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# Preliminary Investigations on the Health Benefits of *Citrullus colocynthis* (L.) Schrad Seeds

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors AAO, UEI and CCI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAO, GOA, BIO and TOA managed the analyses of the study. Author AAO managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** To evaluate the composition of *Citrullus colocynthis* (L.) Schrad seed as a means of assessing its health and possible therapeutic benefits.
**Study Design:** Test-tube Lab Research.
**Place and Duration of Study:** Federal Institute of Industrial Research, Oshodi, Nigeria, between June 2018 and March 2019.
**Methodology:** Intact dehulled *Citrullus colocynthis* (L.) Schrad seeds were analyzed for their proximate and elemental content using standard methods and atomic absorption spectroscopy, respectively. The seed oil was extracted with n-hexane via cold maceration and the extracted oil was analyzed for its physiochemical properties. The fatty acid profile was determined using gas chromatography-mass spectrometry.

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**Results:** The proximate analysis values were determined to be 6.51%, 51.46%, 21.62%, 13.26%, 3.76% and 3.39% for the moisture, crude fat, crude protein, crude fiber, ash and total carbohydrate content respectively. Results of the elemental analysis show the seed contains; 3653.0322 mg/kg Na, 6639.7818 mg/kg K, 2329.0612 mg/kg Ca, 235.6057 mg/kg Fe, 5252.5884 mg/kg Mg, 27.9056 mg/kg Zn and 7.0068 mg/kg Pb. The predominant fatty acid detected with an area percentage of 20.31 was cis-11-octadecenoic acid. Other fatty acids detected include cis-9, cis-12-octadecadienoic acid, cis-9-octadecenoic, hexadecanoic acid, octadecanoic acid and icosanoic acid. Squalene, a biosynthetic precursor of cholesterol, was detected with an area percentage of 8.54.

**Conclusion:** The evaluation of the compositional data provided evidential support for its beneficial health impact particularly in regards to nutritional and cardio-vascular health.

*Keywords: Citrullus colocynthis seeds; elemental analysis; fatty acid profile; physicochemical properties; proximate analysis.*

## 1. INTRODUCTION

*Citrullus colocynthis* (L.) Schrad., also known as Egusi melon, Bitter apple, Colocynth, Wild gourd, is a member of the cucurbit species of the Cucurbitaceae family. The plant is native to regions in Africa, temperate Asia, tropical Asia and Europe [1]. It is widely cultivated in West Africa, the Middle East, Southwest Asia and other African countries for its nutritional, medicinal and environmental value [1,2]. Plants of the Cucurbitaceae family are annual or perennial, climbing or non-climbing, creeping, herbaceous vines that characteristically grow in the tropics and temperate areas. They are known to thrive in arid, temperate, tropical and subtropical locations; some species are extremely sensitive to near freezing temperatures [3,4]. Due to its propensity to grow under challenging environmental conditions, its medicinal properties and its high nutritional value, *C. colocynthis* is a valuable desert plant [3].

*C. colocynthis* has a significant history of medicinal, pharmaceutical, nutraceutical and nutritional use. Several parts of the plant have been used for medicinal purposes and in recent times many of its attributed properties have been validated in animal and human models. Myriad ethnomedicinal literature details the medicinal properties and various bioactives of the *C. colocynthis* plant. The seed extract of *C. colocynthis* has been demonstrated to possess antiulcer and antioxidant potential and its free radical scavenging action is been postulated to be due to its flavonoid content [5,6] while the fruit has been reported to contain a variety of bioactive chemical constituents responsible for its antioxidant, cathartic, carminative, anthelmintic, cytotoxic, antidiabetic, antilipidemic, insecticide, antimicrobial, antipyretic and anti-inflammatory activities [7,3,8]. *In-vitro* and animal model studies validate the ethnopharmacological use of the plant extracts as an anti-microbial [9] and analgesic\ anti-inflammatory agent [10] while several comparative studies have bolstered its use in promoting hair growth [11]. Other studies support its potential in pest control as a green solution to managing dengue vector, *Aedes aegypti* and lymphatic filariasis vector, *Culex quinquefasciatus* [12], as alternative biofuel [13], and as an environmental tool to prevent erosion.

Seeds from the Cucurbitaceae family are cultivated and consumed for their high oil (up to 50%) and protein content (up to 35%) [14] and the *C. colocynthis* seed “Egusi” is no exception. The Egusi seeds are a large part of the diet; used either as the ground whole seed for soups (particularly in West Africa), protein cakes, etc or as the oil extract for cooking. Compositional studies have demonstrated the seed’s mineral, amino acid [15], fatty acid [13,16] and phospholipid profile [17]. High levels of linoleic acid, a polyunsaturated fatty acid (PUFA), has been found in Egusi melon seed [16] and it has also been shown to contain phospholipids; phosphatidylcholine and nly sophos phosphatidylcholine [17]. Additional studies show a favourable saturated to unsaturated fatty acid ratio [13], good quantities of glutamic acid, aspartic acid, arginine, isoleucine, leucine, phenylalanine, phosphorous, potassium, magnesium, zinc and iron [15]. Due to its abundance of macro- and micro-nutrients, it has been put forth as a solution to fight malnutrition in both infants and adults as part of a high-nutrient formulation, particularly in resource-limited settings [18].

The widespread consumption of the *C. colocynthis* seed has been mired by the long-standing notion that diets high in omega-6 polyunsaturated fatty acids (n-6 PUFAs), particularly linoleic acid (LA), result in a predisposition to or an aggravation of inflammatory diseases or diseases with an inflammatory component. However, the theoretical pro-inflammatory risk of n-6 PUFAs, particularly linoleic acid (LA), is yet to be adequately shown clinically [19,20].

Numerous studies and clinical trials have proposed a beneficial effect on human subjects; the cholesterol-lowering effect has been well established in humans [21,22], improvement in insulin resistance [23] and hypertension [24]. Due to its anti-inflammatory and anti-adhesion effect on vascular endothelial cells, possible use in vascular diseases such as atherosclerosis [25] and sickle cell anemia has been hypothesized. In sickle cell anemia, phospholipid and fatty acid content and composition in erythrocyte membrane are significantly altered and decreased due to high oxidative stress which oxidizes the membrane PUFAs, thus it is likely that the role of phospholipids and PUFAs in maintaining erythrocyte membrane integrity, function and regenerative capabilities will magnify its therapeutic potential [26,27,28].

The scope and purpose of this study is a preliminary assessment of the nutritional and health implications of consumption of *C. colocynthis* (L.) Schrad seed and to provide evidence through compositional studies.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The dehulled melon seeds were purchased from a local market (coordinate: 6.507060, 3.369277) in Lagos, Nigeria and authenticated at the Lagos University Herbarium, Department of Botany, University of Lagos, Akoka, Nigeria by Dr. G. I. Nodza. A voucher specimen with voucher number 8382 was deposited for reference purposes.

### 2.2 Sample Pre-treatment

Damaged seeds, dirt and other unwanted debris were removed from the sample. The seeds were allowed to air dry for 48 hrs to achieve a percentage moisture content of less than 8% which was ascertained using an A&D MS-70 Moisture Analyzer (A & D Store, Illinois, USA) at 105°C before they were homogenized using a Solitaire mixer grinder (VTCL, India), weighed on an Explorer analytical balance (Ohaus, Switzerland) and stored in an airtight amber glass container.

### 2.3 Sample Extraction

The seed oil from ground melon seed was extracted with n-hexane (Fisher Scientific, UK) by cold maceration for 72 hours. The sample was filtered with Whatman no 1 filter paper (Sigma-Aldrich, Germany). The solvent was removed using a Rotavapor rotary evaporator (Buchi, Switzerland) at 68ºC and the residual solvent in the oil was allowed to evaporate at room temperature. The oil was centrifuged at 4000 rpm for 20 mins using a CellSpin centrifuge (Hanil Scientific Inc., Korea). The resultant oil was packaged in an air-tight amber glass container and stored at -5ºC.

### 2.4 Proximate Analysis

The ground seeds were analysed for their moisture, crude fat, crude fiber, crude protein, carbohydrate and ash content. The moisture content was taken using an A&D MS-70 Moisture Analyzer at 105ºC. The proximate parameters (crude fat, crude protein, crude fiber and ash value) of the ground *C. colocynthis* seeds were determined using the Association of Official Analytical Chemists [29] method while the carbohydrate content was calculated by difference using the formulae;

$$Total\ Carbohydrate\ (\%) = [100 - \%\,(Protein + Fat + Moisture + Ash + Fiber)]$$

### 2.5 Elemental Analysis

The AOAC [29] official methods for elemental determination in food after dry ashing 999.11 with slight modification was adopted for the digestion and elemental analysis of the sample. The prepared sample solution was analysed with the AA-7000 Atomic absorption spectrophotometer (Shimadzu, Japan) for K, Na, Ca, Fe, Zn, Mg and Pb.

### 2.6 Oil Analysis

Analysis of the melon oil was carried out according to standard methods. The colour, odour and taste of the melon oil were observed. The specific gravity, acid value and peroxide value were determined using standard methods from Pearson's Chemical Analysis of Foods [30] while the iodine value, saponification value and unsaponifiable matter were determined using standard methods from AOAC [29]. The refractive index of the oil was determined using an Abbe-type refractometer (Bellingham + Stanley Ltd, UK) at 30ºC.

## 2.7 Fatty Acid Analysis

The *Citrullus colocynthis* oil sample was methylated using the method described by Wang et al. [31] with slight modification to derive fatty acid methyl esters (FAMEs). 100µL of the oil was placed in a 10mL tube to which 3 mL of 0.5 M methanolic KOH was added. The mixture was heated in a water bath at 60°C for 15 mins. After cooling to room temperature, 3 mL of n-hexane and 2 mL of distilled water were added and mixed thoroughly using a SA7 vortex mixer (Stuart, UK). The n-hexane fraction of the mixture was analysed for its fatty acid profile using a QP2010SE Ultia Gas chromatography-mass spectrometer (Shimadzu, Japan).

## 2.8 Statistical Analysis

The results for the proximate, elemental and oil analysis values are presented as the mean values ± SD; of duplicate determinations in the case of proximate and oil analysis while elemental analysis results were in pentaplicate determinations. The RSD values of all measurements were less than 5%.

# 3. RESULTS AND DISCUSSION

## 3.1 Proximate and Elemental Analysis of *Citrullus colocynthis* (L.) Schrad Seed

The proximate and elemental analysis of the dehulled intact *Citrullus colocynthis* seeds quantified the macro and micronutrients. The moisture, crude fat, crude protein, crude fiber, ash and total carbohydrate content was determined to be 6.51%, 51.46%, 21.62%, 13.26%, 3.76% and 3.39% respectively which was relatively comparable to previously conducted studies [15,32] on other melon seeds. The slight differences observed, are likely due to variations in the seed strain, soil and nutrient composition, pre-purchase and post-purchase processing. The Na, K, Ca, Fe, Mg, Zn and Pb contents were analysed to be 3653.0322 mg/kg, 6639.7818 mg/kg, 2329.0612 mg/kg, 235.6057 mg/kg, 5252.5884 mg/kg, 27.9056 mg/kg and 7.0068 mg/kg respectively and there was an observed significant variation amongst previously reported mineral content values across board suggesting that the mineral content is highly variable and dependent on cultivation soil and conditions. It can be observed from the data that *Citrullus colocynthis* seeds are rich in protein, unsaturated fats, fiber, potassium, calcium, iron, magnesium, zinc and relatively low in total carbohydrates; the proximate total carbohydrate value does not distinguish between simple sugars and complex carbohydrates. This result combined with previous studies and reports on its PUFAs, vitamin [33,34] and calorie content [35] suggests its classification as a nutrient-dense food. According to the National Cancer Institute [36], nutrient dense foods are foods that are high in nutrients but relatively low in calories and typically contain vitamins, minerals, complex carbohydrates, lean protein and healthy fats, with little or no solid fats, refined starches, added sugars and sodium. There are increasing recommendations of a shift to nutrient-dense foods and beverages across and within all food groups in place of less healthy food choices and additionally, the Dietary Approaches to Stop Hypertension (DASH) diet recommends the consumption of foods and beverages low in saturated fats and sodium, and rich in potassium, calcium, magnesium, fiber and protein to manage blood pressure and maintain cardiovascular health, as evidenced from the DASH research trials [37]. Data obtained from the proximate and elemental analysis provides a strong indication of the nutritional relevance of the *Citrullus colocynthis* seeds when prepared with little or no saturated fats, added sugars and sodium in the human diet.

An in-depth analysis of mineral content Egusi seeds demonstrates its beneficial abundance of K, Ca, Fe and Zn which is appropriate to meet the daily recommended value for individuals of age 4 years and above, which specifies 3500 mg K, 1000 mg Ca, 18 mg Fe and 15 mg Zn [38]. The magnesium content of 5252.5884 mg/kg appears disproportionate to the daily value of 400 mg [38], however, it's important to note that though magnesium toxicity is possible, it is unlikely as magnesium intestinal absorption is not directly proportional to intake but depends majorly on cellular magnesium levels [39]. Another major reservation is the seemingly high sodium level (3653.0322 mg/kg). However, low sodium foods have been defined by a sodium content of less than 140 mg per serving while high sodium foods contain more than 400 mg per serving; moderate-sodium foods are between [40]. A typical daily serving of nuts and seeds weighs approximately 30 g [41] which in the case of Egusi seeds contains 109.59 mg of sodium thus classifying Egusi seeds as a low sodium food and thus a suitable component of the DASH dietary plan [37]. A third concern is the lead content of 7.0068 mg/kg. Lead is a naturally occurring toxic metal causing severe toxicity to multiple body systems particularly the neurological, cardiovascular and nephrological system; foetuses and developing children are particularly vulnerable and there is no known level of lead exposure that is considered safe [42]. The widespread use of lead containing products has resulted in environmental contamination. Noting this, several international bodies have placed regulations on the maximum level of lead to be contained in certain edibles, the value ranging from 0.02-3.0 mg/kg wet weight [43,44]. Though none specified a maximum level for nuts and seeds, the range gives a clue as to what is generally acceptable and at a level of above the maximum level (3 mg/kg), the Egusi sample analysed contains a relatively high amount of lead. This suggests the contamination of the soil, water source, fertilizer or other materials or equipment used in the cultivation and/or processing of the *C. colocynthis* and calls for a review and intervention in the cultivation and processing methods of the *Citrullus colocynthis* plant, rather than complete exclusion from the diet.

### 3.2 Physiochemical Properties of *Citrullus colocynthis* (L.) Schrad Seed Oil

The specific gravity, refractive index, acid value, free fatty acid value, peroxide value, saponification value and unsaponifiable matter as shown in Table 3. There is an observed relative conformation of the melon oil physiochemical properties with reported values [45,13]. The quality characteristics of the oil (colour, odour, taste, acid value and peroxide value) were in line with the Codex Alimentarius [46] international food standards for edible fats and oils; free of perceptible indicators of adulteration and rancidity and an acid and peroxide value of less than 4.0 mg KOH/g and 10 meq $O_2$/kg.

The iodine value which reflects the degree of unsaturation of the oil sample was found to be 117.74 g $I_2$ / 100 g which is comparable to 110.93-111.46 reported by Obasi et al. [47]. This value is of particular interest as it is indicative of the relative abundance of fatty acids with double bonds; monounsaturated and polyunsaturated fatty acids (MUFAs and PUFAs) which play multiple physiological roles in the body.

**Table 1. Proximate analysis of dehulled *Citrullus colocynthis* seed**

| S/No | Parameter | Mean value ± SD | Literature value [15] | Literature value [32] |
|---|---|---|---|---|
| 1 | Moisture | 6.51% ± 0.28 | 4.60% ± 0.30 | 4.85% ± 0.04 |
| 2 | Fat | 51.46 % ± 0.18 | 45.70% ± 0.10 | 46.24% ± 0.02 |
| 3 | Protein | 21.62 % ± 0.81 | 23.40% ± 0.20 | 25.73% ± 0.06 |
| 4 | Fibre | 13.26 % ± 0.04 | 12.00% ± 0.10 | 5.00% ± 0.07 |
| 5 | Ash | 3.76 % ± 0.05 | 3.70% ± 0.10 | 4.48% ± 0.02 |
| 6 | Carbohydrate | 3.39% ± 0.27 | 10.60% ± 0.20 | 13.70% ± 0.02 |

** Data are mean of duplicate determinations ± standard deviation*

**Table 2. Elemental analysis of dehulled *Citrullus colocynthis* seed**

| S/No | Element | Mean value ± SD (mg/kg) |
|---|---|---|
| 1 | Sodium | 3653.0322 ± 0.0002 |
| 2 | Potassium | 6639.7818 ± 0.0006 |
| 3 | Calcium | 2329.0612 ± 0.0002 |
| 4 | Iron | 235.6057 ± 0.0006 |
| 5 | Magnesium | 5252.5884 ± 0.0008 |
| 6 | Zinc | 27.9056 ± 0.0026 |
| 7 | Lead | 7.0068 ± 0.0004 |

** Data are mean of five determinations ± standard deviation*

### 3.3 Fatty Acid Profile of *Citrullus colocynthis* (L.) Schrad Seed Oil

GC-MS analysis demonstrated the principal fatty acid to be cis-11-octadecenoic acid, also known as cis-vaccenic acid, with an area % of 20.31. Cis-vaccenic acid is an omega 7 fatty acid [48] and its exact physiological role in the human body has not been as extensively studied as other fatty acids, however, several reports suggest an overall beneficial impact on humans. There was an observed inverse association of erythrocyte membrane cis-vaccenic acid with coronary heart disease [49], of plasma cis-vaccenic acid with heart failure [50] and of cis-vaccenic acid on HT-29 human colon cancer cell growth [51]. Also, the therapeutic potential of cis-vaccenic acid in sickle cell anemia as an inductor of the gamma-globin gene is been evaluated and it was observed that cis-vaccenic acid up-regulated ү-globin expression and increased ү-globin mRNA levels when exposed to primary erythroid progenitor stem cells isolated from transgenic mice bone marrow [52].

Linoleic acid, with an area percentage of 20 for the total fatty acid composition (methyl ester and free form) was second in percentage prominence. Linoleic acid is an essential omega 6 fatty acid with increasing attention for its protective role against cardiovascular disease, coronary artery disease, hypertension, hypercholesteremia and diabetes mellitus and as a result, there are recommendations of an increase in linoleic acid intake from 5-6% of daily energy requirement to 5-10% (up to approx. 22 g/d) to reduce the risk for chronic diseases [53,54].

**Table 3. Physiochemical properties of the *Citrullus colocynthis* oil extract**

| S/no | Parameter | Value | Literature value [45] |
|---|---|---|---|
| 1 | Colour | Pale yellow | Dark yellow with greenish tint |
| 2 | Smell | Mild distinctive nutty odour | Mild odour |
| 3 | Taste | Mild nutty taste | Mild flavour |
| 4 | Specific gravity | 0.88 mg/ml ± 0.02 | - |
| 5 | Refractive index | 1.466 | 1.4655 |
| 6 | Iodine value | 117.74 g $I_2$/100g ± 1.42 | 123.90 g $I_2$/100g |
| 7 | Acid Value | 2.805 mg KOH/g | 2.75 mg KOH/g |
| 8 | Free Fatty Acid | 1.40 % | - |
| 9 | Peroxide value | 1 meq $O_2$/kg | 1.0 – 7.6 meq $O_2$/kg |
| 10 | Saponification Value | 205.47 mg KOH/g ± 2.13 | 202.45 mg KOH/g |
| 11 | Unsaponifiable matter | 1.235 % ± 0.04 | 2.15% |

** Specific gravity, acid value, iodine value, peroxide value, saponification value and unsaponifiable matter are expressed as mean of duplicate determinations ± standard deviation*

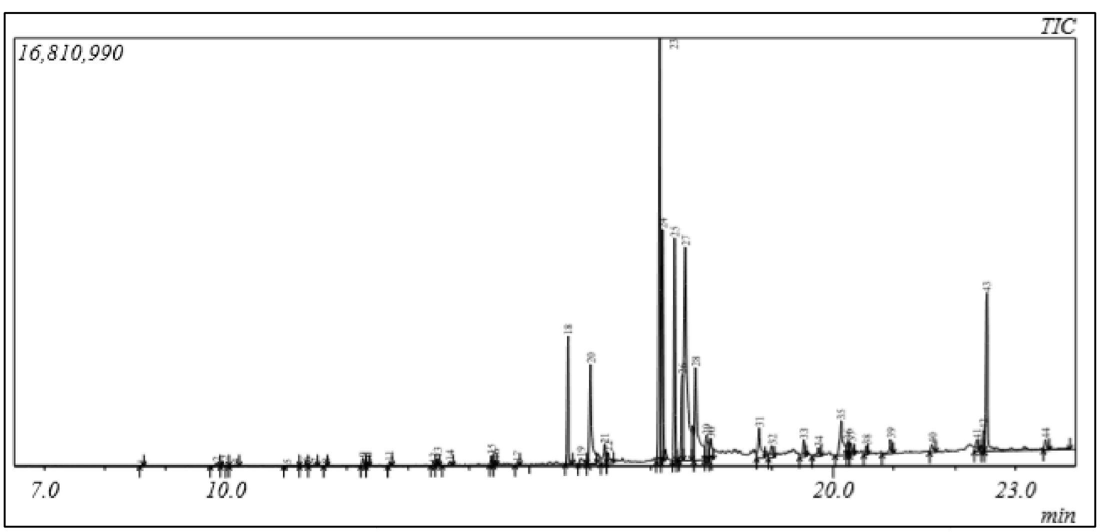


**Fig. 1. GC-MS Total Ion Current (TIC) chromatogram of fatty acid methyl esters of *Citrullus colocynthis* oil extract**

**Table 4. Peaks of interest from GC-MS TIC chromatogram of the fatty acid methyl esters of *Citrullus colocynthis* oil extract**

| Peak number | Retention time | Peak area % | Hit 1 |
|---|---|---|---|
| Peak 18 | 15.630 | 4.18 | Hexadecanoic acid methyl ester (Palmitic acid, methyl ester) |
| Peak 20 | 15.999 | 5.63 | n-Hexadecanoic acid (Palmitic acid) |
| Peak 23 | 17.146 | 15.45 | 9,12-Octadecadienoic acid methyl ester (Linoleic acid, methyl ester) |
| Peak 24 | 17.191 | 7.18 | 9-Octadecenoic acid methyl ester (Oleic acid, methyl ester) |
| Peak 25 | 17.392 | 7.03 | Methyl stearate (Stearic acid, methyl ester) |
| Peak 26 | 17.520 | 4.55 | Cis-9, cis-12-Octadecadienoic acid (Linoleic acid) |
| Peak 27 | 17.566 | 20.31 | Cis-11-Octadecenoic acid (Cis-Vaccenic acid) |
| Peak 28 | 17.733 | 6.78 | Cis-9-Octadecenoic acid (Oleic acid) |
| Peak 31 | 18.781 | 2.09 | Eicosanoic acid (Arachidic acid) |
| Peak 43 | 22.522 | 8.54 | Squalene |

Following is Oleic acid, Palmitic acid, Stearic acid and Eicosanoic acid with a total area percentage of 13.96, 9.81, 7.03 and 2.09 respectively. Oleic acid is a non-essential omega-9 monounsaturated fatty acid and it has been attributed with decreasing low-density lipoprotein while simultaneously increasing high-density lipoprotein, inhibiting cholesterol synthesis, possessing a hypotensive effect, lowering cancer risk, coronary artery disease by 20-40% [55], improving insulin resistance and diabetic risk and modulating inflammation [56]. Also, in the pharmaceutical preparations, oleic acid has been used to enhance the activity of antioxidants due to its high stability to oxidation. In contrast, palmitic and stearic acids are 16-carbon and 18-carbon saturated fatty acids respectively. Though these fatty acids are utilized in the body in important biosynthetic pathways, as a source of energy provision and storage, there is a recommended shift from saturated fatty acids consumption to PUFAs consumption due to their pathological effects on the human system. They have been positively associated with inflammation in chronic diseases [57] particularly coronary heart disease [58], cardiovascular mortality [59], Alzheimer’s disease [60], insulin resistance and glucose intolerance [61], however as an exception stearic acid has been attributed with cardiovascular benefits. Eicosanoic acid, a saturated fatty acid with a 20-carbon chain, is less well studied, its physiological or pathophysiological role is yet to be fully elucidated and results have been inconsistent as to its role in the incidence of diabetes; whether negative [62] or positive [63]. Deducing from its fatty acid profile, Egusi seed oil with a ratio of 1:1.8:1.1 of saturated to monounsaturated to polyunsaturated fatty acids which is relatively comparable to the AHA recommendation of 1:1.3:1 [64], will lead to an overall positive effect on the human body system.

Squalene was, also, detected in the *C. colocynthis* oil sample at a composition of approximately 8% and is an intermediate in the biosynthesis of sterols including steroid hormones and cholesterol in plants and animals. This compound has been attributed with strong oxygen scavenging antioxidant [65] and anti-cancer abilities and has been theorized to be responsible for the protective effect of olive oil observed in ecological studies [66].

## 4. CONCLUSION

This investigation highlights a strong nutritional role and provides evidence of a possible therapeutic role of *Citrullus colocynthis* (L.) Schrad seed intake in the human population. However, it is important *C. colocynthis* cultivation is carried out in farmlands without heavy metal contamination and the mode of preparation for consumption is given due consideration. Analysis of the amino acid and phospholipid profile of the seed will proffer additional evidence to the beneficial impact of *C. colocynthis* consumption.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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