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Physico-chemical and GC-MS Analysis of Calabash (*Lagenaria siceraria)* Seed Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAW designed the study of the overall work, wrote the protocol, fitted the data, wrote the first draft of the manuscript and managed the literature searches. Authors RUU and IGW checked the first draft of the manuscript, the revisions and assisted the experiments implementation. Author AAW gave the results interpretations. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To show the potential of the hexane extract of the seed oil in cosmetics and perfumery.
Study Design: Quality evaluation by physico-chemical analysis and determination of fatty acid composition through GC-MS qualitative analysis were carried out on hexane extract of calabash (*Lagenaria vulgaris)* seed oil.
Place and Duration of Study: Laboratory of Biochemistry Laboratory, Department of Biochemistry, between April and Aug 2016.
Methodology: Dried seeds were crushed into powder using mortar and pestle and were stored in a plastic container before analysis. Seed oil was obtained by complete extraction using the Soxhlet

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extractor (GG-17, SHUNIU). The qualitative fatty acid analysis was done using Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) system.
Results: The oil yield (%) was 29.33±0.01, the colour was dark yellow. The following physico-chemical results were obtained; Acid value mgKOH / g of 0.47±0.01, Iodine value gI_2/100 g of 23.03±0.07 and Saponification value mgKOH / g of 156±0.01. The GC-MS results revealed the following fatty acid composition; Palmitic acid, stearic acid, Eicosadenoic acid, linoleic acid, oleic acid, erucic acid, arachidic acid and behhenic acid respectively.
Conclusion: Quality characteristics through physico-chemical analysis and fatty acid profile qualitatively determined showed the potential of the hexane extract of the seed oil in cosmetics and perfumery.

Keywords: Calabash seed oil; physico-chemical; GC-MS; cosmetics; perfumery.

1. INTRODUCTION

The calabash, bottle gourd *Lagenaria siceraria* (synonym *Lagenaria vulgaris* Ser.), from the family Cucurbitaceae is one of humankind's first domesticated plants, providing food, medicine and a wide variety of utensils. The genus name *Lagenaria* comes from *lagena,* the Latin name for a Florence flask; referring to the fruit of *L. siceraria*. The species name *siceraria* probably also refers to the fruit which is useful when it is mature and dry (*siccus*) [1]. Bottle gourd is a useful crop to include in climate change adaption strategies for agronomy [2]. It is a major source of medicinal agents' science ancient times. Various plants parts including fruits of this family have been established for their pharmacological effect. *Lagenaria siceraria* (Molina) standley (LS) is an annual herbaceous climbing plant Swith a long history of traditional medicinal uses in many countries, especially in tropical and subtropical regions. Since ancient times the climber has been known for its curative properties, and has been utilized for treatment of various ailments, including jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure (CCF), and skin diseases [3].

Lagenaria siceraria and its fruits are widely cultivated in Nigeria from the savannah region of the North to the forest areas of the South. It belongs to the family Cucurbitaceae with the common name of bottle gourd, calabash gourd, etc. It is an herbaceous annual climber and trailer of about 4.5 m long. The seed is used as a substitute for melon. The seeds have a dark brownish testa with its endosperm oily [4]. It is known in Hausa as Kwáryáá [5]. Calabash seed is mainly cultivated in semiarid regions and usually grown in a shrub with its fruit hanging or grown on a flat bed. Matured calabash seeds may be harvested 90 – 120 days after planting. In Africa, the calabash seeds are widely cultivated and traditionally and majorly used as containers and storage vessels by rural dwellers [6]. The seed oil contain various phytochemical constituents like tannins, saponins, alkaloids, steroids and terpenoids [7]. Polyphenols and antioxidant activity of seed oils of bottle gourd cultivars was reported [8]. Production of Biodiesel from Calabash Seed Oil was also reported [9]. This research is aimed at physicochemical characterization and GC-MS analysis of hexane extract of calabash seed oil.

Fig. 1a. Riped calabash gourd

Fig. 1b. Calabash seeds

Fig. 1c. Hexane extract of calabash seed oil

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The calabash seeds were procured from commercial producers at Argungu town of Kebbi State, Nigeria. The dried seeds were crushed into powder using mortar and pestle and were stored in a plastic container for oil extraction.

2.2 Oil Extraction Procedure

The hexane extract was obtained by complete extraction using the Soxhlet extractor (GG-17, SHUNIU). The 10 g of each powdered kernel sample was put into a porous thimble and placed in a Soxhlet extractor, using 150 cm^3 of n-hexane (with boiling point of 40- 60℃) as extracting solvent for 6 hours repeatedly until required quantity was obtained. The oil was recovered by evaporation using Water bath at temperature of 70 degrees Celsius to remove the excess solvent from the extracted oil. The oil was then stored in refrigerator for subsequent physicochemical analysis.

2.3 Percentage Oil Yield

The oil which was recovered by complete distilling of most of the solvent on a heating mantle was transferred to a beaker. The beaker was then placed over water bath for complete evaporation of solvent for about 2 hours and volume of the oil was recorded and expressed as oil content (%) in line with literature report [10].

2.4 Determination of Oil Colour

The colour of the oil was determined by observation using several independent competent individuals. Oil colour was correlated using colour charts [11].

2.5 Physico-chemical Analysis

The physico- chemical analysis of the calabash seed oil was carried out using the methods reported [12-14].

2.6 GC-MS Analysis

The analysis of the fatty acids in the calabash seed oil sample was done at National Research Institute of Chemical Technology (NARICT), Zaria, Nigeria, a Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) system was used. The temperature programmed was set up from 70℃ to 280℃. The carrier gas used was helium. The injection volume was 2 µL with injection temperature of 250℃ and a column flow of 1.80 milliliter per minute for the Gas Chromatography. For the mass spectroscopy, ACQ mode scanner with scan range of 30-700 atomic mass per unit at the speed of 1478 was used. The NIST05 mass spectral library was used for mass spectra comparison [15].

3. RESULTS AND DISCUSSION

The outcomes resulted from the physicochemical parameters are recorded in Table 1. The major fatty acids derived from hexane extract of calabash seed oil are recorded in Table 2.

Table 1. Physicochemical properties of *Lagenaria siceraria* seed oil*

Parameters	Values
Oil yield (%)	29.33±0.01
Colour	Dark yellow
Acid value mgKOH/g	0.47±0.01
Iodine value gI_2/100 g	23.03±0.07
Saponification value mgKOH/g	156±0.01

*Values are expressed as mean and ± tandard deviation of triplicate determinations **

3.1 Discussion

The oil yield (%) was 29.33±0.01 lower than 42.23 ± 0.208 reported for castor bean seed oil [16] higher than 19.23±0.07%, reported for *Ipomoea carnea* seed oil [17] the colour of the oil was dark yellow. It was reported that many consumers preferred the bright color, transparent but close to its natural color of oil [18]. The following physico-chemical results were obtained; Acid value mgKOH / g of 0.47±0.01 was obtained which is lower than 12.97 ± 0.01

reported for *Neocarya macrophylla* seed oil [19]. Lower acid value signifies a maximum purity and made it suitable for soap production. Iodine value gI_2/100 g of 23.03±0.07 lower than 100, oils with iodine value below 100 are classified as non - drying oils and they are good or useful in soap production [20]. Saponification value mgKOH/g of 156±0.01 was obtained higher than 141.12±1.19 reported for l and hexane extracts of shea nut fat [21]. Higher saponification values indicate suitability for soap production. The The spectral analysis from the GC-MS fragments revealed the following fatty acids; palmitic acid, stearic acid, linoleic acid, behenic acid which were reported previously as having potential in cosmetic, perfumery and pharmaceutical industries [22]. Eicosadenoic acid was also obtained an Omega-6 fatty acid which is among_ polyunsaturated fatty acids also referred to as ω-6 fatty acids or *n*-6 fatty acids, they are a family of pro-inflammatory and anti-inflammatory [23]. The seed oil also compose of Oleic acid an unsaturated fatty acid that is the most widely distributed and abundant fatty acid in nature. It is used commercially in the preparation of oleates and lotions, and as a pharmaceutical solvent [24]. The spectra showed Erucic acid which is a major feedstock for the oleochemical industry [25]. It is prevalent in wallflower seed with a reported content of 20 to 54% in high erucic acid rapeseed oil [26]. Spectral results also showed Arachidic acid. It is found in appreciable quantities only in some vegetable fats and oils**,** where it occurs as glycerol ester [27]. The results showed the potential of the hexane extract of the seed oil in cosmetics and perfumery.

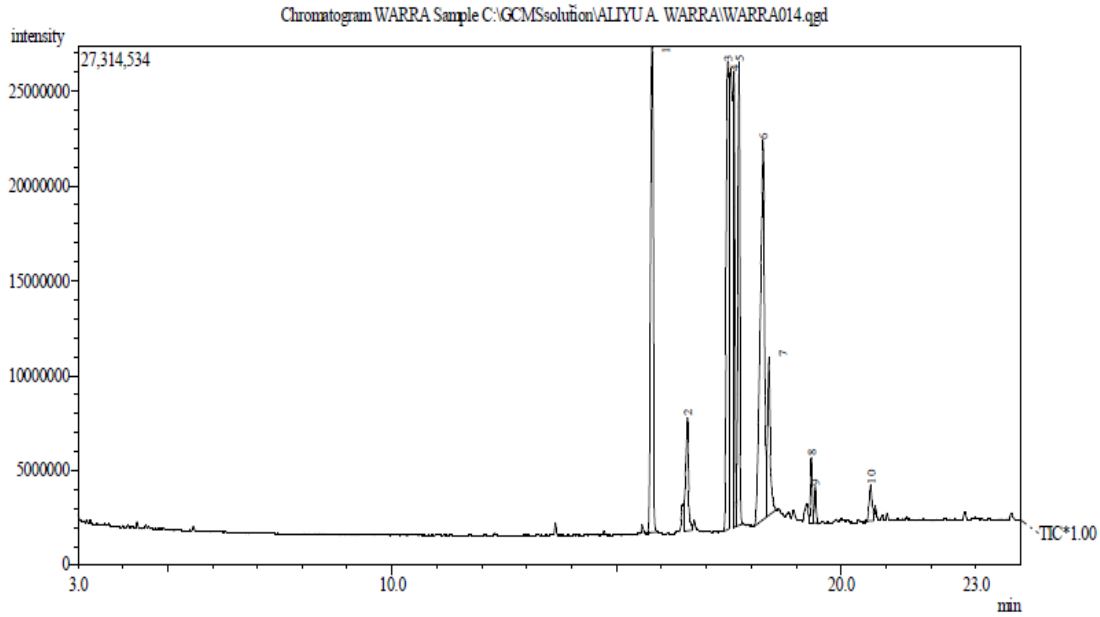

Fig. 2a. Typical GC-MS total ionic chromatogram (TIC) of hexane extract of calabash seed oil

GC-MS Fragments

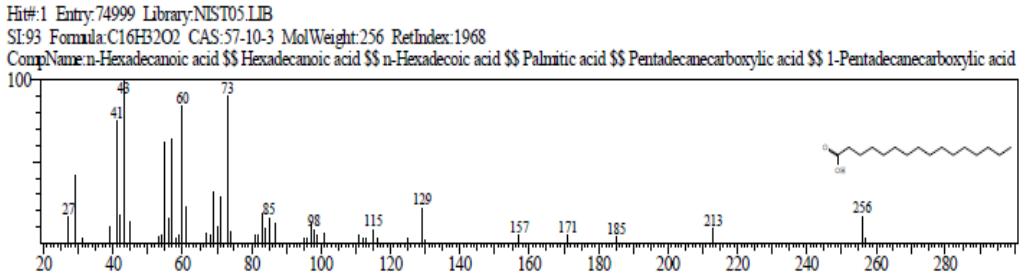

Fig. 2b. GC-MS fragment of palmitic acid

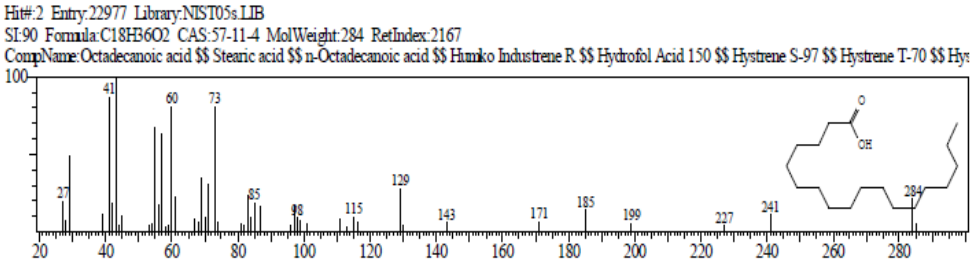

Fig. 2c. GC-MS fragment of stearic acid

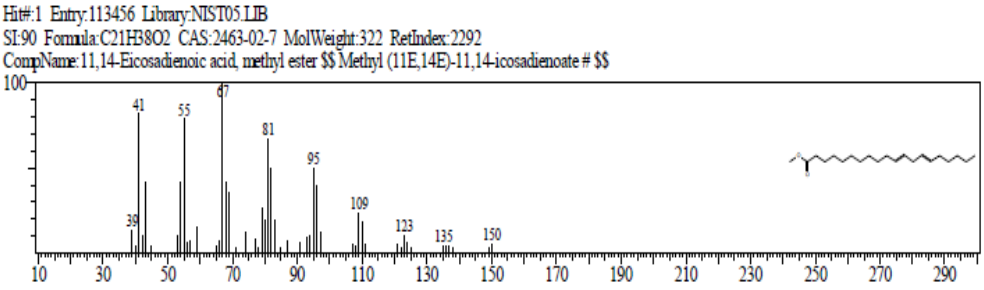

Fig. 2d. GC-MS fragment of eicosadenoic acid

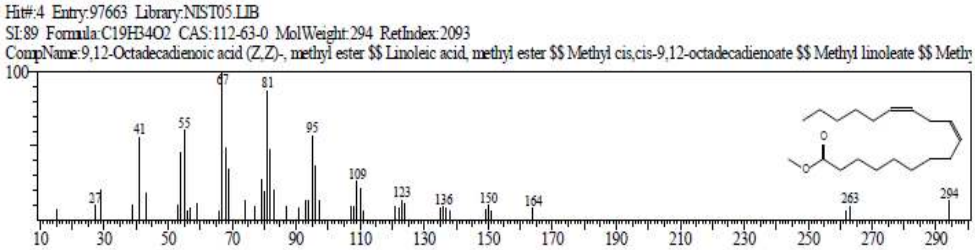

Fig. 2e. GC-MS fragment of linoleic acid

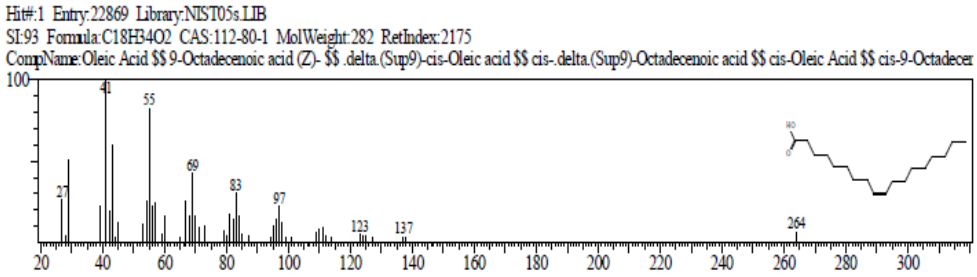

Fig. 2f. GC-MS fragment of oleic acid

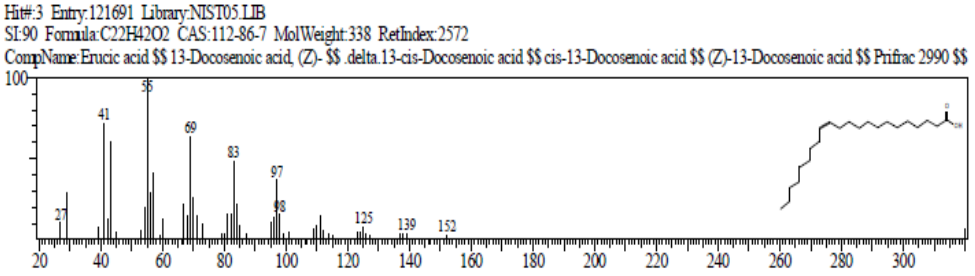

Fig. 2g. GC-MS fragment of erucic acid

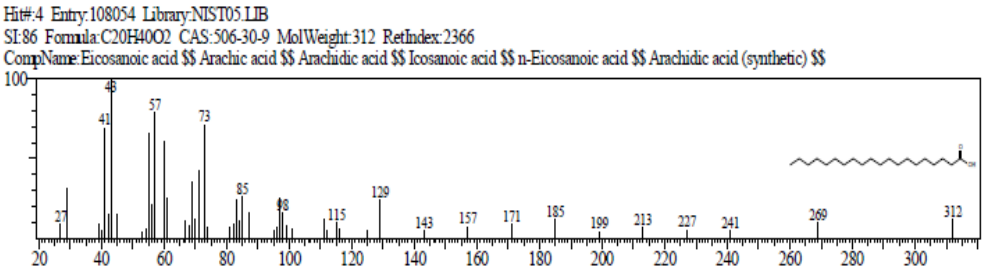

Fig. 2h. GC-MS fragment of arachidic acid

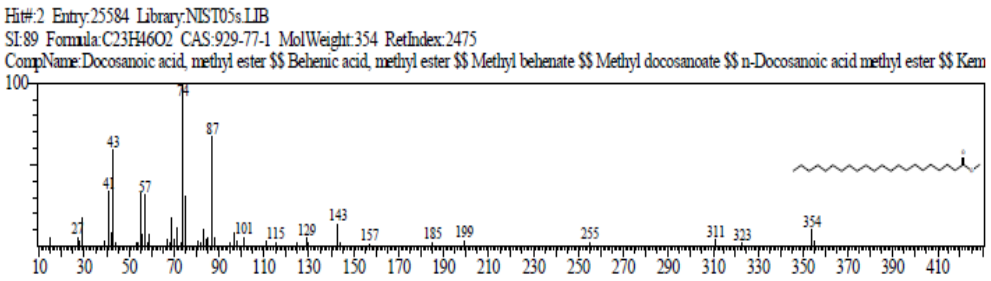

Fig. 2i. GC-MS fragment of behenic acid

Table 2. Major fatty acids derived from hexane extract of calabash seed oil

S/N Name of fatty acid	MF	MW	RI	SI% to T.C.
1. Palmitic acid	$C_{16}H_{32}O_2$	256	1968	93
2.Stearic acid	$C_{18}H_{36}O_2$	284	2167	90
3.Eicosadenoic acid	$C_{21}H_{38}O_2$	322	2292	90
4.Linoleic acid	$C_{19}H_{34}O_2$	294	2093	89
5.Oleic acid	$C_{18}H_{34}O_2$	282	2175	93
6.Erucic acid	$C_{22}H_{42}O_2$	338	2572	90
7. Arachidic acid	$C_{20}H_{40}O_2$	312	2366	86
8.Behenic acid	$C_{23}H_{46}O_2$	354	2475	89

Note: S/N = Serial number, M.F.=Molecular formula, M.W. = Molecular weight, RI= Retention index SI% = Similarity index, T.C. = Target compound

4. CONCLUSION

The results of the physico-chemical and GCMS analysis indicated the potential of the hexane extract of the seed oil in cosmetics and perfumery.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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