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Gas Chromatographic Analysis of Bioactive Compounds in the Seed Oil of *Pentaclethra macrophylla* (African Oil Bean Tree)

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ABSTRACT: This study characterized the bioactive components in the seed oil of *Pentaclethra macrophylla* to establish its usefulness in replacing costly convectional oils in industrial applications. Identification and quantification of the bioactive attributes were performed using gas-liquid chromatography with flame ionization detector (GC-FID) after extraction with n-Hexane. The main bioactive contents per 100 g in the oil were sitosterol (457.58 mg), campesterol (34.54 mg) and stigma-sterol (18.52 mg) been the most abundant sterols. The oil contained per 100 g β-carotene (15.89 mg), xanthophyll (10.32 mg), lutein (9.22 mg), and neo-xanthin (5.46 mg) as the highest carotenoids. The oil has γ- tocopherol (43.83 mg) and α-tocopherol (3.35 mg) also per 100 g. The main terpenes per 100 g in the oil were γ-elemene (17.28 mg), α-caryophyllene (13.06 mg), β-elemene (12.43 mg) and D-limonene (6.11 mg). Terpenoids including β-amyrin, α-amyrin and lupeol were found in trace amounts.

1. INTRODUCTION

Plants produce many bioactive principles that have found use in the pharmaceutical industry, thus impacting the healthcare system in positive ways. These compounds protect against free radical damage by acting as natural antioxidants (Altemimi et al., 2017; Du et al., 2002; Ogunwusi & Ibrahim, 2016). The potentials of edible seed oils have been exploited in the management of diseases. Diets rich in seed oils have provided micronutrients that helped the body defense mechanisms against reactive oxygen species; and generated biologically active metabolites that have played essential roles in the physiological resolution of inflammatory processes (Mazzocchi et al., 2021).

Depending on plant species, seed oils have been demonstrated as a viable and renewable source of bioactive compounds with great health-promoting activities in human nutrition and cosmetic applications (Quilez et al. 2020). Several seed oils, including *Satureja hortensis* (Quílez et al., 2020) ; *Punica granatum* L and *Momordica charantia* L. (Yoshime et al., 2019); Cucurbitaceae (Rezig et al., 2019) and tomatoes (Szabo et al., 2019) , have been studied and presented as alternative plant oils that can serve as raw materials for food applications.

The African oil bean tree (*Pentaclethra macrophylla* Benth) is an indigenous forest fruit tree in Nigeria belonging to the family Leguminosae cultivated in homestead (Omokhua, 2009). The tree can reach a height and width of approximately 21 and 6 m, respectively. Its bark is greyish to dark reddish-brown and used for treating leprosy sores, while the seed helps prepare delicacies when cooked and fermented (Aseogwu et al., 2006). The extract of stem bark, leaf, root bark and seed pulp of *P. macrophylla* oil has anti-inflammatory, and anthelmintic activities, hence used to treat gonorrhoea, convulsions, dysentery, taken as an analgesic, laxative, emollient against itch and is used to induce abortion (Oboh, 2007). It is an exceptionally highly sought-after food supplement for both local consumption and export due to its richness in vitamins and minerals make (Aseogwu et al., 2006; Enujiugha & Agbede, 2000). The seed with 58.15% oil content serves as a source of oils for candle making, cooking and soap) (Ogunwusi & Ibrahim, 2016; Tico, 2005).

Africa seed oil is equally rich in phytochemicals, including alkaloids, tannins, saponin, oxalate, phytate and ascorbic, much as it contains minerals such as potassium, magnesium, sodium, iron, calcium, manganese and zinc (Aladekoyi et al., 2017). The oil of *P. macrophylla* seed had been reported to inhibit the growth of *Bacillus licheniformis*, *B. cereus*, Lactobacillus species, *Escherichia coli* and *Candida albicans*, thus confirming that it is possible its antimicrobial effects and medicinal values (Okoye et al., 2014). The seed oil was further shown to protect against atherosclerosis due to its anti-atherogenic property and is edible because of its low free fatty acid value (Anioke, 2019; Ikhuoria

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et al., 2008). The present study aimed to conduct a detailed characterization of the bioactive compounds in the seed oil of *P. macrophylla* to further its exploitation for industrial uses in cosmetics and sustain pharmaceutical uses.

2. MATERIALS AND METHODS

2.1. Plant materials

A botanical garden at Ilaro Federal Polytechnic's Department of Science Laboratory Technology provided the seeds, which were harvested between July and October of last year. At the Forestry Research Institute of Nigeria (FRIN), voucher number FHI 111205 was used to identify seed samples.

2.2. Chemicals and reagents

Standard tocopherols, cholesterol, stigmasterol, and sitosterol were purchased from Sigma Chemical Co. n-Hexane (HPLC grade) and pyridine were procured from (Merck, Vikroli Mumbai, India) (St. Louis, Mo., USA). It was acquired from Supelco as Sylon BFT (Bis-(trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (Bellefonte, Pa., USA).

2.3. Sample preparation

Following a previously published process, the Soxhlet extractor was used to extract n-Hexan (Oyedeji et al., 2017). Solvent removal was completed in a rotary evaporator (Model RE300, Bibby Scientific Ltd., United Kingdom) at reduced pressure while traces of n-hexane was expelled under nitrogen gas.

2.4. Analytical method

Carotenoids, terpenoids, and sterols in the seed oils were determined using Gas Chromatography-Flame iIonization Detector as Du et al. (2002) described.

2.5. Chromatographic conditions

HP Gas chromatograph 5890 series II, software - HP ChemStation Rev.A 09.01[1206], Column type – HP -5MS and HP INNOWax, Agilent Technologies India Pvt. Ltd, Hyderabad, India. Injection temperature - split injection; split ratio - 20:1; carrier gas – nitrogen, inlet temperature – 250^{o}C; column dimension (30 m $\times$ 0.25 mm $\times$ 0.25 μm); detector and detector temperature – fid (320^{o}C); flow rate – 1.0 ml/min; hydrogen gas pressure – 30 psi; compressed air – 35 psi; oven programs (Carotenoids and sterols - initial temperature @ 60^{o}C. first ramping @ 10 ^{o}C/min for 20 min, maintained for 2 min second ramping @ 15^{o}C/min for 4 min.).

3. RESULTS AND DISCUSSION

Carotene, xanthophyll, lutein and neo-xanthin, were the major carotenoids determined in the seed oil of *P. macrophylla* (**Table 1**). Their respective concentrations were 15.89, 10.32, 9.22 and 5.46 mg/100 g. Carotene was the major carotenoid content with a 15.89 mg/100g (32%) concentration, followed by xanthophylls 10.32 mg/100 g (21%). Other carotenoids include lutein, neo-xanthin, viola-xanthin, and authera-xanthin with 5.46, 3.91, 3.57 and 0.51 mg/100 g, respectively. Others, which occurred in trace amount were β-cryptoxanthin (8.27 x 0^{-3}mg/100 g), malvidin (1.08 x 10^{-4}mg/100 g) and lycopene (3.91 x 10^{-6} mg/100 g).

Table 1
Carotenoids and sterols of *P. macrophylla* seed oil

Carotenoids	(mg/100 g)	Sterols	(mg/100 g)
Carotene	15.89	Sitosterol	457.58
Xanthophyll	10.32	Campesterol	34.54
Lutein	9.22	Stigmasterol	18.52
Neo-xanthin	5.46	Δ^5-avenasterol	3.32
Viola-xanthin	3.91	Cholesterol	2.20 x 10^{-4}
Authera-xanthin	3.57	Cholestanol	7.01 x 10^{-6}
β-cryptoxanthin	8.27 x 10^{-3}	Ergosterol	5.56 x 10^{-6}
Malvidin	1.08 x 10^{-4}	Others	48.22
Lycopene	3.91 x 10^{-6}	**Total**	**513.97**
Total	**48.89**		

P. macrophylla seed oil contained nine different antioxidants, with carotene been the most abundant at about 35% and lower to 94.8% in palm oil Zeb and Mehmood (2004) .

The total concentration of carotenoids in this study, as shown in Table 1, was (48.89 mg/100 g) and is higher than 0.036 and 0.051 mg/100 g in Gordial olive fruit oil and Hojiblanca oil, respectively (Gandul-Rojas et al., 1999). The carotenoid content of sea buckthorn (1167 mg/100 g)is higher than that obtained in this study (48.89 mg/100 g) Zeb and Mehmood (2004) .

In addition to vitamin A activity, carotenoids, particularly -carotene, operate as a scavenger of reactive free radicals and a quencher of chlorophyll and singlet oxygen excited states. Carotenoids can operate as potent peroxidation inhibitors when exposed to light. This function is, however, reduced due to heat-induced degradation with increasing temperature. The antioxidant activities of β -carotene are enhanced in the presence of α-tocopherol (Ghazani & Marangoni, 2013).

Sitosterol, campesterol, stigmasterol and Δ^5-avenasterol were the major sterols determined in the seed oil of *P. macropylla*. Their respective concentrations were 457.58, 34.54, 18.52, and 3.32 mg/100 g (**Table 1**). Cholesterol, ergosterol and cholestanol, were also determined in trace amounts of 2.20 x 10^{-4}, 5.56 x 10^{-6} and 7.01 x 10^{-6} mg/100 g, respectively. The total sterols determined in this study were 513.97 mg/100 g and are much higher than the total sterol content of 295 mg/100 g measured in the seed oil of *Cucurbita maxima* (Montesano et al., 2018). Sitosterol accounted for 89% of the total sterols and was distantly followed by campesterol (7%). Sitosterol percentage was higher in *P. macrophylla* than olive, peanut, sunflower, soya bean, and rapeseed oils with 84, 68, 62, 55 and 52%, respectively (Philips et al., 2002).

The amount of stigmasterol (3%) to other sterols in this study was found to be higher than that reported for different species of berry and kiwi seed oils that ranged between 0.3 - 2.4%. There is evidence that stigmasterol serves as a precursor

of progesterone, an important human hormone that plays a critical role in regulation and tissue repair mechanisms Hoed et al. (2009) . The 7% campesterol of the sterols in this study was lower compared to other vegetable oils such as soya bean (19.8%), rapeseed (31.9%), sunflower (10.5%), sesame seed (18.25%), and Arachis seed oils (17.3%) but higher than other oils that include apricot (3.8%), olive (2.85), and hazelnut seed oils (4.31%). There is evidence that stigmasterol serves as a precursor of progesterone, an important human hormone that plays a critical role in regulation and tissue repair mechanisms (Hilali & Charreuf, 2007).

Vegetable oil sterols have been shown to reduce total and low density lipoprotein (LDL) cholesterol levels in people by inhibiting gastrointestinal absorption of cholesterol (Lim et al., 2010). It has been found that a number of plant steroid compounds have anti-tumor properties, mainly in breast, colon, and prostate cancers. Cancer cell tumour inhibition and an increase in cancer cell apoptosis are linked to their mode of action (Fabrikov et al., 2019). From the preceding, the oil of *P. macropylla* could be deployed to deliver synthesized anti-cancer nanoparticles and drugs to enhance their potency. African oil bean seed in a previous study was demonstrated to have a positive effect on lipid metabolism in rats and showed an anti-atherogenic property indicating that it is protective against atherosclerosis, thus establishing its richness in sterols (Anioke, 2019).

Beta-caryophyllene, Υ-elemene, α-caryophyllene, β-elemene, D-limonene and linalool were the major terpenes determined in the seed oil of *P. macrophylla* (**Table 2**). Their respective concentrations per 100 g oil were 45.67 mg for β-caryophyllene being the major terpene, Υ-elemene (17.28 mg), α-caryophyllene (13.06 mg), β-elemene (12.43 mg), D-limonene (6.11 mg) and linalool (2.90 mg). Other terpenes were also determined in trace amounts at concentrations ranging between 0.02 and 0.59 mg/100 g (Table 2). The terpene content in cannabis seed oil mainly includes caryophyllene and myrcene (740 and 160 mg/L, respectively), which has a higher concentration than the terpene contents in *P. macrophylla* (58.73 and 0.52 mg/100 g, respectively). Terpenes give oils their characteristic smell and are primarily found in the essential oil of cannabis rather than in the seed oil (Adumanya et al., 2014; Bourgou et al., 2010).

Tocopherols are valuable antioxidants for cellular buffer and protection against diseases in humans. The major tocopherols obtained in the seed oil of *P. macrophylla* were Υ-tocopherols, α-tocopherols, δ-tocopherols, β- tocopherols with concentrations of 43.83, 3.35, 0.40 and 2.42 x 10^{-2}mg/100 g, respectively (**Table 2**). The total tocopherol determined was 47.61 mg/100 g. The tocopherols are isomers of vitamin E with γ-tocopherol been the most abundant at approximately 92%, similar to that of soya bean oil (88%) and higher than 10.5, 58, and 46% for Brazilian nut (*Bertholletia excelsa*) oil, olive oil and walnut oil, respectively (Chunhieng et al., 2008; Nehdi et al., 2012). The total vitamin E in the study is greater than 33, and 42.54 mg/100 g for palm kernel and cold-pressed olive oils (Ellwood et al., 2014). Tocopherols are noted for their antioxidant activities; α-tocopherols has been the most active in capacity *in vivo* compared with other homologues. The α-tocopherols level in this study is suitable for the daily recommended level of 5–9 mg when *P. macrophylla* oil is used in food systems (Ghazani & Marangoni, 2013). Due to its high levels of γ-tocopherol, *P. macrophylla* oil can find use in cosmetic and pharmaceutical formulations by adding value and stability to those products because of their inherent antioxidant capacity and improves their emollient effects.

Total terpenoids content in the seed oil was 1.03 mg/100 g, as shown in **Table 2**. Terpenoids such as α- and β-amyrin have been reported to have anti-inflammatory effects in rodents, while some authors have also reported their usefulness as painkillers and for treating inflammation (Ebajo et al., 2015; Okoye et al., 2014). These compounds in the seed oil of *P. macrophylla* suggest their usefulness as a painkiller and an anti-inflammatory agent.

The oil of *P. macrophylla* has chlorophyll at a concentration of 39.4 mg/100 g and above the 30 mg/kg specification for crude vegetable oils in Canada. Chlorophyll, a prooxidant, is involved in autoxidation, and photooxidation mechanisms reduce the shelf-life of oils as it contains pheophytin and pheophorbide as derivatives are therefore undesirable. The level of chlorophyll in the oil of *P. macrophylla* can be reduced to an acceptable level by bleaching it with activated charcoal (Ghazani & Marangoni, 2013; Mraihi et al., 2013). The refractive index (RI) is a physical property that can be related to the structure that includes double bonds in fatty acids, and it increases as a result of an increase in conjugation, the formation of hydroperoxide and polymerization (Arya et al., 1969; Ospina et al., 2016). The RI of *P. macrophylla* seed oil is 1.46 at 40°C, and it is consistent with the literature values of RI for most vegetable oils.

4. CONCLUSION

This study revealed the abundance of tocopherols, tocotrienols, terpenes and terpenoids and sterol in the seed oil of *P. macrophylla*. This study further showed that *P. macrophylla* oil has excellent nutritional potentials that are underexploited. Disease, including coronary disease, could be prevented with oil either directly or through many food products, serving as an antioxidant and as an anti-inflammatory agent. Related to the relative abundance and low cost of *P. macrophylla*, the tree holds good promise concerning the commercial production of oils.

CONFLICTS OF INTEREST

Authors declares that there is no conflict of interest associated in this research work.

Table 2
Terpenes, terpenoids, tocoperols and other components of *P. macrophylla* seed oil

Terpenes (mg/100 g)		Terpenoids (mg/100 g)		Tocopherol (mg/100 g)		Other components	
γ- elemene	17.28	β- amyrin	6.32×10^{-1}	γ- tocopherol	43.83	Chlorophyll (mg/100g)	39.45
α- caryophyllene	13.06	α- amyrin	2.63×10^{-1}	α- tocopherol	3.35	Saponification value (mg KOH/g)	172.0
β-elemene	12.43	Lupeol	1.27×10^{-1}	δ- tocopherol	4.03×10^{-1}	Unsaponifiable matter (%)	0.42
Linalool	2.90	Taraxerol	4.60×10^{-3}	β- tocopherol	2.42×10^{-2}	Refractive index @ 40^{o}C	1.46
D-limonene	6.11	Bauerenol	3.40×10^{-4}	**Total**	**47.61**		
Others	48.22	**Total**	**1.03**				
Total	**100.0**						

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AUTHOR CONTRIBUTIONS

AOO - Research concept and design, AOO - Collection and/or assembly of data, AOO - Data analysis and interpretation, AOO, BAO - Writing the article, BAO - Critical revision of the article, LAA - Final approval of the article.

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