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GC-MS analysis and physiochemical properties of *Enterolobium cyclocarpum* (elephant ear) seed oil

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Abstract

Background: The present study aimed to extract, evaluate the physicochemical properties, and chemical characterization of the seed *Enterolobium cyclocarpum*. *E. cyclocarpum* is a legume of the Leguminosae family. It is used as foliage, for animal feeding and oil is extracted for several application in folk medicine for the treatment of sore throat, colds, diarrhea, headache, intestinal ailments, and stomachache. The leaf infusion is used as a laxative In the West Indies; seeds are chewed for a sore throat.

Methods: Three hundred grams (300 g) of each of the pulverized samples were extracted with 100% hexane using Soxhlet extractor to produce a light-brown oil of low viscosity. The physicochemical properties of the oil were analyzed using AOAC standard methods, while the oil components were characterized on an HP-5MS column Gas Chromatography coupled with Mass Spectrometry (GC-MS).

Results: The yield of the oil is very low (only 4.11 % by weight), Fatty acid composition showed that undecanoic acid methyl ester (36.68%) was the dominant fatty acid, followed by 3-linoleic acid (25.04%), and 2-linolelaidic acid methyl ester (12.98%). Physicochemical properties of themed oil were moisture content (0.85%); refractive index (25ºC), 1.45; Specific gravity, 0.86±0.02; iodine value, 68.55±0.02/100 g of oil; peroxide value, 6.58±0.03 meq. O_2/kg of oil; free fatty acids, 2.63±0.01 mg of KOH/g of oil; acid value, 5.26±0.02 mg of KOH/g of oil; saponification value, 189.00±0.03; Unsaponifiable value, 0.66±0.01%.

Conclusion: This study has shown that the seed oil of *E. cyclocarpum* displayed properties and constituents as a substitute oil for industrial, pharmaceutical, and domestic applications.

Keywords: *Enterolobium cyclocarpum*; oleic acid; linoleic acid; iodine value.

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Background

Over time, interest in plant research has increased all over the world owing to its enormous applications in traditional systems of medicine for treating a wide variety of diseases. Various medicinal plants have been identified and modern scientific approaches is adopted in studying their authenticity, safety, and effectiveness for therapeutic use. The results highlight the great potential of medicinal plants in the field of pharmacology [1].

Enterolobium cyclocarpum is a plant that belongs to the family Leguminosae, and it is traditionally called parota in Mexico and has been deemed as one of the most important legume species in tropical America [2]. *E. cyclocarpum* is also found in the northern part of Africa [3]. *E. cyclocarpum* exhibits a remarkably wide range of chemical diversity and a multiplicity of biological properties [4]. In addition, the pods are reported to contain phytochemicals which give it its medicinal properties ranging from anti-inflammatory to anti-tumor. Genus Enterelobium is closely related to *Albizia* and *Samanea* and is probably only maintained as a separate genus due to its cultivation [1]. The parota tree is one of the most important species and has a very extended crown spread. The enormous amount of biomass obtained from these types of trees and their properties, such as hardness, low- weight, and resistance to decomposition, makes the parota suitable for a large variety of uses. Parota trees also have applications in pharmaceutics and medicine [5].

The use of *E. cyclocarpum* is versatile among different cultures and traditions. The Mexicans use the bark extract medicinally against colds and bronchitis[6]. The root decoction is used in hot bath for treatment of stomach cancer and as a sedative in Venezuela and Colombia respectively. Other known activities include colds, diarrhea, headache, intestinal ailments, and stomachache. The legumes is well known to contain a high amount of protein and is used as a substitute in animal feeds in Nigeria[7]. Koenig et al noted that the addition of *E. cyclocarpum* to feed offered to sheep, reduced the ruminal organic matter and a reduction in the protozoal cell numbers of 25% was sufficient to achieve the beneficial effects of reduced fauna on the bacterial protein supply [8].

E. cyclocarpum have been reported to contain several phytochemicals, including tannin, flavonoids, alkaloids, terpenoids, and cardiac glycosides[1]. Untreated (raw) seeds recorded a high content of secondary metabolites such as saponin, tannin, oxalate, and trypsin. Lupeol, an oxygenated sesquiterpenoids was isolated from the hexane extract, while 3*β*-hydroxy-21*β*-*E*- cinnamoyl-oxyolean-12-en-20-oic acid, 3*β*, 21*β*-dihydroxyolean-12-en-28-oic acid (machaerinic acid) and its lactone (3*β*- hydroxyolean-12-en-21*β*→28-lactone) from the fruits of *E. contorstisiliquum* [10-11]. Metabolites like betulinic acid which inhibits the growth of colon cancer cells and tumors and downregulates Sp transcription factors through activation of proteasome-dependent (SW480 cells) and proteasome-independent pathways (RKO cell) and Machaerinic acid lactone and derivates exhibit several pharmacologic effects such as hypoglycemic, antinociceptive and analgesic effects were isolated from the *E. cyclocarpum* [4].

The proximate analysis of the seed oil of *E. cyclocarpum* reveals constituents such as oil, moisture, crude fibre crude protein, ash and carbohydrates [11]. The fixed oil of *E. cyclocarpum* is indirectly influenced by the physicochemical properties of the oil like color, specific gravity, free fatty acid value, iodine value, saponification value etc. Seeds of *E. cyclocarpum* are rich in protein (up to 36%), and its amino acid composition is comparable to that of wheat or fish flour. The seeds also contain mineral elements like iron, calcium, phosphorus, sodium, magnesium, potassium, copper, and zinc. In some places, they are consumed in sauces, soups and as a coffee substitute, and several medicinal properties have been attributed to them [1]. The seeds of *E. cyclocarpum* is extensively collected and consumed because they are rich in amino acids, carbohydrates, minerals, and proteins[1]. Studies also showed that the oil of the seeds produces higher volatile fatty acids and contains high proportions of linoleic and oleic acid as well as palmitic and linolenic acid [12] further reported that these seeds are edible and contain substantial nutrients to support high productivity in animals [7].

Chemical constituents in plants are influenced by climatic conditions, age of the plant, plant parts, and the extraction methods adopted. The Southwest region of Nigeria is known for its varying climatic changes between the long-wet seasons and very short dry seasons within the year. This climatic condition however impacts diverse quality and features on plant phytochemicals and primary metabolites. Herein, we investigated the physicochemical properties and the chemical components (using Gas-Chromatography Mass Spectrometry) present in the extracted oils of *E. cyclocarpum* seed oils to access their nutritional, medicinal and commercial purposes

Methods

Sample collection and Identification

The sample was collected in the botanical garden of the Federal University of Agriculture, (FUNAAB) Abeokuta, Ogun State, Nigeria (7.483383667699847, 3.434245309629134) on the 25th of January 2019 during the harmattan season (November to January). The dry pods and the whole plant were taken to the Lagos State University (LSH) Herbarium for identification and authentication. The plant materials were identified, and the voucher number, LSH21/0020, were issued as sample markers for ease of reference.

Sample Preparation

Shells of the seeds were manually cracked open. Seeds were then separated from the shells. The seeds were pulverized using a laboratory grinder.

Extraction of oil

Three hundred (300 g) of the pulverized seed was extracted using a Soxhlet apparatus. At each extraction batch, 50 g of the pulverized seed was placed in a 500 mL round bottomed flask and extracted with 250 mL of hexane due to the non-polar nature of the fixed oil. The mixture was concentrated on a rotary evaporator to obtain a pure oil.

Gas Chromatography (GC) analysis of the oils

The analysis was accomplished with an HP-5890 Series II instrument equipped with an HP-Wax and HP-5 capillary columns (both 30 m × 0.25 mm, 0.25 µm film thickness), working with the following temperature program: 60ºC for 10 min, rising at 5 ◦C/min to 220ºC. The injector and detector temperatures were maintained at 250ºC; carrier gas nitrogen (2 mL/ min); detector dual, FID; split ratio 1:30. The volume injected was 0.5 µL. The relative proportions of the oil constituents were percentages obtained by FID peak area normalization without using a response factor.

Gas Chromatography-Mass Spectrometry analysis

An Agilent 7890 GC-FID fitted with an HP5 Column (30 m×0.32 µM). analysis was done in split mode with the injector temperature set at 300ºC. The detector temperature was also set at 300℃. Hydrogen flow and airflow were set at 40mL/min and 300mL/min respectively. Helium was used as carrier gas and the column flow was set at 1mL/min. The oven was programmed at 100℃ initial temperature, which was held for 5mins, oven was then ramped to 225℃ at 7℃/min and then held for 7 mins. Identifications were also made possible by the use of a homemade library of mass spectra built up from pure substances and components of known oils, and MS literature data.

Physicochemical analysis of the seed oils

The physicochemical properties of the oil were evaluated to determine the quality and stability of the oil. These properties include color, refractive index, specific gravity, saponification value, unsaponifiable matter. Others are peroxide value, acid value, free fatty acid, iodine, and moisture content. They were all determined using the standard analytical methods described by AOAC [13].

Color Determination: The color of the respective oils was determined by physical observation in daylight and under ultraviolet radiation of 254 and 366nm using an ultraviolet chamber.

Moisture Content: The constant temperature oven method was used at a low constant temperature using the modified method of Nahar et al, 2009 [14]. 10 g of the finely pulverized *E. cyclocarpum* seeds were placed in a pre-weighed crucible. And place in an oven at a temperature of 103°C for 17±1 hours. At the end of 17 hours, the sample was removed, covered, and placed in a desiccator to cool for 30 to 45 min. The experiment was done in triplicate. Moisture content was calculated using the appropriate formula below;

$$\text{Moisture Content} = \frac{w-d}{w} \times 100 \qquad \text{Equation 1}$$

Where,
w = wet sample d = dry sample

Free Fatty Acid (FFA): 2g of the sample was weighed into a clean dry conical flask and melted. 25 ml of diethyl ether and 25ml ethanol + 1ml phenolphthalein were added to the sample. The solution was titrated against 0.1M of NaOH to obtain a pink endpoint. Titration was done in triplicate. The Acid Value was obtained by multiplying the FFA by 2.

Peroxide Value: 1 g of the sample was weighed into a clean dry conical flask with 1 g of potassium iodide and 20 mL of solvent mixture (2:1 of glacial acetic acid and chloroform). The solution was brought to boil and added to a conical flask containing 20 mL of 5% potassium iodide solution. This was then titrated against 0.002 M of sodium thiosulphate using starch indicator until a grey to colorless endpoint was obtained.

Saponification Value: Alcoholic KOH was prepared by dissolving 20 g of KOH in 10 mL of distilled water and making up to 500 mL mark with ethanol. 2 g of sample was melted in a conical flask and 25 mL of alcoholic KOH was added and the solution was refluxed for 1 hr. 1ml of phenolphthalein was added to the solution and then titrated against 0.5 M of HCl to obtain a pink colorless endpoint.

Unsaponifiable Matter: Analysis from the saponification process + 50 mL of distilled water was transferred into a separating funnel and washed, extracted 3 times with 25ml each of diethyl ether. The aqueous layer was collected into a conical flask while the organic layer was in a beaker. The organic layer was transferred to an empty separating funnel and washed until it was no longer alkaline (pink). The washed solvent was collected into an already weighed beaker and set down to allow for evaporation. The beaker was then dried in an oven at 80ºC. It was allowed to cool, then the final weight was taken.

Specific Gravity: For the determination of the specific gravity of oils, a clean 50 mL specific gravity bottle was weighed (w_0). Then the bottle was filled to the brim with water and a stopper was inserted. Extra water spilled out, the water on the stopper and bottle were carefully wiped off and reweighed (w_1). The same process was repeated but used oil samples instead of water and weighed again (w_2). The specific gravity of the oil was calculated using the formula below.

$$\text{Specific Gravity: } \frac{w2-w0}{w1-w0} \qquad \text{Equation 2}$$

Where,
W_0 = weight of empty specific gravity bottle
W_1 = weight of water + specific gravity bottle
W_2 = weight of test sample + specific gravity bottle.

Refractive Index: The refractive index of the oil samples was determined with the help of Abbey Refractometer model A 82051 (BS). Two drops of respective oil were placed on the prism with the help of a syringe and the prism was firmly closed by tightening the screw head. The apparatus was allowed to stand for 5 min after the reading was recorded from the display screen.

Iodine Value: 0.2 g of respective oil was weighed into a conical flask. 10ml of carbon tetrachloride and 20 mL of the wij solution was added to the flask and the solution was kept in dark for 30min at room temperature. 15ml of 10% potassium iodide solution with 100 mL of distilled water was added to the flask. The resulting solution was titrated against 0.1 M Sodium thiosulphate, using starch as an indicator till the endpoint where the blue-black coloration becomes colorless. A blank titration was carried out at the same time starting with 100ml carbon tetrachloride. Iodine was then calculated by the following formula.

$$\text{Iodine Value} = \frac{(B-S)\times N\times 12.69}{Weight\ of\ sample} \qquad \text{Equation 3}$$

Where,
B = 0.1N sodium thiosulphate required (ml) by blank
S = 0.1N sodium thiosulphate required (ml) by sample
N = Normality of sodium thiosulphate solution.

Results and Discussion

Table 1 is the physicochemical properties of the seed oil of *E. cyclocarpum*. The yield of the oil shows a high content of unsaturated oils. A golden-yellowish color of oil was extracted from the seed of *E. cyclocarpum via* the Soxhlet apparatus.

The moisture content of E. cyclocarpum

The moisture content obtained was 0.85%. This amount is relatively low and will prolong the shelf-life of the oil. The presence of moisture or water contributes to the hydrolysis and oxidation of triglycerides thereby breaking into glycerol and free fatty acids and thus reducing the unsaturation of the free fatty acid. Lower moisture content also reduces the activities of micro-organisms on the oil [15]. When legumes seed attained physiological maturity its moisture content ranges from 40 to 50 percent [16], however, since the *E. cyclocarpum* seed has been thoroughly dried, the value obtained with previous studies.

Refractive index of E. cyclocarpum

The refractive index of 1.45 (40 ^{o}C) shows that the oil contains more double bonds. As reported in previous studies, the refractive index of oil increases with increased unsaturation and chain length of the fatty acid [19]. The refractive index of the oil was within the range of cottonseed, Brazil nut, hazelnut, palm oils, *Moringa oleifera*, and *Lannea kerstingii* [19-21])

Iodine value of fixed oil of E. cyclocarpum seed

The iodine value is expressed as the number of grams of iodine that will add to the double bonds of the oil in 100 grams of fats or oils. It determines the degree of unsaturation or double bonds present in oils or fat. The higher the iodine value, the more unsaturated the oils or fats are [20]. In this study, the iodine value of the *E. cyclocarpum* oil is 68.55 g of I_2/100. This is significantly low when compared to previous values obtained for the fixed oils of olive, groundnut, cotton, *Lannea kerstingii* and sunflower oils, which ranged from 86 to 145 g of I_2/100 g of oil [18], [21]. In other climes, lower iodine value also indicates lower nutritional value, but high oxidative stability.

Saponification value of E. cyclocarpum

The number of milligrams of potassium hydroxide required for converting one gram of the fat into soap and glycerine is known as saponification value (SV). It is used to know the amount of fatty acid present in the oil [15]. In addition, the factor is used to determine if the oil is useful in soap making. The saponification value for the studied oil was 187mgKOH/g. The value has a close similarity to that of *Terminalia belerica* seed oil and seed kernel oil (189.24mgKOH/g). The more conventional oils such as palm oil, vegetable oils with SV between 240-257 mg KOH/g found a great application in soap production due to high foamability. Olive oil, Rubber seed oil, Sesame oil, and Canola oils (170-190 mg KOH/g) have a similar SV value as the *E. cyclocarpum* oil. Their applications is more relevant for food making and for confectionaries[22].

Acid value of E. cyclocarpum

The lower the acid value of oil, the fewer fatty acid it contains which makes it less exposed to rancidity, the low acid value is also an indication that the oil is edible [23]. Acid value is an important index in the determination of the quality and oxidative potential of an oil, it is a measure of the degradation of the triglycerides into free fatty acid. According to previous studies, a very low acid value (< 0.1), indicates a high-quality oil. In comparison to this study, the value (5.26) obtained in this study is higher than the minimum value. A higher value could be attributed to high oxidative degradation of the oil by heat of extraction or methods of drying[24].

Peroxide Value of E. cyclocarpum

The peroxide value indicates the rancidity process. The oil that shows a high amount of PV is more prone to rancidity. Which could affect the quality of the oil. When the peroxide value is less than 10.00 (millieq O_2/kg), hence the oil will be resistant to peroxidation during storage [23]. The low peroxide value of 6.58 (milllieq O_2/kg) indicates that the oil can resist lipolytic hydrolysis and oxidative deterioration.

Chemical Composition Present in E. cyclocarpum

The chemical composition was characterized using GC-MS and the result is given in Table 2 below. The chromatogram representing the peaks of the separated and identified constituents is shown in Figure 1 below. A total of seven (7) constituents were identified which makes up to 97.93% of the fixed oil from the seed. The saturated fatty acid family makes up to 58.76% and the unsaturated family up 41.24%. The most abundant unsaturated fatty acid is the polyunsaturated 3-Linoleic acid methyl ester at 25.04%. In general, fatty acids are the main constituent of seed oils and are known to have a major parameter that distinguishes the physiochemical properties of the seed oils.

The dominant fatty acid was undecanoic acid methyl ester (36.68%), followed by 3-linoleic acid methyl ester (25.04%), 2-linolelaidic acid methyl ester (12.98%) heptadecanoic acid methyl ester (10.18%) and stearic acid (9.06%). The overall results of this analysis showed that the saturated fatty acid (SAFAs) makes up 57.62wt% of the compositions whereby the monounsaturated fatty acid (MUFAs) is 15.22 wt% and polyunsaturated fatty acid (PUFAs) is 25.04 wt%. Ezeagu shas previously reported the presence of high molecular weight fatty acids, such as palmitic, linoleic, and linolenic acids in some tropical seeds from Nigeria including *E. cyclocarpum* [25]. In addition, Babayemi, 2006 [26] reported the presence of steroids, proteins, tannins and saponins in some seed pods of *E. cyclocarpum*, impacting on them some high nutritive values. The present findings align with the previous studies in terms of composition and the reported nutritive values.

Linoleic acid is a proven fatty acid of immense pharmacological and nutraceuticals applications. Linoleic acid is common in most cereals, legumes, and seeds [27]. Studies have shown that Linoleic acid is the primary dietary of omega-6 fatty acid, which serves the function of structural components in membranes, and as precursors of eicosanoids, which modulate renal and pulmonary functions, and inflammatory responses [28].

The higher % of undecanoic acid in the fixed oil shows that the oil is often been used as an antifungal agent to treat ringworm and athlete's foot. The saturated fatty acid (SAFAs) is slightly higher than the unsaturated fatty acid (UASA). The SAFA contents of the oil in the present research compare favorably to the SAFAs of other edible oils in previous studies.

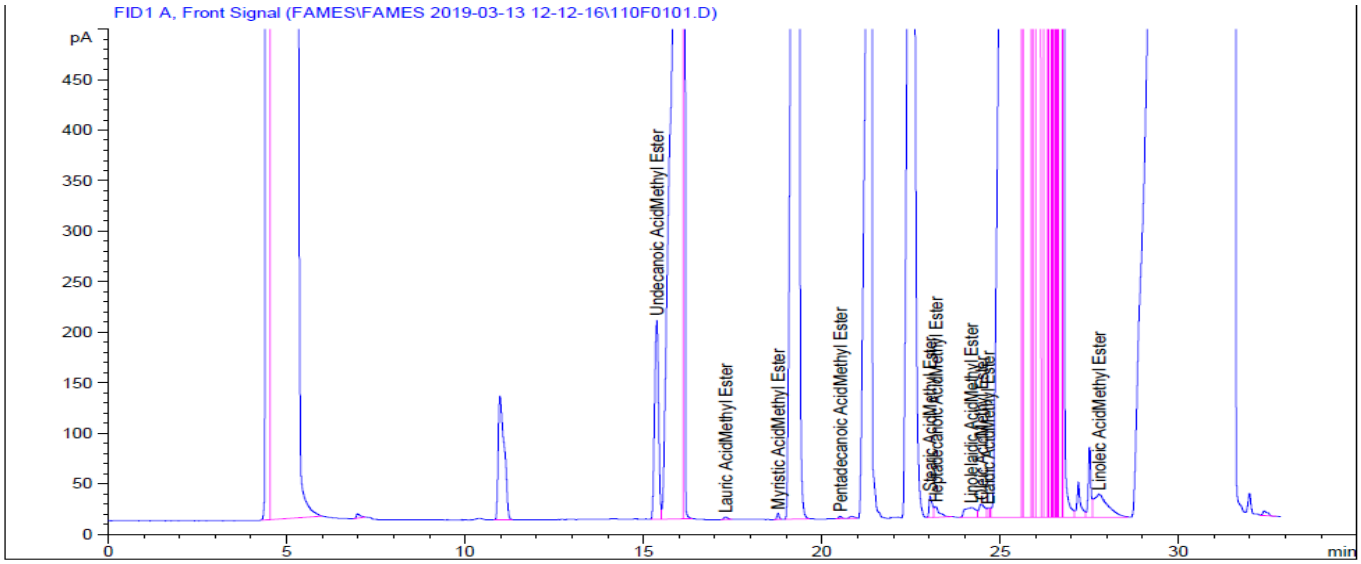

Figure 1. GC chromatogram of the seed oil of *E. cyclocarpum*

Table 1. Physicochemical values of the fixed oil of *E. cyclocarpum*

Physicochemical parameters	Values
Moisture content %	0.85±0.01
Refractive index %	1.45±0.02
Specific gravity %	0.86±0.02
Saponification value mgKOH/g	187.00±0.03
Unsaponifiable matter %	0.66±0.01
Peroxide value (millieq O_2/kg)	6.58±0.03
Free fatty acid mgKOH/g	2.63±0.01
Acid value	5.26±0.02
Iodine content$g1_2$/100g	68.55±0.02

mgKOH/G – milligram per potassium hydroxide per gram, (millieq O_2/kg) – milli equivalent oxygen per kilogram.

Table 2. Some fatty acid content of the fixed oil *E. cyclocarpum*

Fatty acid family	Norm %
Saturated undecanoic acid methyl ester	36.68
Polyunsaturated 3-linoleic acid methyl ester	25.04
Monosaturated 2-linolelaidic acid methyl ester	12.98
Saturated heptadecanoic acid methyl ester	10.18
Saturated stearic acid methyl ester	9.06
Monosaturated Oleic acid methyl ester	2.29
Saturated Lauric acid methyl ester	1.70
Total	**97.93**

Conclusion

In this study, we evaluated the physicochemical properties of the seed oils of *E. cyclocarpum*. It was found that the oil is a non-drying oil with an iodine value of less than 100. Also, the presence of PUFA's in the seed oil suggests that the seed oil is a potential source of Omega-6 fatty acids, which is of immense application in the nutraceutical and pharmaceutical industries. The value obtained from the iodine value, acid value, FFA, and saponification value is indicative of the use of oil in the food and cosmetics industries. The method of drying and extraction could also affect the overall oxidative degradation of the oil as revealed by the acid value. Hence, the use of cold pressing method can be adopted while extracting the oil. Further research involving the isolation of PUFAs from seed oil will be of immense value to the pharmaceutical industry.

Abbreviations

PUFA, Polyunsaturated Fatty Acid; SAFA, Saturated Fatty Acid; UASA, Unsaturated Fatty Acid; FFA, Free Fatty Acid; MUFA, Monounsaturated fatty acid; GC-MS, Gas Chromatography Mass Spectrometry; PV, Peroxide value; KOH, Potassium peroxide; HCl, Hydrochloric acid; NaOH, Sodium Hydroxide; AOAC, Association of Official Analytical Chemists; RKO, SW 480; HP-5MS, Hewlett Packard-5 Mass Spectrometry; SV, Saponification value.

Authors' Contribution

The authors ONA, OLO, IAO, and FMM, designed the study, performed the statistical analysis, wrote the protocol. Authors FTA, and ZAM collected the seed, extracted the oil and the analysis. Authors ONA, FTA, OLO, and ZAM wrote the first and final draft of the manuscript. The supervision of the study was done by authors ONA and FMM. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest

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