FATTY ACID PROFILE OF OIL EXTRACTED FROM AFRICAN PEAR (*Dacryodes edulis*) SEED AND AFRICAN STAR APPLE (*Chrysophyllum albidum*) SEED

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ABSTRACT

The environmental menace posed by waste and poor management, use and reuse of most agricultural materials considered as waste in rural areas of sub-Saharan Africa cannot be overstressed. The study was carried out to determine the fatty acid profile of African star apple (*Chrysophyllum albidum*) and African pear (*Dacryodes edulis*) seeds which many consumers considered as waste materials. The seeds of both fruits were respectively removed, air-dried, pulverized and extracted with hexane. Phytochemical screening was performed on both crude extracts according to standard methods. The oil was isolated using vacuum liquid chromatography (VLC) method and characterized by gas chromatography-mass spectrometry (GCMS). Glycosides, saponins, steroids and terpenoids were present for both seed extract. Fatty acids including oleic acid (28.34%), linoleic acid (39.56%) and palmitic acid (8.03%) were detected as the principal fatty acids in *Dacryodes edulis* seed oil while oleic acid (39.86%), linoleic acid (25.18%) and palmitic acid (13.39%) were detected for *Chrysophyllum albidum*. The results show high percentages of long chain unsaturated acids in both oils which makes them suitable to be used as additives in food.

Keywords: *Chrysophyllum albidum*, *Dacryodes edulis*, fatty acids, oil, phytochemicals.
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INTRODUCTION

Lipids consist of mixtures of organic molecules, which are mainly triacylglycerols, diacylglycerols and monoacylglycerols. Other free fatty acids and minor components like phospholipids, phytosterols and tocopherols are included. Fat consists of a group of chemical constituents which are soluble in organic solvents and insoluble in polar solvents like water. Chemically, fats are described as esters of glycerol and fatty acids. Although the words "oils", "fats" and "lipids" are all used to refer to fats but "oils" are liquid at room temperature, while "fats" are solid at normal room temperature. Seeds are often considered as residues of agriculture-based industries [1]. However, studies conducted over the years have shown that seeds of various plants contain several chemical and bioactive components [2]. There has been increasing interest toward investigations on the bioactive chemical constituents and health benefits of these seed oils. The seeds oils extracted from different plants are rich sources of polyunsaturated fatty acids and antioxidants, such as tocols, bioflavonoids, and phytosterols [2]. Many traditional systems utilize seed oils due to their multiple health benefits and bioactive constituents [3]. The fatty acid contents, tocopherols, phenolics and other phytochemical constituents in seed oils possess several significant activities among which are antioxidant, anti-inflammatory, anti-diabetic, anticancer and wound healing [4]. More so, the knowledge of organic remedy and natural based cosmetics innovation has made seeds oils promising industrial, nutritional and medicinal-promoting oil of the future. Seed oils are rich and renewable sources of healthy poly unsaturated fatty acids (PUFAs) and mono unsaturated fatty acids, MUFAs [4]. Fatty acid profiling, also referred as analysis of fatty acid methyl esters (FAME) determines the quality of oil seeds and processed oil by identifying and quantifying the fatty acids present in a sample. The resultant fatty acid profile indicates important assistance in valuing oil seeds and processed oil for commercial purpose. Fatty acids are commonly analyzed by gas chromatography (GC) after conversion to fatty acid methyl esters (FAMEs) which are more easily separated and quantified compared with either triglycerides or free fatty acids. In most methods the fat is saponified, which releases the fatty acids from triglycerides and phospholipids to liberate free acids. The free acids are then trans-esterified to form fatty acid methyl esters. Most solid samples are hydrolyzed by strong acid and/or alkali, and then extracted with organic solvents. To accurately quantify the fatty acid content of a sample as a weight percentage, a synthetic fatty acid (typically C13:0, C19:0, C21:0 or C23:0) is added to the sample prior to extraction as an internal standard. The use of the internal standard compensates for variability in both the preparation and analysis of the sample. The fatty acid methyl esters are then separated on the GC and quantified using a flame ionization detector (FID). Separations are performed with wax type capillary columns when only basic chain length and saturation are needed. In order to quantify cis versus trans isomers, specialized highly-polar capillary columns are employed. The FID burns the FAMEs producing ions generating an electrical

current which is measured and plotted as the response in the chromatogram.

Dacryodes edulis (African Pear), family-*Burseraceae* is also African plum; 'Ube' in Igbo and 'Ohurum' among the Bini tribe of Nigeria. It is an indigenous evergreen tropical fruit tree grown in the humid and sub humid climate of West African countries. In south-east Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October. It is an annual fruit of about 3cm in diameter and contains a leathery shelled stone surrounded by a pulpy pericarp of about 5mm thick. The pericarp is butyraceous (with the qualities of butter). It is this portion of the pear which is eaten, either raw or cooked that forms a sort of 'butter' [5]. The seeds are dark brown or blackish, obliquely ellipsoid, up to 2.8cm long and 1.2cm wide; its coat is hard, bony, shiny and dark brown and when broken reveal white coloured cotyledons [6]. African star apple is one fruit of great economic importance in tropical Africa due to its diverse medicinal and food uses. *Dacryodes edulis* fruit pulp contains 48% oil [7] and is rich in protein, fat, fibre, minerals and essential amino acids [8]. The seeds have been reported to contain 3.3% protein [7]. Notwithstanding, the seeds are often disposed as waste after consuming the pulp. Previous researches show that the seed of *D. edulis* contains 18-34% oil [9] making it comparable with other oil-bearing seeds such as palm kernel (40%), cotton seed oil (30%) [9]. The pulp of *Dacryodes edulis* contains more of unsaturated fatty acid (69.88%) than the seeds (39.13%) [10].

The use of non- edible seed oil of *D. edulis* as biodiesel feedstock, waste plantain and banana peels as heterogenous catalyst will help mitigate the environmental hazards posed by the improper handling of these wastes, thus contributing to the possibility of sustaining a clean environment. Non edible oils have been used as alternative to edible oils to address food insecurity and reduce fatty acid methyl ester (FAME) production cost [11]. The relatively high oil content of *D. edulis* seed as reported will encourage its usage as feedstock for biodiesel production [9]. However, limited studies have been done on the characterization of the seed oil

Chrysophyllium albidum (African star fruit) family-*Sapotaceae* ('Udara' in Igbo, 'Otien' in Bini), is one fruit of great economic importance in tropical Africa due to its diverse medicinal and food uses [6]. *Chysophyllum albidum* is a forest fruit tree commonly found throughout tropical Africa. The tree grows as a wild plant and belongs to the family of *Sapotaceae*. It is a small-to-medium tree species, up to a height of 25-37 m having a mature girth varying from 1.5 to 2.0 m. The fruits are not usually harvested, but left to drop naturally to the forest floor where they are picked. The use of waste and non-edible oil as medicinal materials and biodiesel feedstock will reduce the cost of biodiesel production since the feedstock costs constitutes approximately 70-95 % of the overall cost of biodiesel production [9]. Hence, the use of waste non-edible oils should be given higher priority over the edible oils as raw materials for drugs, food supplement and biodiesel feedstock. Thus, the research is aimed at determining the fatty acid profile of *D. edulis* and *C. albidum* seed oils.

MATERIALS AND METHODS

Collection of samples: The *C. albidum* and *D. edulis* seeds were purchased from New Benin Market, in Benin City, Edo State, Nigeria. The seed samples were identified in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The seeds were washed with distilled water, sun dried, the pericarp removed and were pulverized.

Extraction of oil from the seed: About 315 g of *D. edulis* seed and 223 g *of C. albidum* were respectively extracted with 500 mL each of n-hexane using Soxhlet extractor for 8 hours. The hexane crude extracts were then dried using Na_2SO_4 and concentrated in a rotary evaporator (RE, 200: USA). The oils were then stored in sample bottles for analysis.

Phytochemical Screening

Phytochemical screening was done on the hexane extract to identify the presence of chemical constituents such as alkaloids, glycosides, steroids, flavonoids, saponins, terpenoids, phenolics, flavonoids and eugenols by using standard procedures of Sofowara and Trease and Evans as outlined in [12] and [13].

Test for Glycosides

To 1mL of the extract was dissolved 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-layered with 1ml of conc.H_2SO_4. A brown ringis observe if glycoside is present.

Test for Saponins

About 0.5g of the plant extract was shaken with water in a test-tube and observed for frothing. Using saponin rein weiss (supplied by Merck) as standard.

Test for flavonoids

About 2 mL of the extract was boiled in 10ml of distilled water and filtered. The filtrate was divided into two different portions A and B of 5ml each.

(i) **To portion A:** 10% Lead acetate solution was added in few drops. A yellowish precipitate is indicative of a positive result.

(ii) **To portion B:** 5ml of 20% NaOH and few drops of dilute HCl were added to the solution. Formation of a colourless solution is indicative of a positive test.

Test for phenolic compounds

About1mL of the plant extract was added to 5ml of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was

added. A pale-yellow colouration is indicative of positive test.

Test for Tannins

To 2mL of the extract, 10ml of distilled water was added and boiled for 5 minutes and then filtered into two equal portions.

(i) To about 2 drops of the filtrate, ferric chloride ($FeCl_3$) solution was added; formation of a bluish precipitate is observed for hydrolysable tannin.

(ii) To about 5 drops of the filtrate, 2ml dilute HCl was added and boiled for 5 minutes. Red precipitate is observed for condensed tannin.

Test for Eugenols

To 2mL of the extract was mixed 5mls of 5% KOH solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A pale-yellow precipitate is indicative of positive test.

Test for Steroids

About mL of acetic anhydride was added to 0.5g plant extract in 2ml of dilute H_2SO_4. A colour change from violet to blue or green is required for the presence of steroids.

Test for Terpenoids (Salkowski test)

About 5mL of each extract was mixed in 2ml of chloroform and 3mls of conc. H_2SO_4 was carefully added down the side of the inner wall of test tube to form a layer. A reddish-brown colouration of the inter-phase is required for the presence of terpenoids.

Test for alkaloids

Dragendoff's, Wagner's reagent and Picric acid were used to test for alkaloids. About 1ml each of the plant extract was transferred into three different test tubes labeled A, B and C.

(i) **To portion A**: 2mls of Dragendoff's reagent (made of a mixture of Potassium Bismuth Iodide salt) was added. Reddish brown precipitate is required for a positive test.

(ii) **To portion B**: 2mls of Wagner's reagent was added. Reddish brown precipitate is indicative of a positive test.

To Portion C: 2mls of Picric acid was added. A yellowish precipitate is a positive test

Isolation of oil from seed extracts using Vacuum-Liquid Chromatography (VLC)

The hexane crude extract was subjected to vacuum liquid chromatography (VLC) using silica gel (particles size: 200 - 425 mesh) as the solid phase while hexane: methanol (3:1) was used as the mobile phase. The vacuum liquid chromatography (VLC) apparatus was set-up with a sintered funnel and the adsorbent (silica gel) were sufficiently poured up to three-quarter (3/4) and then tapped while the vacuum was on for about 10-15 minutes for thorough packing. Whatman's No 4 filter papers were cut accordingly to fit in between the packed silica gel (particle size: 200-425 mesh) and the crude seed extract. The oily phase obtained was passed over $Na_2SO_4$for drying and concentrated using a rotary evaporator. A characterization was done by gas chromatography-mass spectrometry (GC-MS).

Fatty acid analysis: 50mg of the extracted oil sample of both seed were saponified (esterified) for 5 minutes at 95°C with 3.4ml of the 0.5M KOH in dry methanol. The mixture was neutralized by using 0.7M HCl and 3ml of the 14% boron trifluoride in methanol was added. The mixture was heated for 5 minutes at the temperature of 90°C to achieve complete methylation process. The fatty acid methyl esters (FAME) were thrice extracted from the mixture with redistilled n-hexane. The content was concentrated to 1ml for gas chromatography analysis and 1µl was injected into the injection port of GC.

RESULTS

Phytochemical screening

The results of the phytochemical screening of the seed extract of *D. edulis* and *Chrysophyllum albidum* are are in Table 1 below:

Table 1: Phytochemical screening of the seed extract of *D. edulis* and *C. albidum*

S/N	Phytochemicals	*D. edulis*	*C. albidum*
1	Glycosides	+	+
2	Steroids	+	+
3	Terpenoids	+	+
4	Alkaloids	-	-
5	Saponins	+	+
6	Flavanoids	+	-
7	Tannins	-	-
8	Phenolics	+	+
9	Eugenols	-	-

(+) detected (-) not detected

GC-MS analysis of isolated oil of African star apple seed oil (*Chrysophyllum albidum*)

The GC-MS chromatogram of the isolated dark brown oil given in Figure 1 revealed 16 peaks. The chemical compounds identified in the oil fraction are presented in Table 2.

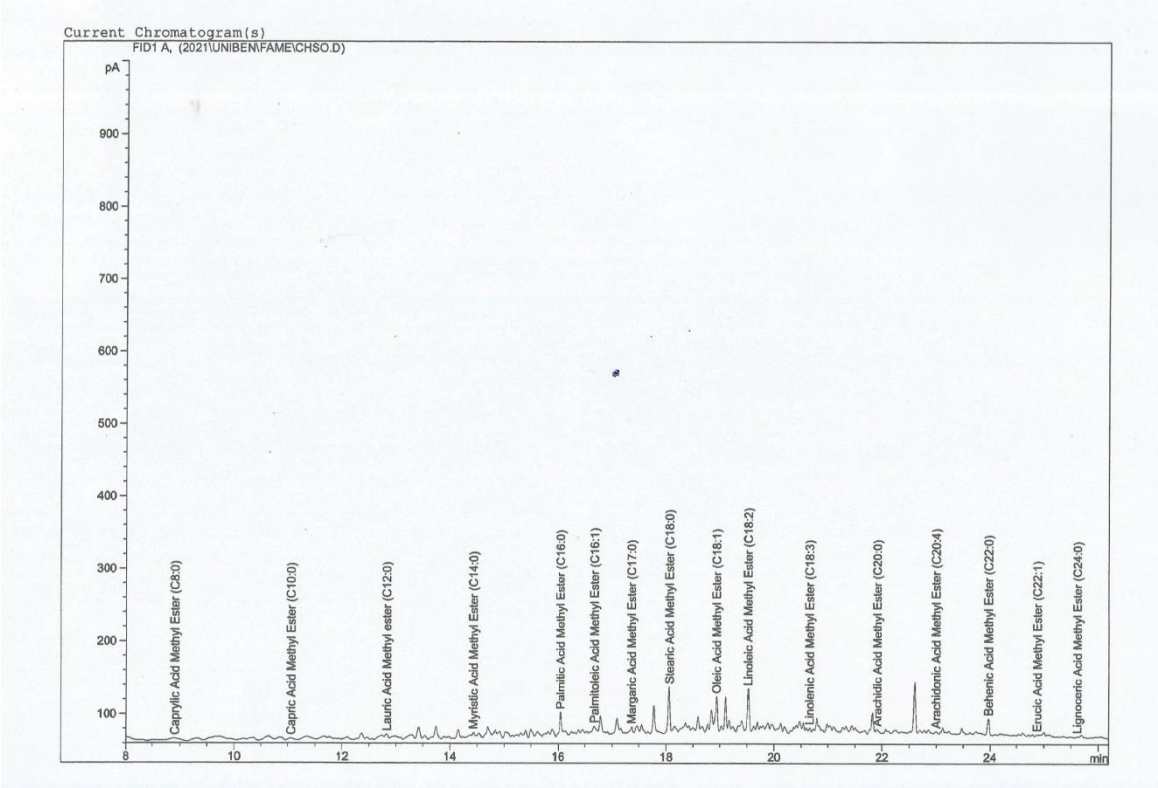

Figure 1: GC-MS spectrum of *Chrysophyllum albidum* seed oil

Table 2: GC-MS results of Fatty acid of *Chrysophyllum albidum* seed oil

Peak No.	Retention Time (Rt), (Minutes)	Fatty acid	Amount/Area
1	8.902	Caprilic acid methyl ester C8:0	ND
2	11.055	Capric acid methyl ester C10:0	ND
3	12.824	Lauric acid methyl ester C12:0	ND
4	14.435	Myristic acid methyl ester C14:0	3.28
5	16.040	Palmitic acid methyl ester CC16:0	13.40
6	16.672	Palmitoleic acid methyl ester C16:1	0.62
7	17.365	Magric acid methyl ester C17:0	0.04
8	18.056	Stearic acid methyl ester C18:0	6.13
9	18.937	Oleic acid methyl ester C18:1	39.86
10	19.522	Linoleic acid methyl ester C18:2	25.18
11	20.654	Linolenic acid methyl ester C18:3	9.46
12	21.906	Arachidic acid methyl ester C20:0	0.531
13	22.994	Arachidonic acid methyl ester C20:4	0.057
14	23.968	Behenic acid methyl ester	0.33
15	24.879	Erucic acid methyl ester C22:1	0.24
16	25.616	Lignoceric acid methyl ester	0.89
TOTAL			100.0

ND = Not detected

GC-MS analysis of isolated oil of *D. edulis* (African pear seed oil)

The GC-MS chromatogram of the isolated yellow oil given in Figure 2 revealed 16 peaks. The chemical compounds identified in the oil fraction are presented in Table 3.

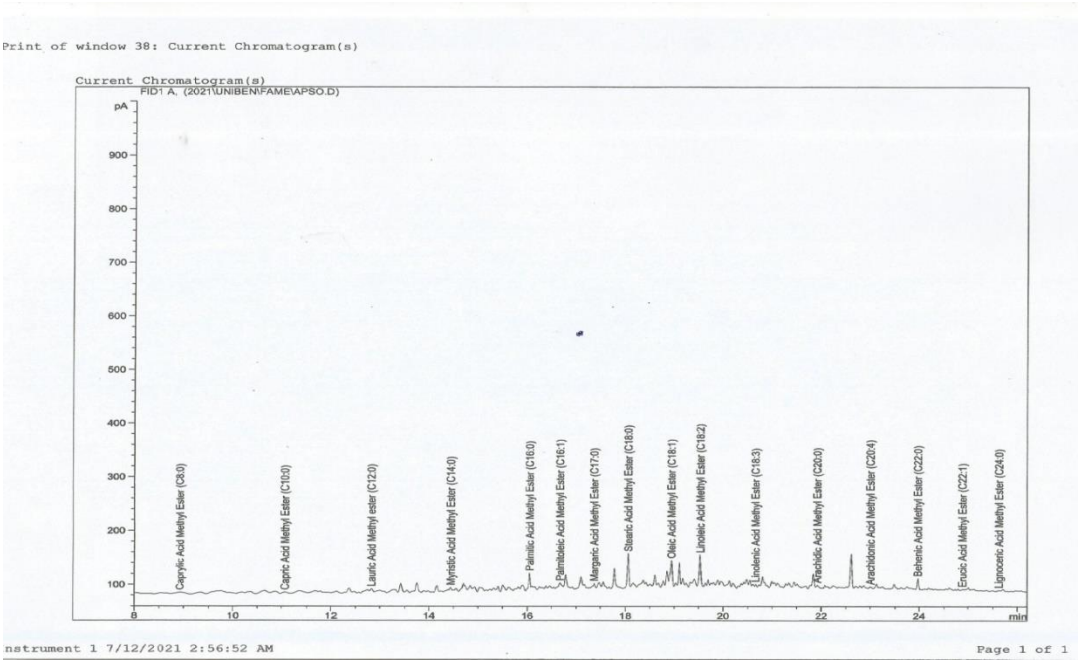

Figure 2: GC-MS spectrum of *D. edulis* seed oil

Table 3: GC-MS results of Fatty acid of *D. edulis* seed oil

Peak No.	Retention Time (Rt), (Minutes)	Fatty acid	Amount/Area
1	8.922	Caprilic acid methyl ester C8:0	ND
2	11.065	Capric acid methyl ester C10:0	ND
3	12.830	Lauric acid methyl ester C12:0	ND
4	14.440	Myristic acid methyl ester C14:0	0.24
5	16.044	Palmitic acid methyl ester CC16:0	8.03
6	16.677	Palmitoleic acid methyl ester C16:1	0.68
7	17.369	Magric acid methyl ester C17:0	0.14
8	18.060	Stearic acid methyl ester C18:0	2.10
9	18.941	Oleic acid methyl ester C18:1	28.34
10	19.526	Linoleic acid methyl ester C18:2	39.56
11	20.658	Linolenic acid methyl ester C18:3	18.81
12	21.911	Arachidic acid methyl ester C20:0	0.50
13	22.998	Arachidonic acid methyl ester C20:4	0.06
14	23.972	Behenic acid methyl ester	0.37
15	24.885	Erucic acid methyl ester C22:1	0.27
16	25.623	Lignoceric acid methyl ester	0.99
TOTAL			100.0

ND = Not Detected

DISCUSSION

The phytochemical screening of both seed extract showed the absence of alkaloids, tannins and eugenols which could be ascribed to polarity level (Table 1). The presence of others like glycosides, terpenoids, saponins, phenolics and steroids suggest that the seed extract have useful bioactive agents that have physiological effects in humans [14]. In medicine, these phytochemicals are used as antioxidant, anticancer and anti-inflammatory agents [14]. The presence of glycosides, steroids terpenoids also corroborate the findings of Offunein [15] for *D. edulis* while flavonoid and saponins were reported by Adekanmi and Olowofoyeku in [16] (2020) for *C. albidum* seed oil. The major fatty acid detected for both seed was oleic acid with *C. albidum* (39.86%) and *D. edulis* (28.34%) respectively. Linoleic acid was also detected at a high percentage when compared to other fatty acids. The result of Omejein [17], on the characterization of the *Chrysophyllum albidum* seed oil gave similar results with oleic acid showing the largest abundance of 30.21%. Thus, both seed oils are rich in unsaturated fatty acids, which have dietary, nutritional and medicinal benefits to man.

CONCLUSION

The findings from the research indicates useful phytochemicals in both seed oils which are highly implicated in most nutritional and medicinal additives or drugs examples of which are phenolics, saponins and steroids. The high levels of unsaturated fatty acid contents present in both oils also make them suitable as additives in food and medicine. More research is recommended for isolation of the phytochemical constituents, nutritional composition and bioactive activity of the seed oils.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this work.

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