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Vitamin E, Phospholipid, and Phytosterol Contents of *Parkia biglobosa* and *Citrullus colocynthis* Seeds and Their Potential Applications to Human Health

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ABSTRACT: Vitamin E, phytosterol, and phospholipids are classes of lipids that are also referred to as nutraceuticals. These lipids are components of foods, which have nutritional as well as numerous other health benefits, and consumption has been shown to prevent certain deadly diseases. These compounds can only be obtained from plant products; there is therefore a need for more research on the availability of these compounds from common food sources. Oils extracted from *Citrullus colocynthis* and *Parkia biglobosa* seeds were analysed for vitamin E, phospholipid, and phytosterol contents using a chromatographic technique. The seeds had total vitamin E contents of 53.47 and 42.57 mg/100 g, phytosterol contents of 260 and 451 mg/100 g, and phospholipid contents of 409 and 1,603 mg/100 g for *C. colocynthis* and *P. biglobosa*, respectively. Thus, consumption of these two plants as condiments will help people consume these essential lipids and could serve as dietary supplements to prevent and combat occurrence of certain deadly diseases; this is important as the world is revolving towards disease prevention rather than curing, which is often more expensive and difficult.

Keywords: vitamin E, phytosterol, phospholipid, health benefit

INTRODUCTION

Nutraceuticals are substances derived from food sources, which could provide extra health benefits alongside the nutritional benefits of food. Nutraceuticals may also be used in medicine (Nasri et al., 2014). Examples of nutraceuticals are vitamins, sterols, phospholipids, omega 3, and stilbenoids. Vitamin E, phospholipids, and phytosterols are classes of lipid, a large group of molecules present freely in nature that have various biological functions (Bruce, 2012).

Vitamin E majorly comprises of tocopherols and tocotrienols, which are generally referred to as tocols. Tocols are a group of amphiphatic and lipid soluble compounds that have been found to possess numerous health benefits (Kamal-Eldin and Appelqvist, 1996; Eitenmiller and Lee, 2004). Tocols act as antioxidants and could therefore help neutralize substances that can damage the genetic materials by oxidation (Rudzińska et al., 2016). Phytosterols and phospholipids have also been found to have numerous health benefits: they have impact on proper cognition and brain development, and have anticancer properties (Jayaraman et al., 2008; Berger et al., 2004). Including these lipids in diets may go a long way in improving human health and in preventing diseases that may arise from their absence. Vegetable oils, nuts, and seeds are the major sources of these lipids, and supply them to the body systems when they are consumed. The numerous health benefits of these lipids have therefore generated a need for readily affordable sources of these lipids in food. This will be of great importance to health and the economy, especially in developing countries because the global world is gradually revolving toward the use of dietary supplements to combat and prevent occurrence of certain deadly diseases.

Parkia biglobosa (African locust bean seed) is the seed of a matured fruit that comes from the Parkia tree. When these seeds are harvested, they undergo fermentation to form what is called '*Iru*', '*Ogiri*', or '*Dadawa*' in Yoruba, Igbo, and Hausa languages, respectively, in Nigeria (Augustine et al., 2013). This contains high quantities of protein, lipid, soluble sugars, carbohydrate and ascorbic acid (Augustine et al., 2013; Alabi et al., 2005). The cotyledon is very nutritious and contains lower amount of

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fibre and ash contents; this could also serve as an alternative source of protein in the diets of poor families because it is rich in essential acids and vitamins (Akintayo, 2004).

Citrullus colocynthis (melon) belongs to the family of Cucurbitaceae. *C. colocynthis* seeds are grown for food and are less expensive and widely distributed (Egbebi, 2014). *C. colocynthis* is an annual plant common in countries including Europe, Asia, and Africa. The seeds have therapeutic effects, including antioxidant and anti-inflammatory effects, and can also be used as analgesics (Azhari et al., 2014). *C. colocynthis* seeds are used to prepare different delicacies when ground; however, these seeds are usually boiled or cooked before being consumed as they contain some toxic compounds that may be harmful if consumed uncooked or in a large quantity (Bnouham et al., 2006).

P. biglobosa and *C. colocynthis* are commonly used as condiments because of their availability and affordability and believed nutritional value. Therefore, we carried out a comprehensive analysis of the nutraceuticals present in these plants to help further elucidate and establish the nutritional and health benefits of these seeds.

MATERIALS AND METHODS

Materials

P. biglobosa (PKB) and *C. colocynthis* (CTC) seeds were obtained from farms in the outskirts of Ado-Ekiti, Nigeria in September 2017. The seeds were identified at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. Samples were washed thoroughly and air dried before grinding.

Extraction of oil

Powdered sample (200 g) was loaded into Soxhlet extractors and the oils were extracted with diethyl ether for 8 h. Extracted oils were then concentrated using a rotary evaporator.

Determination of vitamin E content

To quantify the tocols in the vegetable oils, we followed the methods of Du and Ahn (2002) and Ahsan et al. (2015) with slight modifications. Oil samples (100～150 mg) were weighed into 50 mL Erlenmeyer flasks, and were esterified with 10 mL of a freshly prepared mixture containing ethanol, 33% KOH solution, and ascorbic solution (in order to prevent oxidation of the tocols during esterification). Sample were homogenized and incubated at 50°C for 1 h then cooled with ice water for 10 min, before addition of 5 mL deionised water and 5 mL redistilled hexane the mixture was then shaken thoroughly and capped. The mixture was then allowed to stand for about 15 h, the phase was separated and the unsaponifiable matter was transferred to a scintillation vial under nitrogen. To derive tocopherols, we then added a pyridine and a mixture of 99% bistri fluoro acetamide and trimethyl chlorosilane or, to derive tocotrienols, we added trimethyl siliane. We then added 2 mL of internal standard solution and 7 mL of hexane and the lipids were derived overnight at room temperature before analysis by gas chromatography (GC).

GC equipment and conditions: Hewlett-Packard Packed 6890 Gas Chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with flame ionization detector. The capillary column used was 30 m×0.25 mm ×0.25 μm for tocopherol and 15 m×0.25 mm×0.15 μm supported on $BaCO_3$ for tocotrienol. The chromatographic conditions were as follows: initial temperature 180°C (10 min); increased from 8°C/min to 260°C, then to 280 °C at 2°C/min, maintained at 13 min. The injector and detector temperatures were 290°C and 300°C, respectively. Helium was used as the carrier gas at a flow rate of 1.2 and 2.5 mL/min for tocopherols and tocotrienols, respectively. Peak areas were measured using a Hewlett-Packard Packard 7860 integrator (Agilent Technologies, Inc.).

Determination of phytosterol content

The composition of the phytosterols was determined according to the method of the International Organization for Standardization (ISO, 1998). After addition of 1.0 mL of internal standard solution, approximately 250 mg of oil was saponified with an ethanolic potassium hydroxide solution; the unsaponifiable fraction was isolated by solid-phase extraction on an aluminum oxide column, and the steroid fraction was obtained after thin layer chromatography. Bands were visualized using *n*-hexane/diethyl ether (1:1, v/v) as the developing solvent. The sterol profile was analysed using GC analysis carried out with a HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] software (Agilent Technologies, Inc.) fitted with a HP-INNOWax column (30 m×0.25 mm×0.25 μm, Agilent Technologies, Inc.) equipped with flame ionization detector. Nitrogen carrier gas was used at a flow rate of 35 mL/min and a pressure of 22 psi, 1 mL/min. The injector and detector temperatures were 250°C and 320°C, respectively, and the oven was programmed to decrease in temperature from 60 to 15°C at 4°C/min. The injection volume was 1 L, with a split ratio of 20:1. The total sterol content was determined by considering all peaks of sterols eluted between cholesterol and Δ^7-avenasterol. Peaks were identified by comparing the relative retention times of samples with those obtained from standards.

Determination of phospholipid content

The phospholipid content of the extracted oil was deter-

mined using the method of Raheja et al. (1973), with slight modification. Extracted fat (0.01 g) was added to the test tubes, and nitrogen was passed over the oil to completely remove the solvent. Chloroform (0.40 mL) was then added to the tubes, followed by 0.10 mL of a chromo-genic solution. The content of the tube was heated to 100°C in a water bath for 80 s, and cooled to room temperature; 5 mL of hexane was then added and the tube was gently shaken several times. The solvent and the aqueous layer were allowed to separate; the hexane layer was recovered and concentrated to 1.0 mL for GC analysis using a gas chromatography instrument (HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] software, Agilent Technologies, Inc.) equipped with pulse flame photometric detector. The conditions were as follows: stainless steel column 30 m×0.25 mm×0.25 μm (HP-INNOWax, Agilent Technologies, Inc.); column temperature of 250°C; carrier gas N_2, 35 mL/min and H_2, 30 mL/min. Oven temperature program: initial temperature of 50°C; first ramping at 10°C/min for 20 min and maintained for 4 min; second ramping at 15°C/min for 4 min and maintained for 5 min.

Determination of fatty acid composition

The fatty acid composition was determined according to Cocks and van Rede (1996) with slight modification. Extracted oil (0.5 g) was mixed with 3 mL of dimethyl ether and 0.2 mL of sodium methoxide to form a colloidal solution. The solution was allowed to settle and was centrifuged to precipitate. The solid was filtered and the filtrate was kept for GC analysis. 1 μL of the filtrate was injected into the gas chromatography instrument (HP 6890 Powered with HP ChemStation Rev. A 09.01 [1206] software, Agilent Technologies, Inc.) equipped with a flame ionization detector. The conditions were the same as described for determination of phospholipid composition above. Individual fatty acids were identified by comparing their retention times with a certified fatty acid methyl esters. The relative percentage of each fatty acid was quantified as the percentage of total fatty acids.

RESULTS AND DISCUSSION

Vitamin E content

Analysis of the content of vitamin E in the samples are presented in Table 1. CTC had the highest content of vitamin E (53.47 mg/100 g), while both samples had a very high amount of γ-tocopherol. Tocotrienols were detected in minute quantities in the investigated seeds; α- and β-tocotrienols were measurable, both at levels of 1.0 mg/100 g and less. The γ-tocopherols recorded for these seeds are higher than those previously reported for canola, sunflower, corn, and soybeans (12.0, 9.23, 25.92, and 27.3 mg/100 g, respectively) by Grilo et al. (2014). Among the various isomers of vitamin E, γ-tocopherol is regarded as the most powerful free-radical reactive nitrogen oxide species remover due to its ability to trap electrophiles (Wechter et al., 1996). γ-Tocopherols act as anti-inflammatory agents, aid cell signaling, and have the ability to lower cancer risk (Wechter et al., 1996; Ju et al., 2010). Furthermore, γ-tocopherols have been reported to be stronger than α-tocopherols for reducing platelet aggregation, delaying intra-arterial thrombus γ-aggregation, and oxidizing low-density lipoproteins (Li et al., 1999; Saldeen et al., 1999; Ju et al., 2010). The investigated plant seeds have high amounts of γ-tocopherol and could therefore be superior sources of γ-tocopherol when consumed.

Moreover, it is noteworthy to mention that the four homologous series of tocopherols are present in these seeds, thus suggesting they are of high nutritive value. The presence of all the tocopherols isomers has been found to give food synergetic activities and assist their array of beneficial biological functions (Hammond, 2003; Ahsan et al., 2014).

Of importance, γ- and δ-tocopherols have synergistic roles in counteracting pro-oxidant effects of α-tocopherol and prevent lipid peroxidation (Saldeen and Saldeen, 2005). Likewise, all tocopherols, except β-tocopherol, inhibit smooth muscle proliferation (Saldeen and Saldeen, 2005).

Lack of vitamin E and natural tocopherols, such as γ- and δ-tocopherols, in some diets may reduce health promoting abilities. Given the presence of tocopherol isomers in these seeds, routine consumption could provide all the required tocopherol isomers to maintain balanced biological functions.

Although fat and vitamin E coexist in many dietary sources, γ-tocopherol-rich nuts often contain high levels

Table 1. Vitamin E content of the vegetable oils

(unit: mg/100 g)

	PKB	CTC
Tocopherols		
α-Tocopherol	2.43±0.005	3.86±0.008
γ-Tocopherol	39.6±0.082	47.9±0.047
β-Tocopherol	$1.56\times10^{-5}\pm4.71\times10^{-8}$	$3.53\times10^{-5}\pm4.71\times10^{-8}$
δ-Tocopherol	$1.22\times10^{-1}\pm5.2\times10^{-4}$	$3.16\times10^{-1}\pm4.71\times10^{-3}$
Total	42.15	52.08
Tocotrienols		
α-Tocotrienol	$1.20\times10^{-1}\pm0.0047$	$4.31\times10^{-1}\pm0.0012$
γ-Tocotrienol	$2.98\times10^{-1}\pm0.0012$	$8.93\times10^{-1}\pm4.7\times10^{-4}$
β-Tocotrienol	$5.76\times10^{-5}\pm4.71\times10^{-8}$	$6.15\times10^{-2}\pm4.7\times10^{-5}$
δ-Tocotrienol	$1.50\times10^{-5}\pm4.71\times10^{-7}$	$1.95\times10^{-5}\pm4.71\times10^{-8}$
Total	4.18×10^{-1}	1.39
Total vitamin E	42.57	53.47

Mean±SD (n=3).
PKB, *Parkia biglobosa*; CTC, *Citrullus colocynthis*.

of polyunsaturated fatty acids (PUFAs), while many α-tocopherol rich plant oils tend to have more monounsaturated fatty acids than PUFA (Jiang, 2014; Rudzińska et al., 2016). Respective attributes in the studied seeds are likely to be of immense interests to food and other manufacturing industries.

Phytosterols

Results showed that seed samples had high contents of phytosterols (Table 2). These seeds are rich in β-sistosterol. PKB showed the highest quantity of 377 mg/100 g, and the content for CTC were within the range 52.9 ~70.8%, which has been reported for some varieties of melon by Petkova and Antova (2015).

Studies has shown that β-sistosterol may help normalize natural killer cell function (Rothschild, 1999), which may be linked to its ability to reduce pain levels by controlling formation of inflammation and inflammatory cytokines, and modulating immune functions (Berger et al., 2004). The most abundant sterols in plants are: β-sistosterol, campesterol, and stigmasterol. These phytosterols lower absorption of cholesterol, which is linked to various diseases like coronary heart disease (Nguyen, 1999), and possess anticancer, anti-atherosclerosis, anti-inflammation, and antioxidation activities (Cantrill and Kawamura, 2008). The presence of appreciable amount of these phytosterols in the investigated seeds further underlines their importance as sources of these sterols and the associated health benefits. The PKB and CTC seeds had total phytosterol contents of 451 and 260 mg per 100 g, respectively, which is higher than 149 and 117 mg, respectively, reported for soy beans and kidney beans by Higdon et al. (2017). Consumption of less than 300 g of these samples can therefore supply the daily dietary intake (150~400 mg) of phytosterols to the human system to help lower blood cholesterol levels (Cantrill and Kawamura, 2008). The actual daily dose needed to lower blood cholesterol levels is 2~3 g, which translates to 3.4~5.2 g of the esterified form (Cantrill and Kawamura, 2008; Kmiecik et al., 2011).

Dietary sterols have also been found to reduce serum cholesterol levels, thus slowing down growth and spread of cancer cells; moreover, sterols are beneficial in reducing symptoms of benign prostatic hypertrophy (Berger et al., 2004). Therefore, these seeds could be natural sources of plant sterols, which can only be obtained from dietary sources, and consumption should be encouraged for all persons due to the numerous health benefits.

Phospholipids

The phospholipid profile of the vegetable oils is presented in Table 3. PKB seeds showed very high amount of phosphatidylserine (PS) and phosphatidylcholine (PC), whereas CTC seed showed very high amount of phosphatidylinisitol. Phospholipids are essential lipid molecules found in cellular membranes that make up the lipid bi-layers. Phospholipids are also important for optimal brain health (Jayaraman et al., 2008). High amount of PS in PKB seeds reflects its use in traditional medicine for treatment of eye diseases; PS is an important phospholipid in the nervous system and in vision. PC and PS aid proper cellular function to help the brain cope with stress and depression, brain cognition, memory revitalization, increases in learning skills, development of vocabulary skills, and improved sight (Weihrauch and Son, 1983; Jayaraman et al., 2008).

There is also high amount of PC in PKB seeds. PC has been shown to be effective in ameliorating and curing liver disease (Küllenberg et al., 2012), and useful for normal brain development (Zeisel, 2004). PC is the most abundant phospholipid in mammalian cell membranes, comprising 30 to 50% of total phospholipids (Zeisel, 2004); this further supports the importance of these seeds as sources of PC for human health.

The phospholipid analysis showed that PKB is rich in total phospholipids; with concentrations of 1,603 mg/100 g, PKB contains higher amounts of phospholipids than peanut and beef (620 and 660 mg/100 g, respectively),

Table 2. Phytosterol composition of the vegetable oils (unit: mg/100 g)

Phytosterol	PKB	CTC
Cholesterol	$1.41\times10^{-6}\pm8.16\times10^{-9}$	$1.15\times10^{-8}\pm4.71\times10^{-11}$
Cholestanol	$1.83\times10^{-3}\pm4.71\times10^{-6}$	$6.93\times10^{-4}\pm8.16\times10^{-7}$
Ergosterol	$1.84\times10^{-3}\pm4.71\times10^{-6}$	$6.88\times10^{-4}\pm4.71\times10^{-7}$
Campesetrol	22.5±0.471	42.8±0.047
Stigmasterol	48.6±0.331	30.4±0.082
5-Avenasterol	2.92±0.011	19.0±0.471
β-Sistosterol	377±0.471	168±0.816
Total	451	260

Mean±SD (n=3).
PKB, *Parkia biglobosa*; CTC, *Citrullus colocynthis*.

Table 3. Phospholipid composition of the vegetable oils (unit: mg/100 g)

Phospholipid	PKB	CTC
Lysophosphatidylcholine	24.43±0.05	5.74±0.02
Sphingomyelin	2.57±0.03	1.93±0.02
Phosphatidylcholine	585±1.00	38.4±0.06
Phosphatidylglycerol	48.0±1.00	1.99±0.01
Phosphatidylserine	617±0.58	16.0±0.58
Phosphatidylinositol	206±1.15	269±1.00
Phytoglycolipid	0.02±0.01	0.033±0.001
Phosphatic acid	64.9±0.06	26.9±0.06
Phosphatidylethanolamine	54.5±0.21	49.4±0.45
Total phospholipid	1,603	409

Mean±SD (n=3).
PKB, *Parkia biglobosa*; CTC, *Citrullus colocynthis*.

which are common sources of phospholipids (Cohn et al., 2010). Therefore, PKB could be regarded as better sources of phospholipids and dietary phospholipids, which are extracted from food products (Küllenberg et al., 2012). Moreover, consumption of both seeds may adequately supply all the required phospholipids. These seeds could therefore provide a ready source of the essential lipids needed for some human medical conditions, and consumption should be encouraged. For example, targeting phospholipid metabolism through drug therapy or dietary supplementation reduces symptoms of depression and bi-polar disorders in human (Küllenberg et al., 2012); given that these conditions are common in people in developing countries due to the economic crises and hardship faced by inhabitants, routine use of these plant products could be a cheaper source of nutraceuticals and help mitigate these health problems.

Fatty acids

The PKB and CTC seeds showed oil yields of 30.5% and 40.3%, respectively (data not shown). The fatty acid profile showed the seed oils majorly contain PUFA, with linoleic acid being the most predominant (Table 4). This also supports previous studies showing that γ-tocopherol-rich plants contain more PUFA (Jiang, 2014; Rudzińska et al., 2016). The fatty acid profile of these seeds is also in agreement with previous studies (Akintayo, 2004; Alabi et al., 2005; Augustine et al., 2013; Azhari et al., 2014; Egbebi, 2014), although the PUFA contents are higher than those reported for rapeseed and almond seed oils (20.9 and 22.8%, respectively) by Orsavova et al. (2015). Consumption of large amounts of PUFA is linked to a lower incidence of depression, a lower risk of Alzheimer's disease, and decreased prevalence of age-related memory loss (Olatunya et al., 2017) since the human brain requires high levels of PUFA (Needlman, 2004) for normal function. Lack of dietary PUFA is characterized by rough and scaly skin, dermatitis, increased transepidermal water loss, and reduced growth (Jeppesen et al., 2000). Thus, regular consumption of PUFA-rich foods will help prevent these diseases and keep the body healthy. These plants could therefore represent readily available sources of essential fatty acids, and therefore help prevent diseases associated with their depletion.

In conclusion, this study shows that, these seeds possess high amounts of vitamin E, phytosterol, and phospholipid and, therefore, may be good dietary sources of these nutraceuticals and help combat certain deadly and age-related health problems associated with their insufficiency. In addition, these seeds may be used as food supplements, which have been proposed as preventive measures against such diseases.

Table 4. Fatty acid composition of the vegetable oils (unit: %)

Fatty acid	PKB	CTC
Caprylic acid [C8:0]	<0.001	<0.001
Capric acid [C10:0]	<0.001	<0.001
Lauric acid [C12:0]	<0.001	0.26±0.008
Myristic acid [C14:0]	<0.001	0.57±0.005
Palmitic acid [C16:0]	34.0±0.08	13.1±0.124
Palmitoleic acid [C16:1]	0.80±0.008	0.07±0.012
Margaric acid [C17:0]	<0.001	0.01±0.005
Stearic acid [C18:0]	2.85±0.008	7.15±0.008
Oleic acid [C18:1]	21.6±0.008	15.4±0.047
Linoleic acid [C18:2]	37.5±0.047	62.4±0.047
Linolenic acid [C18:3]	3.12±0.008	0.66±0.012
Arachidic acid [C20:0]	<0.001	0.07±0.016
Arachidonic acid [C20:4]	<0.001	0.03±0.005
Behenic acid [C22:0]	0.19±0.005	0.21±0.012
Erucic acid [C22:1]	<0.001	0.04±0.017
Lignoceric acid [C24:0]	<0.001	0.04±0.016
SFA	37.0	21.4
MUFA	22.4	15.51
PUFA	40.6	63.1
P/S	1.10	2.94

Mean±SD (n=3).
PKB, *Parkia biglobosa*; CTC, *Citrullus colocynthis*; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; P/S, ratio of polyunsaturated fatty acid to saturated fatty acid.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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