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**Physicochemical, Nutritional and Antioxidant Properties of
Momordica charantia and *Citrullus vulgaris* seed oil extracts.**

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Abstract

Two underutilized tropical seeds were investigated for their nutritive, physicochemical and biological potentials. Oils were extracted from the seeds with n-hexane using Soxhlet extractor. The fatty acid of the oils was determined by gas chromatography-mass spectrometry (GC-MS). Amino acid profile was elucidated from defatted seed cake by alkaline hydrolysis using PTH 120 A model. Proximate analysis was done and the *in vitro* antioxidant potential of the crude oil were investigated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) against Ascorbic acid as standard by zones of inhibition methods. Proximate analysis shows that the seed cake

was rich in lipid (25.49-26.55%), protein (18.28-34.24%) and carbohydrate (25.61-34.83%) with high energy density (441.85-478.35 kcal/kg). polyunsaturated fatty acid (PUFA) 9.cis.,11. trans.t,13. trans.-octadecatrienoic (39.16 %) was the most abundant fatty acid in *M. charantia*. Oleic acid, a monounsaturated fatty acid appears in both seed oil. The oils also contain saturated fatty acids such palmitic acid and stearic acid in appreciable quantity. The quality assessment showed that *C. vulgaris* has high percentage of PUFA than *M. charantia* compared to WHO/FAO standard. Twenty amino acids were detected in each of the seed cake, glutamic acid (15.59 vs 14.84), arginine (10.58 vs 12.21), aspartic acid (9.30 vs 8.71), glycine (7.86 vs 6.25), alanine (6.25 vs 5.19) and leucine (5.25 vs 7.59) in *M. charantia* and *C. vulgaris* respectively. The oil from seed also demonstrated significant antioxidant potential compare to ascorbic acid. Both seeds could be a good source of vital fatty acids and amino acids. The seed cake can also serve as viable source of amino acids which could help the world to fight protein deficiency.

Keywords: Proximate analysis, Amino acid profile, Antioxidants, *Momordica charantia*, *Citrullus vulgaris*.

Introduction

Seeds from plants are rich source of bioactive natural compounds, many of which can be found in their oils. Physicochemical characterization of oils from seed is necessary to determine vis a vis edibility and industrial applications. Oils from seed with high acid value is regarded as not edible, because the high acid value is attributed to oil with extensive hydrolysis by lipolytic and oxidizing enzymes (Fakhri and Qadir, 2011). High acid value may also indicate the level of toxicity of the oil (Aremu *et al.*, 2006).

Low acid value, low peroxide value, presence of antioxidants and low free fatty acids provide physicochemical properties that favours the use of an oil for cooking and other culinary activities. These descriptions determine the overall quality of the oil such as the shelf life and extent of adulteration. However, parameters such as high acid and saponification values of oil are usually of low nutritional benefit to man but makes oil suitable for industrial applications especially in soap production.

Momordica charantia (Karela) commonly known as Bitter melon or Bitter gourd is a tropical and subtropical climber of the family Cucurbitaceae. It

is widely distributed in China, Malaysia, India and tropical Africa. The Latin name *Momordica* means “to bite”(referring to the jagged edges of the leaf, which appear as if they have been bitten). All parts of the plant, including the fruit, taste very bitter, as it contains a bitter compound called momordicin that is believed to have a stomachic effect (Scartezzini and Speroni, 2000).

In Ayurveda, various parts of *Momordica charantia* (Karela) are recommended for many diseases like; cholera, bronchitis, anemia, blood diseases, ulcer, diarrhea, dysentery, sexual tonic, vaginal discharge, constipation, food, diabetes, hyperglycemia, jaundice, stones, kidney, liver, fever (malaria), gout, eczema, fat loss, hemorrhoids, hydrophobia, intestinal parasites, skin, leprosy, pneumonia, psoriasis, rheumatism, scabies, snakebite, and as a cure for gonorrhea (Grover and Yadav, 2004; Beloin et al., 2005).

The main constituents of bitter melon (Karela) are triterpenes, proteins, steroids, alkaloids, and phenolic compounds (Grover and Yadav, 2004). *Citrullus vulgaris*, water melon, also belongs to family Cucurbitaceae cultivated throughout the earth. The watermelon fruit has deep green or yellow coloured smooth thick exterior rind with gray or light green vertical stripes. Inside the fruit is pink, red or even yellow in colour with small black seeds embedded in the middle third of the flesh. Generally, watermelon flesh is the main consumable portion; however, outer rind is also used in some parts of the world (Levi et al., 2001).

Watermelon with red flesh is a significant source of lycopene. Its seeds are cooling, diuretic and strengthening, aphrodisiac, seeds are reported also as demulcent, vermifuge and nutritive. Fruit contains carotene, lycopene, mannitol, 20-40 % of oil from seeds. Seeds are rich source of the enzyme urease. Juice contains citrulline to the extent of 0.17 % (Dane and Liu, 2007).

Vegetable oils are one of the major components of human diets, comprising as much as 25 % of average caloric intake. Vegetable oils play important functional and sensory roles in food products and they act as carriers of fat-soluble vitamins (A, D, E, and K). They also provide an essential linoleic and linolenic acid, responsible for growth (Fasina et al., 2006).

Seed oils have been known to have therapeutic and nutraceutical properties as evidence from the works of Burt, 2004; Tabassum *et al.*, 2013 and Shrestha and Tiwari, 2009, who reported that oil from seeds shows significant health benefits, antioxidant activity as well as antimicrobial activity on the human pathogenic bacteria viz. *B. subtilis*, *E. aerogenes*, *Escherichia. coli*, *Pseudomonas. aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella. typhi*, and *Dermatophytes*.

Reports on the seed from plants were scanty, there was little or no information on the chemical composition, antioxidants and antimicrobial activities of the derived oils and fats. hence this work investigates the physicochemical, fatty acid composition and biological activity of oil extracts from the seeds of *M. charantia* and *C. vulgaris*.

Materials and Methods

Collection of Plant Material and Preparation

The seeds of *Mormodica charantia* and *Citrullus vulgaris*, were collected within Ilorin metropolis, Nigeria. The plant materials were identified at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria where voucher specimen numbers UILH/003/916 and UILH/002/1007 were obtained for *Mormodica charantia* and *Citrullus vulgaris* respectively.

The seeds were dried at ambient temperature, de-shelled and pulverized. The ground samples were kept refrigerated prior to analysis. The samples were divided into two parts. One portion was used to analyze the proximate composition while crude oil was extracted from the other portion using n-hexane and the defatted samples were used for amino acid analysis.

Chemicals and Reagents

The reagents used include: n-hexane, methanol, dichloromethane, methanolic KOH, 1,1,-diphenyl-2-picrylhydrazyl (DPPH), Edman reagent (phenylisothiocyanate), NaOH. All reagents were of analytical grade but all solvents were redistilled before use.

Extraction of oil from the seeds of *M. charantia* and *C. vulgaris*

The pulverised seeds of *M. charantia* and *C. vulgaris* (300 g each) were subjected to Soxhlet extraction using n-hexane as the extracting solvent at 55 °C for 4 h. The crude extracts were concentrated using rotary evaporator to obtain the oil (Atolani *et al.*, 2015). The percentage oil yield was calculated from the expression thus:

$$\% \text{ crude fat} = \frac{\text{Weight of the Oil}}{\text{Weight of Sample (g)}} \times 100 \%$$

Determination of the Physicochemical Parameters of Extracted Oil from the Seeds

The physicochemical analysis of the oil was carried out using standard procedures with slight modification where applicable (Ibeto *et al.*, 2012; Atolani *et al.*, 2015). The percentage (%) yields of the oils were calculated. Odour, colour, and physical state were determined by sensory evaluation.

Determination of Saponification Value

Each oil (1 g) was weighed into a conical flask. 25 ml of 0.5 N KOH was added using a pipette. The mixture was warmed in a water bath for 5 minutes, 3 drops of phenolphthalein was added to it while warm and then titrated against 0.5 N HCl until the pink colour disappeared. The discolouration was taken as an indication of the end point. A blank was conducted simultaneously (Abdulhamid *et al.*, 2013).

Saponification value (SV) was then calculated using the equation:

$$SV = \frac{(b-s) \times M \times 56.1 \text{ g/mol}}{\text{Weight of Sample (g)}}$$

Where b= blank titre value; s= sample titre value; Molecular weight of KOH=56.1 g/mol, M= Molarity of HCl

Determination of Iodine Value

Each oil (0.2) g was weighed into 500 ml conical flask and the oil was dissolved with 15 ml of dichloromethane. 25 ml of Wiji's solution was added and the flask was stoppered and slightly shaken. The resulting mixture was allowed to stand in the dark for two hours. The flask was removed and 20 ml of 10 % potassium iodide (KI) solution was added followed by an addition of 150 ml distilled water. The liberated iodine was

then titrated with 0.1M sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) shaking it constantly and vigorously until the blue-black colour had almost disappear using starch as indicator. A blank titration was also carried out (Van Gerpen, 2005; Ibeto *et al.*, 2012; Atolani *et al.*, 2015).

The iodine value (I.V) is given by the expression:

$$\text{I.V} = \frac{V_1 - V_2 \times M \times 12.69}{\text{Weight of Sample (g)}}$$

Where M = Molarity of Sodium thiosulphate; V_1 = Volume of Sodium thiosulphate used for blank; V_2 = Volume of Sodium thiosulphate used for samples.

Determination of Acid Value

Each oil (1 g) was added into a flask with 25 ml of Ethanol and 3 drops of phenolphthalein as indicator was added to it. The mixture was warmed in a bath for 5 minutes and titrated against 0.1 N KOH until the pink colours appears which indicates the end point. A blank was performed alongside. Acid value was calculated using the expression:

$$\text{Acid value (AV)} = \frac{V_1 - V_2 \times N \times 56.1 \text{ g/mol}}{\text{Weight of Sample (g)}} = \text{mg of KOH}$$

Where: V_1 = vol. of KOH used in sample, V_2 = vol. of KOH used in the blank, N= normality of KOH

Determination of Free Fatty Acids

One (1 g) of oil was boiled with 50 ml of ethanol and allowed to cool, 2 drops of phenolphthalein indicator was added and titrated against 0.1 N KOH until pink colour was obtained (Van Gerpen, 2005; Ibeto *et al.*, 2012; Atolani *et al.*, 2015). The free fatty acid was calculated using the expression

$$\% \text{ Free fatty acids (\% oleic)} = \frac{\text{mL OF KOH} \times N \times 56.1 \text{ g/mol}}{\text{Weight of Sample (g)}}$$

Where w = Weight of sample, 56.1= Molecular mass of KOH, V = Average titre value, N=Normality of KOH

Preparation of Fatty Acids Methyl Ester (FAMES)

Oil from each seeds (1 g) was weighed into a round bottom flask. 20 ml of 0.1 M methanolic KOH was added. The mixtures were refluxed for 1 h. After refluxing, the supernatant obtained was cooled, transferred to a separating funnel and extracted with n-hexane in twofold. The oil layer was concentrated in warm water bath and the oil derived kept in a vial and refrigerated until analysed using GC-MS (Van Gerpen, 2005; Ibeto *et al.*, 2012; Atolani *et al.*, 2015).

Analysis of Oil from the Seeds by GC-MS

The oil from seeds of *Citrullus vulgaris* and *Momordica charantia* was analysed using an Agilent Technology 7890 A gas chromatographic GC-FID, equipped with a fused silica capillary column HP-5MS (30 m by 0.32, 0.5µm film thickness) on ultra-pure helium gas and coupled to a mass spectrometer (MS). The chemical compositions of the product obtained were identified by comparison of their MS spectral data with data obtained from National Institute of Science and Technology (NIST, 2008) database. The relative percentages of the constituent compounds were percentages from the GC peak areas based on the total ion chromatogram (Atolani *et al.*, 2015).

Determination of Proximate Analysis

Standard methods of Association of Official Analytical Chemists (AOAC, 2006 and Ratnayake *et al.*, 2006) were used to determine the moisture content, total ash, crude fat and crude protein content of the oil samples. Crude protein (Total nitrogen (%) × 6.25) was determined by Kjeldahl method, using 2 g of sample. Crude fat was obtained by exhaustively extracting 5 g of sample in Soxhlet extractor using n-hexane. Ash content was determined by the incineration of 2 g sample in a Muffle furnace maintained at 550 °C for 5 h.

Moisture content was determined by heating 2 g of sample to constant weight in a crucible placed in an oven maintained at 105 °C. The total carbohydrate content was calculated by difference, i.e. 100- total value. Energy value (kcal/kg) was calculated by multiplying the values obtained for carbohydrate, protein and fat by 4, 4 and 9 respectively, and adding up the values. All the proximate analyses were carried out in duplicate and reported in percentage.

Determination of Amino Acid Profile

The Amino Acid profile in the seed cake was determined using methods described by Benitez, 1989. The defatted samples were hydrolyzed, evaporated in a rotary evaporator and loaded into the phenylthiohydantoin (PTH) derivative Amino Acid Analyzer (PTH Model 120 A) Applied Biosystems

Defatting Sample: The samples were defatted using chloroform/methanol mixture, ratio 2:1. Four gram (4 g) of the sample was put in extraction thimble and extracted for 15 h in Soxhlet extractor (AOAC, 2006).

Nitrogen Determination: A small amount (200 mg) of ground sample was weighed, wrapped in Whatman filter paper (No.1) and put in the Kjeldahl digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate (CuSO_4) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldahl digestion apparatus for 3 h until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45 % sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2 % boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a.	=	Titre value of the digested sample
b.	=	Titre value of blank sample
v.	=	Volume after dilution (100 ml)
W.	=	Weight of dried sample (mg)
C.	=	Aliquot of the sample used (10 ml)
14.	=	Nitrogen constant in mg.

Hydrolysis of the seed cake

Elimination of Oxygen from defatted sample

Each seed cake (100 g) of the defatted sample was weighed into glass ampoule. 7 ml of 6 N HCL was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cysteine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 22 h. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins.

Evaporation

The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved using 5 ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer.

Loading of the hydrolysate into analyzer

The amount loaded was 60 microlitre. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Method of Calculating Amino Acid Values from the Chromatogram Peaks.

An integrator attached to the Analyzer calculates the peak area which is proportional to the concentration of each of the amino acids.

Determination of Antioxidant Activity of Oil Extracts

The antioxidant potential of the oil extracts was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH). The scavengers react with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with a characteristic absorption at 517 nm wavelength and converted to 1,1-diphenyl-2-picryl hydrazine, due to its hydrogen accepting ability. Ascorbic acid was used as a positive control in this assay.

Free Radical Scavenging Activity by DPPH Assay

The ability of *M. charantia* and *C. vulgaris* oil extracts from seeds to scavenge DPPH free radicals was assessed by the method of Negi *et al.*, 2003; Zubair *et al.*, 2018 with little modification. The stock solution of the oil was prepared in dichloromethane to achieve the concentration of 100

µg/ml. Dilutions were made to obtain concentrations of 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195 and 0.098 µg/ml. Diluted solutions, 2 ml each was mixed with 2 ml of dichloromethane solution of DPPH in concentration of 10 µg/ml. After 30 mins incubation in darkness at room temperature (25 °C), the absorbance was measured at 517 nm. Control solution contains equivalent concentrations of Ascorbic acid and all other reagents except the extract. Percentage inhibition was calculated using equation below, whilst IC₅₀ values were estimated from the % inhibition versus concentration plot, using a linear graph in Graph padprism 7. The data were presented as mean values ± standard deviation [n=3]

$$\% \text{ inhibition} = \left(\frac{A \text{ of control} - A \text{ of sample}}{A \text{ of control}} \right) \times 100$$

Statistical Analysis

Results were calculated from duplicate data and were expressed as mean ± standard deviations. The data were compared by least significant difference test using Statistical Package for Social Sciences (SPSS, version 20).

Results and Discussion

Seed from plant are rich source of bioactive natural compounds, many of which can be found in the oil from seed Physicochemical characterization of oil from seed is necessary to determine for edibility and industrial applications. oil from seed with high acid value are generally regarded as not edible, the high acid value is attributed to oil with extensive hydrolysis and will indicate shorter shelf life (Fakhri and Qadir, 2011). High acid value could also indicate toxicity of edible oils (Aremu, *et al.*, 2006).

Physicochemical characteristics that favour the use of oil for cooking and other culinary activities are low acid value, low peroxide value, presence of antioxidants and low free fatty acids. These properties determine the overall quality of the oil such as the shelf life and extent of adulteration.

The Physicochemical properties of *Momordica charantia* and *Citrullus vulgaris* are shown in Table 1. Both oils show appreciable yield, 25.49 % and 26.55 % for *M. charantia* and *C. vulgaris*.

C. vulgaris. shows an acid value of 3.33 ± 0.53 mgKOH/g that is below the value of recommended value 4 mgKOH/g by WHO/FAO for edible oil. This implies the oil is edible. *M. charantia* shows a value of 14.52 ± 0.89 mgKOH/g that is above the recommended value for edible oil. The value obtained for *M. charantia* is however comparable to that of 11.60 mgKOH/g reported for palm kernel oil by Atasie and Akinhanmi, (2009). The saponification value of *Citrullus vulgaris* (22.17 ± 0.39 mgKOH/g) is far lower compared to 192 ± 43.7 mgKOH/g of oil reported for *Citrullus lanatus*, specie of the plants (Oluba et al., 2008). The saponification value obtained for the oil was within the standard value of 181.41 ± 2.60 for edible oil by WHO/FAO.

The % Free fatty acid of *Citrullus vulgaris* ($2.80 \pm 0.21\%$), is within the value of 5.8-7.28 % standard value for Edible Oil, *Mormodica charantia* shows slightly higher value of $8.40 \pm 0.78\%$. The low free fatty acid value of the oil indicated that oil extracts have a higher shelf live, and are generally of good quality. The low % free fatty could also be attributed to the reduced hydrolysis and enzymatic degradation of the oil. This is possible when phytochemicals such as antioxidant are present in the oil.

The presence of natural antioxidant phytochemicals in the oil may contribute to improved shelf live as well as health benefit of the oil (Aremu et al., 2015). The low peroxides value of oil from seeds of *Momordica charantia* (8.03 ± 0.18 mEq/kg) and *Citrullus vulgaris* (7.16 ± 0.23 mEq/kg) were below the recommended value (10 mEq/kg) for food according to FAO and WHO. This further support the claims of the presence of natural antioxidants in the oil as low peroxide value has been attributed to low level of rancidity which is caused by oxidation of oil (Kayode, 2015).

Both oils exhibited a higher iodine value range between 126.55 ± 0.49 – 153.59 ± 0.55 . This indicated that both oils are highly unsaturated, and therefore contain polyunsaturated fatty acids, which was confirmed using the GC-MS analysis. The total unsaturated fatty acids were found to be 50.04 and 55.28 % respectively for *Momordica charantia* and *Citrullus vulgaris*

Linoleic acid (55.28 %), palmitic acid (27.24) and stearic acid (17.40) were the predominant fatty acids in *Citrullus vulgaris*, similar to reported

values for *Citrullus lanatus* which is other specie of the plants by Raziq *et al.*, (2012), Albishri *et al.*, (2013), Edidiong and Ubong, (2013).

9. cis.,11.trans.t,13.trans.-octadecatrienoic acid a rare polyunsaturated fatty acids (PUFA is the most abundant acid in *Momordica charantia* (Table 2).

The result indicated that both oils can be exploited as a source of essential fatty acids due to high presence of PUFA. *Citrullus vulgaris* can be explored as another source of vegetable oil for cooking due to high yield and owing to its low acid value. The result also indicated that the oil can be explored for natural antioxidants and could be employed in food supplement.

Linoleic acid is an essential fatty acid reported by American Health Association to reduce mortality caused by coronary artery disease (Saravanan *et al.*, 2010), another benefit lies in the prevention of atrial fibrillation (Arbex *et al.*, 2015).

The ratio of unsaturated to saturated fatty acids in edible oils and fats is very important for human nutrition. While high levels of saturated fatty acids is desirable to increase oil stability (physical properties), on the other hand nutritionally they become undesirable, because high levels of saturated fatty acids are frequently considered to have influence in increasing the concentration of low density lipoproteins (LDL), affecting the ratio of LDL to HDL (high density lipoproteins), which is one of the lipid indices considered in predicting cardiovascular risk factor in coronary arterial diseases, promoting clotting and vascular smooth muscle proliferation. Diet with increasing intake of linoleic and linolenic acids increase HDL-cholesterol and decreases LDL-cholesterol, while higher intake of oleic acid decreases LDL-cholesterol, but does not affect HDL cholesterol levels (Lawton *et al.*, 2000; Dzisiak *et al.*, 2004).

Table 1: Physicochemical Analysis of the oil from seeds

Parameter	<i>Mormodica charantia</i>	<i>Citrullus vulgaris</i>	WHO/FAO (Edible oil)
% Yield of oil from seed	25.49	26.55	
Saponification value (mgKOH/g)	77.67±0.66	22.17±0.39	181.4±2.60
Acid value (mgKOH/g)	14.52±0.89	3.33±0.53	4
% Free fatty acid	8.40±0.78	2.80±0.21	5.78-7.28
Peroxide value (meqKg ⁻¹)	8.03±0.18	7.16±0.23	10
Iodine Value	126.55±0.49	153.59±0.55	80-106
Colour	Cream colour	Golden yellow	
Physical state at ambient temperature (25°C)	Solid	Liquid	

Table 2: Fatty Acids Composition in the Seeds of *Citrullus vulgaris* and *Momordica charantia*

Peak no.	Fatty acids	Saturation	Relative Abundance	
			<i>Mormodica charantia</i>	<i>Citrullus vulgaris</i>
1	Myristic acid	14:0	-	0.06
2	Palmitic acid	16:0	12.36	27.24
3	Gamolenic Acid	18:3	1.50	-
4	Linoleic acid	18:2	0.67	5.28
5	Oleic acid	18:1	8.71	-
6	Stearic acid	18:0	37.60	17.40
7	9.cis.,11.trans.t,13.trans.-octadecatrienoic	18:3	39.16	-
Total Saturate			49.96%	47.72%
Monounsaturate			8.71%	0%
Polyunsaturate			41.33%	55.28%
Total Unsaturate			50.04%	55.28%

Proximate Analysis

Nutrients derived from food are of great significance and determine the quality of the food Protein, an essential nutrient in diet is utilized for body building, maintenance of body tissues, and production of hormones and enzymes. Fats and carbohydrates supply energy and heat to the body. Fats are also involved in structural cell membrane and hormone synthesis and transportation of lipid soluble vitamins. The ash component is essential for maintenance of homeostasis. The results of the protein, ash, moisture and fat contents of the seeds are shown in Table 3.

The proximate composition of *Momordica charantia* seed were 18.1, 3.1, 25.49, 18.28, 0.2 and 34.83 while *Citrullus vulgaris* seed were 9.9, 3.3, 26.55, 34.24 and 0.4 % for moisture content, ash, lipids, protein and fibre respectively. Table 3 shows the result of the proximate chemical composition (% dry weight) of *Momordica charantia* and *Citrullus vulgaris* seed. The results revealed the seeds contain an oil with a potential of high oil and protein content to satisfy calorie and protein demand when consumed. The very high protein content suggested that the seeds can be exploited as potential source of amino acids for commercial activities.

The amount of crude protein in *Momordica charantia* and *Citrullus vulgaris* seed were 18.28 and 34.24 % respectively which were comparable to protein rich foods such as bambara groundnut (Yagoub and Abdalla 2007), cowpeas (Ragab *et al.*, 2004), seeds ranging between 13.5-26.8 %. Cashew nut, 25.5 % (Aremu *et al.*, 2006), kidney beans, 20.9 % and lentils, 22.9 % (Perez-Hidalgo *et al.*, 1997) have less amount of proteins in comparison with seed protein of *Momordica charantia* and *Citrullus vulgaris*.

Table 3: Percentage Composition of Nutrients in the Seeds of *Citrullus vulgaris* and *Momordica charantia*

Parameters	<i>Mormodica charantia</i>	<i>Citrullus vulgaris</i>
Moisture Content	18.1	9.9
Ash	3.1	3.3
Lipid	25.49	26.55
Protein	18.28	34.34
Fiber	0.2	0.4
Carbohydrate	34.83	25.61
Calorie Kcal/kg	441.5	478.35

Protein from plant provides inexpensive source of proteins that are widely recognized due to their nutritional values (Amadou *et al.*, 2010). Protein is the resource nutrients used to build and repair worn out cell in the body. It plays a vital role in most metabolic activities (Zaman *et al.*, 2010).

Amino acids (AA) are a class of organic compounds that play a major role in the less developed organisms and in human. It is considered as block components in protein synthesis 14. AA have been classified as essential and non-essential for animals and humans (Talole *et al.*, 2013).

Nutritionally, essential amino acids (EAA) are those whose carbon skeletons are not synthesized by animal cells and therefore must be provided from the diet. In contrast, nonessential amino acids (NEAA) are those AA that are synthesized in a species-dependent manner.

EAA are threonine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine and tryptophan. NEAA are aspartic acid, serine,

glutamic acid, proline, glycine, alanine, cystine and tyrosine (McKee, 2003).

Table 4 confirms the amino acid profile of *Momordica charantia* and *Citrullus vulgaris*. The result shows that both seed cakes contain 20 Amino acid, including Tryptophan. The results supported Arginine as the most abundant EAA composition in both seeds with value range between 10.58 – 12.21 g/100g, followed by Leucine (5.25 – 7.59 g/100g) and lysine (3.12 – 4.88 g/100g). Other EAA appears in appreciable quantity in both seed cakes. Tryptophan was found to be 1.01 g/100g in *M. charantia* and 1.52 g/100g in *C. vulgaris*. Aspartic acid and Glutamic acid were the most abundant NEAA acids with value range of 8.71 – 9.30 and 14.84 – 15.59 g/100g respectively. The results are similar to some of the reported works of Ogundele *et al.*, 2012 and Adebisi *et al.*, 2017 for *C. vulgaris*.

Table 4: Amino Acids Present in the defatted Seeds (cake) of *Citrullus vulgaris* and *Momordica charantia*

Functional Group	Amino acids	Concentrations(g/100g)	
		Momordica Charantia	Citrullus Vulgaris
Alphatic side chain	Glycine	7.86	6.24
	Alanine	6.52	5.19
	Valine	4.41	4.15
	Leucine	5.25	7.59
	Isoleucine	3.66	2.88
Hydroxylic (OH) Group	Serine	3.35	2.99
	Threonine	2.11	2.38
	Tyrosine	1.72	2.06
Sulfur Atoms	Cysteine	1.45	2.05
	Methionine	2.08	2.03
Acidic Groups	Aspartic acid	9.30	8.71
	Glutamic acid	15.59	14.84
Basic Group	Arginine	10.58	12.21
	Lysine	4.88	3.12
	Histidine	3.06	2.36
Aromatic Rings	Phenylalanine	4.08	3.90
	Tyrosine	1.72	2.06
	Tryptophan	1.01	1.52
Imidazole group	Histidine	3.06	2.36
Imino acid	Proline	4.16	3.04

Antioxidants are free radicals' scavenger that could reduce the level of oxidative stress and prevent the oxidation of biomolecules in the body (Ebadi, 2001). They react with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with a characteristic's absorption at 517 nm and covert it to 1,1-diphenyl-2-picryl hydrazine, due to its hydrogen accepting ability Hristea *et al.*, (2006). The use of DPPH provides easy, rapid and convenient methods to evaluate antioxidants and radical scavengers (Soler-rivas *et al.*, 2000).

The results of the free Radical Scavenging potential of both oils from the seeds of *Mormodica charantia* and *Citrullus vulgaris* compared well to Ascorbic acid, a known antioxidant compound as presented in **Table 5**.

Scavenging activity at various concentrations compared well to the standard, Ascorbic acid in each table. *Mormodica charantia* shows an optimum inhibition rate of 82.34 % at 50 µg/ml to 76.99 % at 1.562 µg/ml compared to 89.02 % at 50 µg/ml for Ascorbic acid and 64.18 % at concentrations of 0.391 µg/ml. *Citrullus vulgaris* shows optimum antioxidants potential of 76.59 % at 50 µ/ml concentration, with sustained antioxidant potential (74.59 %) even at the lowest concentration tested of 0.09 µg/ml compared to 21.66 % of Ascorbic acid.

The DPPH antioxidant result of both oils shows that the radical scavenging activity are dose dependent (Figure 1), *Mormodica charantia* had the highest activity (82.34 %) with an IC₅₀ value of 0.207 µg/ml compared to *Citrullus vulgaris* (76.75 %) with IC₅₀ value of 0.217 µg/ml. The standard, Ascorbic acid shows a maximum inhibition of 89.02 % with IC₅₀ values of 0.190 µg/ml concentration.

Table 5: Antioxidant Potentials of Both Extracted Oils and Ascorbic acids

	<i>Momordica charantia</i>	<i>Citrullus Vulgaris</i>	Ascorbic Acid
Conc(µg/mL)	Percentage	Inhibition (%)	
50	82.34	76.05	89.02
25	81.73	76.59	88.14
12.5	77.67	76.54	87.26
6.25	80.18	76.75	85.79
3.125	80.10	76.51	84.26
1.562	76.99	76.08	80.67
0.781	36.52	76.56	75.44
0.391	34.57	76.02	64.18
0.195	34.28	75.64	38.26
0.097	33.77	74.59	21.66
IC ₅₀	0.21	0.22	0.19

Values are average of triplicate reading.

Conclusion

The fatty acid profile and antioxidant activity of two underutilized tropical seeds were examined, the amino acid profile and the potential of the seed cake to serve as source of protein in order to curb the incidence of malnutrition were equally accessed.

The results indicated that both oils can be exploited as a source of essential fatty acids due to high presence of PUFA. *Citrullus vulgaris* can be explored as another source of vegetable oil for cooking due to high yield and owing to its low acid value. The result also indicated that the oil can be explored for natural antioxidants and could be employed in food supplement.

The amount of crude protein in both seeds of *Momordica charantia* and *Citrullus vulgaris* was well comparable to protein rich foods such as bambara groundnut and cowpeas seeds.

The Amino acid profile result shows that both seed cakes contain 20 Amino acid, including Tryptophan.

The results of the free Radical Scavenging potential of both oils of *Mormodica charantia* and *Citrullus vulgaris* are comparable to Ascorbic acid, as a known antioxidant

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