disappeared. Few drops of starch were then added and titration continued until the blue colour disappeared after vigorous shaking. The same procedure was used for the other samples and the blank:

$$IV = \frac{12.69C\,(V1 - V2)}{M}$$

Where C is the concentration of sodium thiosulphate used, V_1 is the volume of the sodium thiosulphate solution used for the blank, V_2 is the volume of the sodium thiosulphate used for determination, and M is the mass of the test sample.

2.2.4 Gas chromatography and Mass spectroscopy of ripe and unripe *ackee* seed oil : The seed oil was extracted by Soxhlet extraction method as described by (Onwuka, 2005) using petroleum ether as the extracting solvent. 50 g of the pulverized seed were extracted in 300 ml of the solvent, the extraction continued until enough oil were extracted for the analysis, which was carried out using GCMS-QP2010 Plus Shimadzu, Japan, column oven temperature (70^{o}C), injection temperature and mode (250^{o}C and split), flow control mode (linear velocity), pressure (116.9 kPa), column flow (1.80 ml/minute), linear velocity (49.2 cm/second) ion source temperature (200^{o}C), interface temperature (250^{o}C), solvent cut time (2.50 minute), detector gain mode (relative), detector gain (0.00kV), threshold (2000) and petroleum ether as the methylating solvent.

III. RESULTS AND DISCUSSION

Table 1: Qualitative Phytochemical constituents of ripe and unripe *Blighia sapida* seeds

Parameters	Ripe *Blighia sapida* seeds	Unripe *Blighia sapida* seeds
Tannins	+ +	+ +
Phlobatannins	+ +	---
Saponins	+ +	+ +
Flavonoids	+ +	+ +
Alkaloids	+ +	+ +
Steroids	---	---
Terpenoids	+ + +	+ + +
Phenols	+ +	+ +
Cardiac glycosides	+ +	+ +
Anthraquinones	+ +	+ +
Reducing sugar	+ + +	+ +

Strongly Present +++, moderately Present ++, Absent –

Table 2: Quantitative Phytochemical constituents of ripe and unripe *Blighia sapida* seeds

Parameters ($mg100g^{-1}$)	Ripe *Blighia sapida* seeds	Unripe *Blighia sapida* seeds
Tannins	4662.83 ± 15.37^a	3622.33 ± 3.61^a
Saponins	3068 ± 26.46^a	4208.33 ± 17.61^b
Phenols	317.20 ± 0.89^a	278.13 ± 0.68^b
Flavonoids	5.17 ± 0.09^a	2.92 ± 0.03^b
Alkaloids	0.35 ± 0.01^a	0.48 ± 0.02^b

Values are means of triplicate determination ± SEM.
Rows with different superscripts are significantly different

The qualitative and quantitative phytochemical constituents of the seeds of ripe and unripe *ackee* are as presented in Table 1 and 2. The result revealed the presence of bioactive constituents; alkaloids, saponins, flavonoids, tannins, phenols, phlobatannins, anthraquinones, cardiac glycosides, terpenoids and reducing sugar. However, reducing sugar and terpenoid, were more concentrated in the ripe *ackee* seed. Although, some of these constituents may be completely harmful to both man and farm animals, some are specie specific as observed in the case of tannins and they have also been demonstrated to possess antinutritional effects due to their ability to reduce palatability and digestibility of food stuffs [16]. Both tannins and saponins were found in high amount in the ripe and unripe *ackee* seed. The ripe *ackee* seed contained a higher concentration of tannins. These values are much higher than those reported for papaya, apple, water melon, guava, orange, prickly pear, apricot and paprika which are in the range of ($0.24 – 10.60 mg100g^{-1}$) [17]. Tannins are dietary anti-nutrients responsible for the astringent taste of foods and drinks [18]. They bind to both proteins and carbohydrates and their presence can result to browning or other pigmentation problems in both fresh foods and processed products. Tannins are known to quicken the healing of wounds and burns [19]. *Ackee* seeds may therefore be exploited for tannins for wound treatments. Saponins have the property of precipitating and coagulating red blood cells. Some of their characteristics involve the formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties [20] and bitterness [21]. A higher concentration of saponin was obtained from the unripe *ackee* seed. Saponins are also used as expectorants and emulsifying agents in medicine [22, 23]. They inhibit Na^+ efflux from the cell, thereby leading to higher Na^+ concentration in the cells, and activating a Na^+ - Ca^+ antiporter in cardiac muscle. The increase in Ca^+ influx through this antiporter, strengthens the contraction of the heart muscle [24]. Therefore, *ackee* seed saponin should be exploited for soap production and therapeutically for their hypolipidemic and anticancer activity.

The ripe *ackee* seed had the highest concentrations of phenols and flavonoids. Phenols are antimicrobial agents which inhibit the growth of pathogens [22]. Flavonoids are antioxidants with anti-inflammatory activity [23]. The large amounts of flavonoids in the analysed seeds confers the seeds with biological functions such as protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumor [22]. Flavonoids are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect against the different levels of carcinogenesis [22]. A higher concentration of alkaloid was obtained from the unripe *ackee* seed. *Ackee* seed alkaloids may find use in pharmaceuticals as narcotics, analgesics, antispasmodic and anti-bacterial agents, as anesthetics and central nervous system (CNS) stimulants [24].

Table 3: Physicochemical properties of *Blighia sapida* seed oil

Parameter	Ripe *Blighia sapida* seeds	Unripe *Blighia sapida* seeds
Specific gravity (g/cm^3)	0.91 ± 0.01^a	0.90 ± 0.00^b
Saponification value (mgKOH/g)	92.57 ± 0.01^a	96.77 ± 0.03^b
Acid value (mg KOH/g)	39.49 ± 0.02^a	66.09 ± 0.01^b
Ester value (mg KOH/g)	18.23 ± 0.02^a	33.45 ± 0.23^b
Iodine value (mgI_2/g)	2.07 ± 2.26^a	2.26 ± 0.01^b
Peroxide value (mg reactive O_2g^{-1})	1.00 ± 1.73^a	2.05 ± 1.15^b

Values are means of triplicate determination ±SEM
Rows with different superscripts are significantly different

The ripe and unripe *ackee* seeds yielded an average amount of 15.61±0.01% and 14.05±0.02% ackee oil respectively. The oil yield was lower than those reported for well-known oil seeds such as sunflower (35%), safflower (30%), olive (30%), and soybean (20%), but compares well with that of cotton seed (15%) and more than that of corn (3.0 to 6.5%) [25]. The oil obtained was a bright yellow, clear, viscous liquid, with a characteristic roasted *ackee* scent. The unripe *ackee* seed oil had the highest saponification value, acid value, ester value, iodine value and peroxide value in contrast to the values obtained for the ripe *ackee* seed oil (Table 3). However, there was no significant ($P>0.05$) difference in the specific gravity of the oil extracted from both the ripe and unripe *ackee* seeds. The specific gravity of a substance is indicative of its comparative miscibility [26] with water and other oils. The specific gravity of the oils in this study indicates that it is less dense than water [27]. Acid value can be used to check the level of oxidative deterioration of oil by enzymatic or chemical oxidation. It is a measure of the degree of unsaturation of oil and corresponds to the amount of potassium hydroxide required to neutralize free fatty acids [28]. The lower the acid value of oil, the fewer free fatty acids it contains which makes it less exposed to rancidity. The acid values obtained for the oils in this study are higher than those reported for bean seed oil (2.77 mg KOH/g and 2.74 mg KOH/g [29], *Plukenetia conophora* (11.5 mg KOH/g) [30], *Blighia sapida* oil (14.2 mg KOH/g) [31] but agrees with that of beniseed (47.6 mg KOH/g) [32].

Iodine value measures the degree of unsaturation in a fat or vegetable oil. It determines the stability of oils to oxidation, and allows the overall unsaturation of the fat to be determined qualitatively [28]. The oil analyzed in this study has a lower iodine value when compared with that of *Citrulus vulgaris* (38.5 mgI_2/g) and *Blighia sapida* oil (65.4 mgI_2/g) [33]. Thus, the oils in this study may not be suitable as alky resins for paint formulation or used as varnishes. They may, however, be useful in conjunction with amino resins as finishes for certain appliances, and in this case, the oils can also act as plasticizers. Saponification value determines the quantity of potassium hydroxide (in mg) needed to neutralize the acids and saponify the esters contained in 1 g of the lipid [28]. The saponification value of the ripe *ackee* seed and unripe *ackee* seed agrees with that of *Plukenetia conophora* (92.2 mg KOH/g), but lower than *Adenopus breviflorus* [30]; cotton seed oil, soybean, butter fat and coconut oil [34], rubber seed oil [35], peanut oil in the range of 187 – 250 mg KOH/g. The higher the saponification value of oil, the higher the lauric acid content of that oil. The lauric acid content and the saponification value of oil serve as important parameters in determining the suitability of oil in soap making. The saponification value obtained in this study (Table 3) projects the oil as good in areas of soap making.

The ester value obtained from the ripe *ackee* seed oil and unripe *ackee* seed oil in this study is much lower than those reported for rubber seed oil (191.93 mg KOH/g) [35], shear butter oil (183.4 mg KOH/g), and castor oil (174.09 mg KOH/g) [28]. Ester value represents the number of milligrams of potassium hydroxide required to saponify the esters present in 1 kg of the oil. It is obtained as the difference between the saponification value and the acid value. The peroxide value obtained for the ripe and unripe *ackee* seed oil respectively in this study were less than the (14.4mg reac. O_2g^{-1}) obtained for rubber seed oil [35]. Peroxide value indicates the deterioration of oils. Oils with higher peroxide values are more unsaturated than those with lower peroxide values. Highly unsaturated oils are known to absorb more oxygen and develop higher peroxide values, and oils with higher peroxide values are prone to rancidity [36, 37).The WHO/FAO stipulated a permitted maximum peroxide level of not more than 10 M equivalent of peroxide oxygen/Kg of the oils [37]. Therefore, the oil in this study may be suitable for consumption since it has a peroxide value that is less than 10.

Table 4: Fatty acid composition of ripe and unripe *ackee* seed oil

Parameters (%)	**Ripe *ackee* seeds**	**Unripe *ackee* seeds**
n-hexadecanoic acid (16:0)	3.57	3.14
9-octadecenoic acid (19:9)	1.73	1.92
Octadecanoic acid (18:0)	4.52	3.53
9-Octadecanoic acid (19:9)	---	1.92
11-eicosenoic acid (21:11)	2.12	2.18
Eicosanoic acid (20:0)	6.75	5.60
Erucic acid (22:13)	7.01	6.90
Octadec-9-enoic acid (18:9)	6.58	---
Undecane (11:0)	1.71	---
Decane (10:0)	2.14	---
Benzene (9:0)	2.15	---
MUFA	17.44	12.92
SFA	14.84	12.27

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid.

The gas chromatographic (GC) spectroscopy of the *ackee* seed oil in this study showed several peaks resolved at elution times between 3.0 and 6.0 minutes and between 14.5 and 20 minutes (Figure 1 and 2) reflecting the numerous fatty acids present in *ackee* seed oil. The major peaks observed in both oils have an elution time between 16.5 minutes and 20 minutes. The ripe and unripe *ackee* seeds oil predominantly contained monounsaturated and saturated fatty acids in very low percentages, while the ripe *ackee* seed oil also contained straight chain hydrocarbons and aromatic compound. However, there was no significant ($P>0.05$) variation in the fatty acid content of both seed oils. The percentage total monounsaturated fatty acids (MUFAs) and saturated fatty acids were higher in the ripe *ackee* seed oil. The percentage ratio of unsaturated to saturated fatty acid obtained in this study for the ripe *ackee* seed is (1.18) while that of the unripe *ackee* seed is (1.05). MUFAs contributed only 54.03 and 51.29% of the total fatty acids in the ripe and unripe *ackee* seed oil respectively in contrast to the higher percentages reported for Virginia peanut (81.49%) and hazelnut (83.10%) [38, 39]. Oxidation of fatty acid increases with the relative increase in the amount of unsaturation [41]. Thus, *ackee* seed oil is moderately safe from oxidative rancidity. The MUFAs obtained in this study for *ackee* seed oil, correlates with those reported for cotton seed, linseed, soya (boiled in unsalted water), safflower seed and olive (brine) which are in the range of 1.4 – 6.9% [43, 43], but lower than those obtained for peanut (plain), sesame seed and sunflower seed which are in the range (9.8 – 22.0 %). The saturated fatty acids obtained for *ackee* seed oil in this study correlates with those reported for linseed, safflower and sunflower in the range (3.2 – 4.5 %) but higher than those of soya and olives (0.9 – 1.7 %) and lower than (8.3 – 9.7 %) for sesame seed, peanut and cotton seed. The variability in the fatty acid profile of the seed oils may result from several factors including difference in soil, processing, storage methods, time of harvest and age of the sample. Some fatty acids for example linoleic acid, linolenic acid and erucic acid are temperature responsive during flowering to maturity. MUFAs example oleic, palmitoleic acid helps to lower the levels of "bad cholesterol" (low density lipoprotein cholesterol) and increase "good cholesterol" (high density lipoprotein cholesterol) thereby preventing coronary artery disease, strokes and favouring healthy blood lipid profile. Polyunsaturated fatty acids (PUFAs) also decreases "bad cholesterol" however, intakes of n-6 PUFAs above 10% energy may have adverse effects on the "good cholesterol" [40].

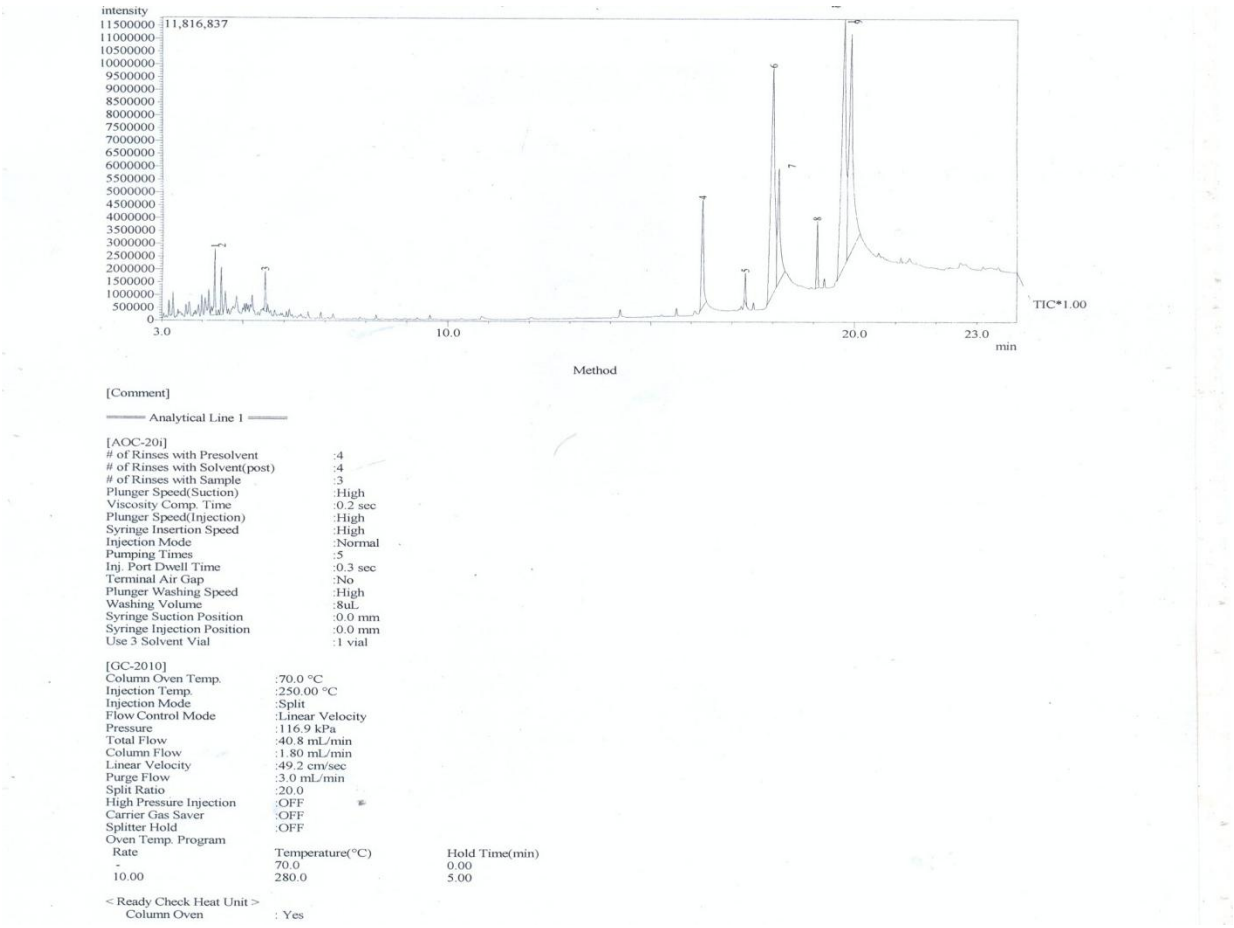

Fig. 1: Gas chromatogram of ripe *ackee* seed oil

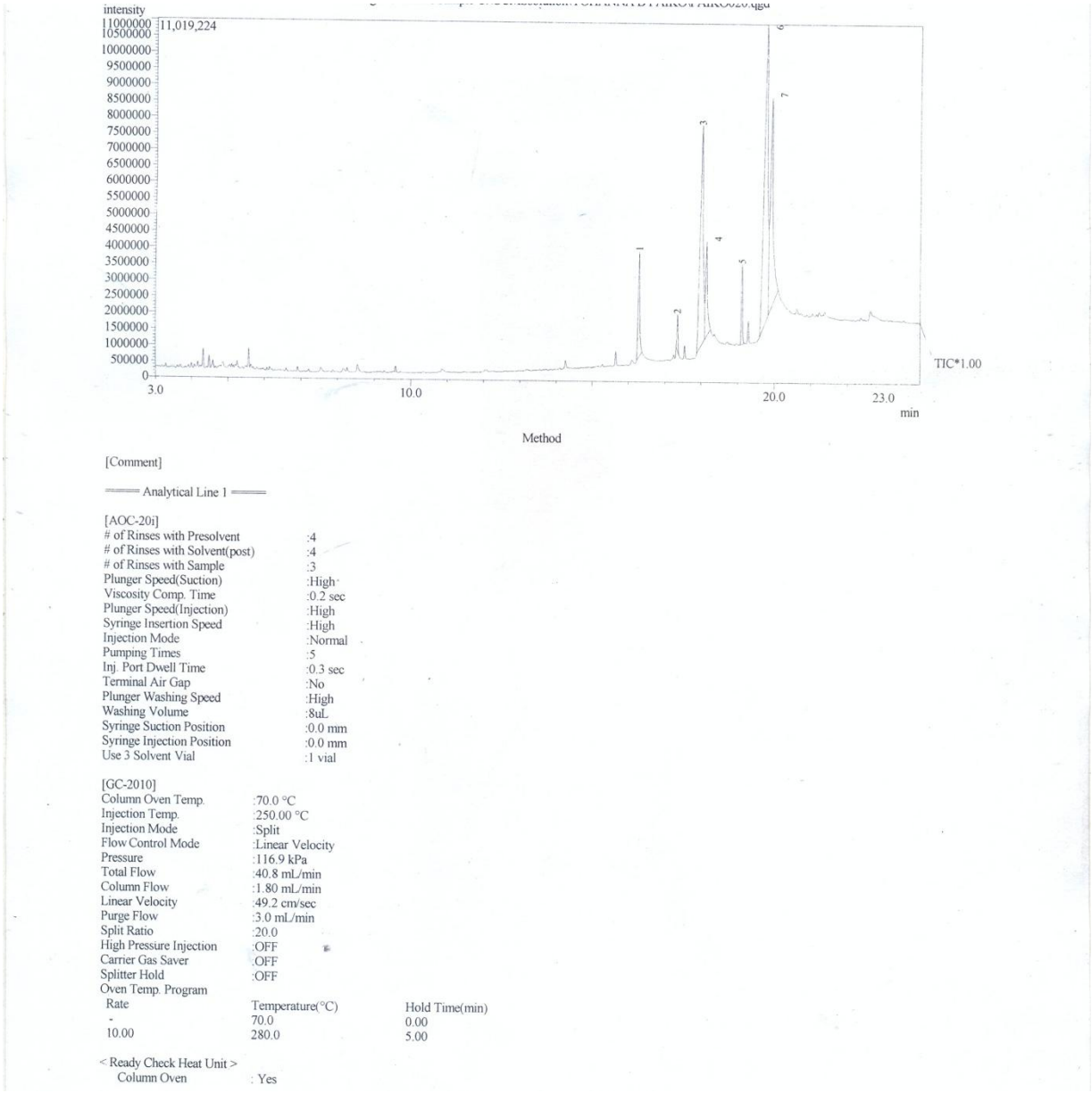

Fig. 2: Gas chromatogram of unripe *ackee* seed oil

IV. CONCLUSION

The findings in this study confirms that *ackee* seed is a moderate oil seed that could be exploited with proper refining for the production of consumable oil and other industrial products and because of the presence of the bioactive components it may also find use in pharmaceutical industries for drug formulation.

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