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PHYSICOCHEMICAL PROPERTIES, FATTY ACIDS COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOME CUCURBITS SEED OILS

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ABSTRACT

The physicochemical properties, fatty acid profiles and antioxidant activity of *Lagenaria siceraria* (short hybrid bottle gourd), *Lagenaria breviflora* and *Luffa cylindrica* (Cucurbitaceae) were studied. The yield, refractive index (n_D 25°C), specific gravity (25°C), free fatty acids, unsaponifiable matter, acid, iodine, saponification and peroxide values of the oils were obtained in the range 22.5-28.3%, 1.45-1.49, 0.92-0.93g/cm^3, 1.9-2.88%, 1.10-2.41%, 2.20-2.50 mgKOH/g, 106.0-153.0 mgI$_2$/g, 202.0-238.5 mgKOH/g, and 4.83-5.37 meq.O$_2$/Kg respectively. The oils have four (4) main fatty acids: linoleic acid (50.3-69.7%), oleic acid (10.4-27.3%), stearic acid (6.4-15.0%) and palmitic acid (10.3-13.4%). The seed oils exhibited significant *in vitro* antioxidant activity on DPPH radical. Their chemical properties are similar to corn and sun flower oil, suggesting their potential use as good table and cooking oils which can increase HDL and reduce serum cholesterol and LDL levels.

Keywords: *Cucurbitaceae*, Seed oils, Fatty Acids, Antioxidant Activity, DPPH Method

INTRODUCTION

Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite radicals have been reported to play a significant role in oxidative stress related to the pathogenesis of various important diseases **[1, 2]**. Lipid peroxidation is an important factor in the deteriorating reaction in food during storage and processing, and is believed to be associated with some diseases such as carcinogenesis, mutagenesis, ageing and arteriosclerosis **[3]**. The role of ROS and free radicals in tissue damage in such diseases is becoming increasingly recognized **[4]**. There is a list of ongoing research on such plant for their potential usefulness as dietary supplements and as adjuvant for use in prevention of disease. Vegetable oils are essential in meeting global nutritional demands and are utilized for many food and other industrial purposes **[5]**. Despite the broad range of sources for vegetable oils, the world consumption is dominated by soybean, palm, rapeseed, and sunflower oils with 31.6, 30.5, 15.5, and 8.6 million tons consumed per year, respectively **[6]**. These conventional sources of vegetable oil no longer meet the ever increasing demands of domestic and industrial sectors **[5]**. Therefore, the need exists to look for other sources to supplement the supplies. From this view point, non-conventional oil seeds are of much concern to cope this challenge. More recently, research activities have focused on examining and characterizing new sources of edible oils. Esuoso *et al.*, **[7]** reported that seeds of some species of *Cucurbitaceae* can be the edible oil sources to meet the increasing demands for vegetable oil. *Cucurbitaceae* is a plant family, also known as gourd family, which includes crops like cucumbers, squashes, luffas and melons. Cucurbits form an important and a big group of vegetables crops cultivated extensively in the subtropical and tropics countries. The family consists of about 118 genera and 825 species **[8]**. *Lagenaria siceraria* (Mol.) Standley fruit is one of the important plants commonly known as bottle gourd and belongs to cucurbitaceae family. The plants contain triterpenoid, cucurbitacins, flavones, glycosides and have been used in traditional system of medicine. It has been used as antihyperglycemic, antihyperlipidemic, analgesic and anti-Inflammatory, antibacterial and diuretic **[9]**. *Lagenaria breviflora* Robert (Cucurbitaceae) is a perennial climber. The leaves are very scabrid and sandpapery. The fruits are dark green with creamy blotches, and are ovoid. Its seeds and fruits have been used in folk medicine since antiquity. *Luffa cylindrica* (Linn) M. Roem. is a climber with slender, slightly hairy, furrowed stem. The fruit is the loofah or vegetable sponge which is the hard fibro-vascular network found within the ripe fruit **[10]**. The plant is reputed to have antitubercular and antiseptic properties. The fruit is used in dropsy, nephritis, chronic bronchitis. The seeds are considered as emetic and cathartic. The seed oil is reported to be used for skin infections. In the form of tincture, the fruit is used in the treatment of ascites, jaundice, biliary and intestinal colitis and also in enlarged spleen and fever **[11]**. Many oil seeds of Cucurbitaceae are widely employed in domestic activities and have high nutritive value. These oil seeds are good sources of lipids and proteins with defatted cakes capable of being used as a protein supplement in human nutrition **[12]**. Several studies have reported the nutritive value, chemical composition and oil characteristics of some *Lagenaria* and *Luffa* seeds from different regions and varieties **[13-16]**. The four fatty acids present in significant quantities are palmitic, stearic, oleic and linoleic acids. *Luffa acutangular* (var. amara) seed oil was found to have high amount of myristic acid and low linoleic acid content **[17]**. The antioxidant and antimicrobial activity of the ethanol and aqueous extracts of these seeds have been reported **[18, 19]**. However, there has been paucity of information on the antioxidant activity of the studied seed oils. Due to the differences among the species and/or varieties of *L. siceraria*, *L. breviflora* and *L. cylindrica* grown in different regions of the world, the present study was undertaken to determine the oil characteristics and *in vitro* antioxidant activity of these Cucurbitaceae species.

MATERIALS AND METHODS

Sample Collection and Extraction

Mature fruits of *L. siceraria* (short hybrid bottle gourd), *L. breviflora* were collected in September, 2012 and *L. cylindrica* in January, 2013 from Ikono Local Government Area of Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. (Mrs.) M. E. Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria where voucher specimens were deposited. The seeds were washed, dried, pulverized with seed coat intact, in a coffee grinder and oil was extracted with *n*-hexane using a Soxhlet apparatus. Oil content was determined gravimetrically. All chemicals, solvents and fatty acid methyl esters (FAMEs) standards used in this study were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO).

Physicochemical Properties Analysis

The physicochemical indices (colour, yield, viscosity, refractive index, specific gravity, acid, iodine, peroxide, free fatty acid, unsaponifiable matter values) were carried out according to the methods described by AOAC **[20]**.

Fatty Acid Composition Analysis

The fatty acid composition of the oils was evaluated by Gas Liquid Chromatography of fatty acid methyl esters (FAMEs) prepared by boron trifluoride-catalyzed transesterification, according to the method of Morrisson and Smith **[21]** and AOAC **[20]**. FAMEs were analyzed on a HP 6890 Powered with HP ChemStation Rev. A09.01 [1206] Software and equipped with a hydrogen flame ionization detector (FID). Separation was performed using a fused capillary column (HP INNOWax, 30m x 0.25mm x 0.25µm) as stationary phase. The oven temperature was programmed as follows: initial temperature at 60ºC, first ramping at 12ºC/min for 20min; second ramping at 15ºC/min for 3min, maintained for 8 min. The injector and detector temperatures were 250ºC and 320ºC respectively. The carrier gas was nitrogen and a split ratio of 20:1was used. The FAMEs were identified by comparing their retention times to those of a standard mixture of fatty acids and the peak areas were integrated.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the cucurbit seed oils was evaluated by method as described by Kumaran and Karunakaran **[22]** with slight modification. Briefly, 0.5 ml DPPH solution (0.05% w/v in methanol) was mixed with serial dilution of (20 to 100 µg/ml, in methanol) of oil samples and mixture was incubated for 30 min at room temperature. Absorbance of reaction mixtures were measured at 517 nm against the blank, which contained all reagents except the test compound. DPPH radical scavenging activity was calculated by using following formula:

% Inhibition = Control - Test/Control x100

RESULTS AND DISCUSSION

The results of the physicochemical properties of *L. siceraria*, *L. breviflora* and *L. cylindrica* seed oils are presented in **Table 1**. The percentage yield of the oil ranged between 22.5 and 28.35%. The low yield could be attributed to the fact that the studied seeds were processed with the hulls. The oil yields will drastically improve with dehulled seeds. Elemo *et al*., **[23]** observed a similar trend for yields of dehulled (44.8%) and whole seed (25.7%) for *L. aegyptica* seed oils. The refractive index (1.45-1.49) and specific gravity (0.92-0.93) of the oils is in close congruence with values indicated for seed oils of three varieties of bottle gourds and *L. acutangula* **[17, 24]**. The highest saponification value (238.5mgKOH/g) and iodine value (153.0 mgI_2/g) were obtained for *L. siceraria* and *L. cylindrica* oils respectively. These values are also high compared with saponification (221.0 mgKOH/g) and iodine (98.7mgI_2/g) values reported by Olaofe *et al*. **[24]**. The iodine value and saponification indices of *L. cylindrica* in the study are proximate with the values presented by Elemo *et al*., **[23]** for oil of whole seed of *L. aegyptica*. **Table 1** also shows that the iodine value of *L. breviflora* (110.7mgI_2/g) is slightly higher than that reported for *A. breviflorus* oil (100.6mgI_2/g) **[25]**.

Table 2 indicates the fatty acids profile of the oil samples. The results reveal that the oils contain significant quantity of unsaturated fatty acids (74.4-80.3%) compared with the saturated acids (19.7-25.6%). The oils are predominated significantly with palmitic acid (10.3-10.4%), stearic acid (6.4-15.0%), oleic acid (10.4-27.3%) and linoleic acid (50.3-69.7%). It is observed that the unsaturated fatty acids (oleic and linoleic) account for most of the cucurbitaceae seed oils. It is also noted that linoleic and oleic acids are the major fatty acids in peanut, soy beans and lentil **[24]**. Olaofe *et al*., **[24]** showed that the three *Lagenaria* (bottle gourds) contained lauric acid (0.11-9.12%), however not detected in their *Citrullus colocynthis* seed oil. Lauric acid was not found as a component of the three Cucurbitaceae oils in the study. Table 2 also shows a less significant amount of arachidonic acid (2.2%) in *L. cylindrica* oil and its non-occurrence in *L. siceraria* and *L.breviflora*. This value is comparable with arachidonic acid (2.02%) detected in *L. aegyptica* **[23]**. The oleic /linoleic acid ratio of *L. cylindrica* oil (0.54) is the highest among the samples studied while *L. siceraria* oil has the lowest value (0.15). This index helps in determining the detrimental effects of dietary fats. The higher the ratio, the more nutritionally useful the oil **[26]**. The amount of linoleic acid (61.1%) in *L. breviflora* oil **(Table 2)** is comparable with that reported for *Adenopus breviflorus* oil (60.73%) **[27]**. The significant quantity of polyunsaturated fatty acids (50.4-69.8%) in the sample oils investigated may have additional advantage over soy beans oils as these unsaturated fatty acids are essential constituents of human diets **[28]**.

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various extracts and is considered a model of lipophilic radical. A chain reaction was initiated by lipid auto oxidation. Antioxidants, upon interaction with DPPH, either transfer an electron or a hydrogen atom to DPPH, thus neutralizing its free radical character **[29]**. The scavenging effects of the concentrations of oil and BHT in the DPPH are illustrated in **Table 3**. The scavenging effects of the seed oils were lower than BHT. The DPPH assay was concentration dependent. *Luffa cylindrica* oil exhibited the highest antioxidant potential (48.75 % inhibition at 100µg/ml). The least activity was exhibited by *L. siceraria* seed oil. This activity may have been partly contributed by some constituents other than fatty acids, for example, tocopherols and phenolic compounds **[30]**.

Table 1: Physicochemical Properties of Some Cucurbits Seed Oils

Oil Characteristics	*L. siceraria*	*L. breviflora*	*L. cylindrical*
% Yield	26.80	22.50	28.30
Odour	Agreeable	Slightly pungent	Agreeable
Colour	Light yellow	Greenish brown	Greenish
State (28°C)	Liquid	Liquid	Liquid
Refractive Index (n_D 25°C)	1.45	1.49	1.46
Specific gravity	0.92	0.95	0.93
Acid value (mg KOH/g)	2.20	2.50	2.47
Iodine value (mgI_2/g)	106.00	110.70	153.00
Saponification value (mg KOH/g)	238.50	211.78	202.00
Unsaponifiable matter (%)	2.41	2.32	1.10
Peroxide value (meq. O_2/Kg)	4.83	5.60	5.37
Free fatty acids (% oleic acid)	1.90	2.20	2.88

Table 2: Fatty Acid Composition of Some Cucurbit Seed Oils

Parameter (%)	*L. siceraria*	*L. breviflora*	*L. cylindrica*
Myristic acid (C14:0)	0.2	0.2	0.2
Palmitic acid (C16:0)	10.4	10.3	13.4
Palmitoleic acid (C16:1)	0.1	0.1	0.1
Stearic acid (C18:0)	9.0	15.0	6.4
Oleic acid (C18:1)	10.4	13.0	27.3
Linoleic acid (C18:2)	69.7	61.1	50.3
Linolenic acid (C18:3)	0.1	0.2	0.1
Behenic acid (C22:0)	0.1	0.1	0.1
Arachidonic acid (C20:0)	-	-	2.2
Saturated acids	19.7	25.6	22.3
Unsaturated acids	80.3	74.4	77.8
Unsaturated/Saturated	4.1	2.9	3.5
Monounsaturated acids	10.5	13.1	27.4
Polyunsaturated acids	69.8	61.3	50.4
Oleic/Linoleic acid ratio	0.2	0.2	0.5

Table 3: Free Radical Scavenging Activity of some Cucurbit Seed Oils on DPPH Radical

Conc. (µg/ml)	*L. siceraria*	*L. breviflora*	*L. cylindrical*	BHT
20	17.10	21.81	19.32	48.52
40	21.37	29.11	26.81	57.84
60	25.68	33.51	31.73	65.18
80	29.14	41.30	33.91	73.44
100	35.13	43.15	48.70	85.65

CONCLUSION

The underutilized seed oils of *L. siceraria, L.breviflora* and *L. cylindrica* are predominant in essential fatty acid (linoleic acid) and total unsaturated fatty acids. The high saponification and iodine indices of the oils may be exploited by the soap and surface coating industries respectively. Furthermore, the proportionate abundance of linoleic and oleic acids which may impute on the suitability of these oils for reducing serum cholesterol, hence the fight against cardiovascular illness; and the significant antioxidant activity exhibited by these oils suggest potentials for future human food supplements and formulations.

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