EFFECT OF BOILING ON THE ANTIOXIDANT PROPERTIES OF THE SEED OIL OF *PENTACLETHRA MACROPHYLLA* (AFRICAN OIL BEAN)

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Abstract

This study was designed to investigate the antioxidant characteristics of the oils of raw and cooked Pentaclethramacrophylla seeds. Oil of P. macrophylla seeds extracted with n-hexane was characterised for their total phenolic contents, total flavonoid contents, DPPH scavenging ability, hydrogen peroxide scavenging ability and ferric reducing properties. The raw seed oil showed higher total phenolic (41.83±2.71 mgGAE/g) and flavonoid (41.83±2.71 mgQE/g) contents compared with the boiled seeds oil, and the difference was significant (p<0.05). The boiled seed oil, however, had higher DPPH, hydrogen peroxide and ferric reducing values compared with raw seed oil, and the difference was significant except in hydrogen peroxide scavenging ability. Boiling can be used as a tool for increasing the antioxidant activity of P. macrophylla seed oil prior to extraction.

Keywords: Antioxidant properties; *Pentaclethra macrophylla;* boiling; seed oil; less free fatty acids

Introduction

Interest in non-conventional sources of oils and fats has increased owing to increased domestic and industrial demand. This is heightened by the low purchasing power of majority of population from low and medium income countries of the sub-tropical region. The sub-tropical region is privileged and rich in genetic resources such as oilseeds, which are potentially important for commercial exploitation. Edible vegetable oils are reduced triglycerides of plant origin that include olive, palm, soya bean, canola, and sunflower oil. Oil and fat are important nutritional components with various roles in our body as components of structural membranes, an energy source, regulating body temperature and insulate organs (Negash, Amare, Bitew & Dagne, 2020).

Antioxidant activity adds to the oxidative stability and bioactivity of components present in vegetable lipids, which may arouse the interest of the cosmetic, pharmaceutical and food industries since antioxidant compounds bring improvements to aesthetics and human health. Additionally, oils with high antioxidant activity alleviated depression in animal experiments (Bristy, Khanam, Sadi & Islam, 2020). Antioxidants, including butylatedhydroxyl anisole (BHA), butylatedhydroxyl toluene (BHT), propyl gallate (PG) and tert-butyl hydroxyl quinone (TBHQ) synthesised from petroleum-based products because of their low cost, are added to food to retard lipid oxidation and stabilise oils and fat (Alves, de Almeida, Souto & de Oliveira, 2021; Bartekova et al., 2021). However, *synthetic antioxidants are not without toxicity risks. They* have been linked to carcinogenesis, cancer, DNA damage and other *health safety* issues including skin allergies and gastrointestinal tract problems, thus calling for the use of natural *antioxidants* to replace them in food and other products (Lourenco, Moldao-Martins & Alves, 2019). Extracts from blackcurrant seeds, bahera seed coat, garlic, sesame seed extract, pomegranate peel, *Pandanus amaryllifolius*, olive and turmeric tree leaves and many others have been used as natural antioxidants in oils and food processing (Permana, Wiradjaja, Sutanto & Surya, 2021; Taghvaei & Jafari, 2015; Patil, 2013). The interest in natural plant components of biological value has shifted attention to under-exploited plant species. Many of these underutilised plants are grown in the subtropics, tropics, and temperate regions and they possess distinct genetic traits, and convey several health advantages.

Pentaclethra macrophylla (African oil bean) belongs to the family *Leguminosea* and the sub-family of *Mimosoideae* and isnative to tropical Africa, with no known varietal characterisation. Its dorso-ventrally flattened, hard, brown seed when mature is dispersed from the pod by an explosive mechanism. The seed has high protein content (48%), and its oil (53.98%) is edible, rich in vitamins and several bioactive compounds (Oyedeji, Azeez & Odeyemi, 2021; Enujiugha & Akanbi, 2005; Ikediobi, 1981). The seed of *P. macrophylla* is used for treating wounds, while the extracts of the stem bark and leaf have been used to treat gonorrhea and convulsions, and used as analgesic, anti-inflammatory, anti-diarrhea and anthelmintic agents and its oil is a potential feedstock for biodiesel production (Iwu et al., 1990). This study, is therefore, designed to study the effects of boiling on the antioxidant components of the oil of *P. macrophylla.*

Methodology

Plant material and sample preparation

The previously identified *P. macrophylla*(FHI 111205) seeds were collected from the botanical garden of the Department of Science Laboratory Technology, The Federal Polytechnic, Ilaro, between January and March 2021. The raw *P. macrophylla* seeds were dehulled and cut into chips. The sample was divided into two portions. While the first portion was sun-dried, the other was heated in a pressure cooker for an hour with distilled water in the ratio 1:5 (sample: water). The boiled seeds after draining out the discoloured water were also sun-dried. The two sample portions were pulverised separately in an electric coffee grinder (Panasonic NC-ZA1), and the obtained powder was stored at 4°C for further analysis. Oil was extracted from the dried, pulverized seed sample with n-hexane as described in a previous study by Oyedeji et al. (2021)

Chemicals and equipment

All the reagents used in this study were of analytical grade. n-Hexane, $NaHCO_3$, methanol, ethanol, $NaCO_3$ and $AlCl_3$ were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). Folin- Ciocalteu (FC), quercetin, DPPH (2,2- diphenyl- 1-picryl hydrazyl), acetate buffer, 2,4,6-tripyridyl-s-triazine (TPTZ), HCl, $FeCl_3{\cdot}6H_2O$, $K_3Fe(CN)_6$, $NaNO_2$, NaOH and saline phosphate buffer and hydrogen peroxide were purchased from Merck (KGaA Darmstadt, Germany). UV-visible spectrophotometer (Shimadzu, UV-1900i, Tokyo, Japan), pH meter (Mettler Toledo), rotary evaporator (IKA-RV10 Basic), oven Memmert UN 110 (Memmert, Germany), vortex mixer (Velp Scientifica Europe) and waterbath (Julabo SW 22).

Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteu (FC) reagent colorimetric method as described by Tenyang, Ponka, Tiencheu, Djikeng and Womeni (2021). Briefly, 0.2 mL of FC reagent, and 2 mL of distilled water were added to 0.5 mL of oil sample in an ethanolic solution, and incubated at room temperature for 3 min. Afterwards, 1 mL of 20% solution of Na_2CO_3 was added to the mixture and re-incubated in a thermostat waterbath at 45ºC for 45 min. The absorbance of the resulting blue coloured complex was measured at 765 nm. The procedure was repeated on all gallic acid standard solutions, and standard curve was obtained. The content of phenols in the test samples was determined from a standard curve, and the results were expressed as milligrammes gallic acid equivalents per gram of oil (mgGAE/g). Three replicate analyses were performed.

Determination of total flavonoid content

The $AlCl_3$ method as described by Phuyal, Jha, Raturi and Rajbhandary (2020) was used for the determination of the total flavonoid content of the oil extracts. Briefly, 0.3 mL of 5% $NaNO_2$ was added to 1.5 mL methanolic oil sample in a test tube, 0.3 mL of 10% $AlCl_3$ and 2 mL of 1M NaOH were added at 6 min intervals after each addition. The mixture was vigorously shaken on an orbital shaker for 5 min at 200 rpm, and the absorbance was read at 510 nm. Sample blank was similarly prepared using 4-mg quercetin in 1-mL methanol that was diluted serially, and read on the spectrophotometer to obtain the calibration curve. Readings were taken in triplicate, and the total flavonoid content was expressed as quercetin equivalent (mg QE/g) using the linear equation obtained from the graph.

Determination of DPPH (2,2- diphenyl- 1-picryl hydrazyl) scavenging ability

The scavenging activity of DPPH free radicals by the oil extracts was measured with some modifications. One millilitre of 0.1 M purple-colored DPPH solution was added to 1.5 mL oil sample in methanol, and incubated at room temperature for 30 min in the dark. A control was prepared by mixing equal volumes of methanol and DPPH solution. Finally, the absorbance of the solutions was measured at 517 nm spectrophotometer. The percentage of inhibition was calculated using the expression

$$I\% = \frac{AC-AO}{AC} X\ 100\% \qquad \text{Eqn. 1}$$

where AC = absorbance of the control (1 mL methanol + 1mL DPPH solution), AO = absorbance of the sample solution, and I% = percentage of inhibition (Phuyal et al., 2020).

Determination of ferric reducing antioxidant ability

The reducing ability of the oil of *P. macrophylla* was determined as described by Wintola, Olajuyigbe, Afolayan, Coopoosamy and Olajuyigbe (2021). Here, 0.5 mL each of 0.2 M phosphate buffer adjusted to pH 6.6 and 0.1% $K_3Fe(CN)_6$ were introduced into 0.5 mL of oil samples ranging from 0.025 – 0.5 mg/mL. The mixture was incubated at 50ºC in a waterbath for 25 min. The reaction was terminated upon removal from the waterbath by the addition of 0.5 mL of 10% trichloroacetic acid and centrifuged at 1000 rpm for 10 min. Finally, 1 mL of the upper layer was transferred into a test-tube and mixed 0.1 mL of 0.01% $FeCl_3$ solution and 1 mL of distilled water and allowed to stand for 10 min at room temperature. The absorbance was measured at 700 nm. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated by plotting the absorbance against the corresponding sample concentration. BHT and quercetin were used as reference compounds.

Determination of hydrogen peroxide scavenging ability

The H_2O_2 inhibition activity of the oil samples was assessed as described by El-Jemli et al. (2016). In this method, 0.6 mL of (4 mM) H_2O_2 solution prepared in 0.1M saline phosphate buffer adjusted to pH 7.4 and incubated for 10 min was added to 1 mL of the oil samples, ascorbic acid and BHT at concentrations of 0.025 to 0.5 mg/mL, respectively. The absorbance of the H_2O_2 at 230 nm was determined after 10 min against a blank solution containing saline phosphate-buffer solution without H_2O_2. BHT and ascorbic were used as positive controls. The percentage scavenging o H_2O_2 in samples was calculated using

$$H_2O_2 \text{ inhibition capacity (\%)} = 1 - \left(1 - \frac{H_2O_2 \text{ conc. of sample}}{H_2O_2 \text{ conc. of blank}}\right) \times 100 \quad \text{Eqn. 2}$$

Statistical Analysis

Data were analysed by one-way analysis of variance using Statistical Package for the Social Sciences (SPSS version 16.0). Comparison was made between species and treatments. Significance level was set at $p < .05$.

Results

The calibration equations for the determination total phenolic and flavonoid contents of the samples are y = 0.0017x - 0.0083 and y = 0.0098x + 0.016, respectively with an excellent R^2 values of 0.998 and 0.996 in the same order. The antioxidant characteristics of the oil of raw and boiled *P. macrophylla* seed as shown in Table 1. The raw seed oil showed higher total phenolic (TPC) and flavonoid content (TFC) than the boiled sample, and the difference was significant at 5% confidence limit. The boiled seed oil, however, had higher DPPH, hydrogen peroxide and ferric reducing values compared with raw seed oil. While there was a significant difference in their DPPH and ferric reducing properties, the two samples were not significantly different in their hydrogen peroxide scavenging ability.

Table 1: **Antioxidant Characteristics of the** oil of Raw and Boiled *P. macrophylla* Seeds

	Raw seeds	Boiled seeds		
	Mean±SD	Mean±SD	t-stat	p-value
Total phenolic content (mgGAEg^{-1})	41.83±2.71^a	36.66±2.67^b	-4.26	0.00
Total flavonoids content (mgQEg^{-1})	41.83±2.76^a	36.77±2.15^b	+4.58	0.00
DPPH scavenging assay (%)	37.62±2.57^a	63.46±7.54^b	-10.25	0.00
Hydrogen peroxide scavenging assay (%)	0.38±0.24^a	0.42±0.09^a	-0.51	0.62
Ferric reducing properties	0.08±0.02^a	0.15±0.07^b	-3.16	0.01

Mean±standard deviation; mgGAEg^{-1} - milligram quercetin equivalent per gram of oil; mgQEg^{-1} - milligram quercetin equivalent per gram of oil. Mean values in the same row having different superscripts showed a significant difference at ($p<0.05$) taking both directional and non-directional hypotheses into consideration.

Discussion

The processing method in this study, however, had no effect on the hydrogen peroxide scavenging ability of the oil. The results are consistent with the study by Tenyang et al. (2022) that reported reduced phenolic content in boiled sunflower seed oil compared to that obtained from the raw seeds, the reduced antioxidant activities of grape seed extracts subjected to different heating conditions, and decreased antioxidant activity of flaxseed hull oil during the heating process (Herchi et al., 2016; Kim et al., 2006). However, this contrasts the study by Maimako, Awakan, Olaniran, Olasunkanmi and Oluba (2022) and Samba et al. (2022) on different accessions of *Psophocarpus*

tetragonolobus and *Momordica charantia* seeds' oils, respectively. While boiling increased the DPPH scavenging activity of the oil in this study, it had no significant effect on *P. tetragonolobus* seed oil obtained by cooking the seed before oil extraction (Maimako et al., 2022).

The total phenolic content values were 41.83±2.71 and 36.66±2.67 mgGAE/g for raw and boiled African oil bean seed oil, respectively. The decrease in total phenolic content of the oil of *P. macrophylla* after boiling is consistent with the works of Emelike, Ujong and Achinewu (2020) and Gomez-Alonso, Fregapane, Salvador and Gordon, (2003) that showed reduced phenolic content and antioxidant activities of oils during frying operations. The total phenolic content of the oils in this study are greater than the 0.133 – 1.596 mgGAE/mL TPC for vegetable oils from Turkey, and 1.76 – 39.16 mgGAE/g for commercial edible vegetable oils sold in Japan (Guzel, Herken & Erel, 2009; Xuan, Gangqiang, Minh, Quy & Khanh, 2018). The oil of boiled and raw seed of *P. macrophylla* has higher TPC value compared to sesame seed oil (26.00mgGAE/g), nutmeg (3.21 mgGAE/g), aniseed (2.52 mgGAE/g), coriander (0.20 mgGAE/g), caraway (0.78 mgGAE/g), and mustard (0.56 mgGAE/g) oils noted for their numerous health benefits. *Eruca sativa* oil, however, has TPC (44.22 mgGAE/g) comparable to what was obtained in this study (El-Fadaly, El-Kadi, El-Moghazy, Soliman & El-Haysha, 2017; Janu et al., 2014). The oil of boiled and raw seed of *P. macrophylla* had a lower phenol content than olive (124 - 516 mgGAE/g) and date (215 – 526 mgGAE/g) seed oils that are considered as rich sources of phenolic compounds in the Mediterranean diet. The TPC in this study is also lower compared to that obtained by supercritical extraction fromlimon seed oil (Park et al., 2021). Heating decreased the TPC and TFC, but resulted in increased free radical scavenging activity of Java plum seed extracts and the antioxidant activity of citrus peel extract (Rohadi, Iswoyo & Larasati, 2020; Jeong et al., 2004). It can be safely concluded that boiling the seeds of *P. macrophylla* before oil extraction significantly reduced the total phenolic and flavonoid contents of the oil, and oppositely enhanced the DPPH and ferric reducing properties. Greater TPC in oil leads to a more potent radical scavenging effect. The oils of raw and boiled *P. macrophylla* seeds are richer in TPC and antioxidant capacities than *Curcuma longa* leaf extract used in the prevention of oxidation of soya bean oil in Brazil (Alves, 2021). Phenols contribute to the oxidative stability of oils and also participate in conferring specific flavour to oils. Phenols have a positive effect on the prevention of coronary heart disease and cancer (Besbes et al., 2005).

Oil from *Citrus limon* obtained by supercritical fluid extraction had greater DPPH scavenging ability than the results of this study (Park et al., 2021). *Amaranthus spinosus* seed oil extracted with ethyl acetate and *Caryodendron orinocense* seed oils showed higher antioxidant activity than the oils of raw and boiled *P. macrophylla* (De Azevedo et al., 2020; Barku, Opoku-Boahen, Owusu-Ansah & Mensah, 2013). The DPPH radical-scavenging activity and ferric reducing properties of the oil of *P. macrophylla* are, however, greater than the 8.65–19.21 mg/mL and 0.11 to 0.30 mmol from the oil of *Thlaspi arvense* from different provinces of China (Liu et al., 2022) and the antioxidant activity of oil extracted from orange (*Citrus sinensis*) (Neuza, Da Silva& Aranha, 2016). The oil of *P. macrophylla* has greater antioxidant activity than *Satureja hortensis* essential oil (Chambre et al., 2020), and poppy, hemp, and sesame seed oils (Senila et al., 2020). Similarly, *Azeratum conyzoides*, *Ipomea carnea*, *Elaeocarpus sphaericus* and *Ficus benghalensis* noted for their rich phytochemicals and used as medicines, have lower antioxidant capacities compared to the seed oil of *P. macrophylla* (Pandey & Rajbhandari, 2014). Peanut and cottonseed oils from Burkina Faso had lower antioxidant activities than the oil in this study (Zio et al., 2021).

Oils rich in antioxidant activities are usually thermally stable and deteriorates less easily because they produce less free fatty acids during heat processing (Banu, Prasad &Siddaramaiah, 2016). Consequently, the use of the oil of *P. macrophylla* will find great advantages as an emollient in cosmetic and pharmaceutical preparations and it will prolong the shelf life of packed foods. The oil in this study has higher total flavonoid contents than *Eruva sativa* seed oil (11.82 mgQE/g) used as an anti-cancer, antifungal and anti-inflammatory agent (El-Fadaly et al., 2017). The total flavonoid contents of *Juniperus oxycedrus* (20.81 6.69 μgQE/mg), *J. thurifera* (14.93 6.69 μgQE/mg), *J. phoenicea* (6.69 μgQE/mg) and *Tetraclinis articulate* (11.78 6.69 μgQE/mg)species investigated as natural antioxidants are much lower than the results of this study (El-Jemli et al., 2016). The oil of *P. macrophylla* seed has comparable flavonoid content with *Ficus benghalensis* (78.2 mg quercetin/g extract) an important medicinal plant in Nepal (Pandey & Rajbhandari, 2014). The TFC of *P. macrophylla* seed oil is higher than the TFC of local and branded frying oils used by local vendors in Port-Harcourt, Nigeria (Emelike et al., 2020). Flavonoids are known for their antioxidant, anti-inflammatory, anti-atherosclerotic and anti-oxidative damage.
The oil of *P. macrophylla* seed showed greater ferric reducing antioxidant power and hydrogen peroxide scavenging activity than the essential oil of *Erythrina caffra* noted for its ethnobotanical importance in some African countries (Wintola et al. 2021). Oils rich in antioxidant activity increased healthy characteristics, including low levels of

atherogenic and prothrombogenic index and preventive lipid score in cheese (Ivanova, Teneva, Dushkova, Vlaseva & Stoyanova, 2020).

Conclusion

Boiling the seeds of *P. macrophylla* before oil extraction significantly reduced the total phenolic and flavonoids contents of the oil, and oppositely enhanced the DPPH and ferric reducing properties. Asides from its healthy nutritional profile, *P. macrophylla* seed oil also possesses antioxidant characteristics comparable with popular seed oils, and therefore will be thermally stable and deteriorates less easily. The oil of *P. macrophylla* seed because of its antioxidant characteristics, is deemed a good alternative to preserve the sensorial and nutritional properties of food against oxidative degradation.

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