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Physiochemical properties and antioxidant potential of Persea Americana seed oil

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A B S T R A C T

Oil extracted from *Persea Americana* seed was assayed for its physiochemical properties and antioxidant potential using various standard methods. The oil content of the seed was found to be < 10%. Brownish-red color oil was liquid at room temperature, with specific gravity of 0.91±0.02 g/mL. Other physiochemical parameters determined were; acid value (4.51±0.08 mgKOH/g), %FFA (2.26±0.08), peroxide value (2.40±0.57 mgO_2/Kg), ester value (31.26±0.03 mgKOH/g), saponification value (35.76±0.07 mgKOH/g) and iodine value (23.5±0.07). The results of the antioxidant activities of the seed oil showed that the flavonoid content (80.00±1.41 mgQE/g) was ~10 folds higher than the phenolic content (8.27±0.06 mgGAE/g). The DPPH radical scavenging value was found to be 51.54±0.25% with an IC_{50} value of 4.68±0.02 mg/mL and reducing power with an average absorbance of 0.85±0.01 and an IC_{50} value of 0.001±0.02 mg/mL. Gallic acid showed better antioxidant activities than the oil studied. The results obtained in this study showed that *Persea Americana* seed oil has nutritional, industrial as well as medicinal potentials.

Capsule Summary: *Persea Americana* seed oil was evaluated for its physiochemical properties and antioxidant potential and it was found that *P. Americana* seed oil has nutritional, industrial as well as medicinal potentials.

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INTRODUCTION

Persea Americana generally known as the avocado or alligator pear is one of the fruits with known excellent nutritional as well as medicinal qualities. It is a climacteric fruit, which implies that it matures on, but ripens off the tree (Orhevba and Jinadu, 2011). It contains high amount of fats and oils, protein and fibre. Its possession of high amount of digestible oil and low sugar content makes avocado pear a rich source of energy and an essential component of diabetics' diets. It is also a rich source of vitamins such as vitamins C, E, K, B1, B2, B6, B9 and minerals such as phosphorus, sodium, magnesium, potassium, iron and zinc (Orhevba and Jinadu, 2011; Oluwole et al., 2013 and Maitera et al., 2014). The possession of many essential nutrients and various potential disease-curing phytochemicals may be responsible for numerous health benefits of plants (Elsayed and Lobna, 2013; Ashraf et al., 2015; Asif, 2015a, b, c, d, 2016; Hussain et al., 2016).

The seed of avocado pear represents one of the under-utilized non-edible parts of the fruit which are usually discarded as residues. The exploitation of non-edible parts of fruits is an emerging trend which may prove to be very profitable in the near future mostly because it involves an important reduction in the production of wastes and the fact that the non-edible parts of many fruits concentrates high levels of valuable bioactive compounds, particularly natural antioxidants (Vinha et al., 2013; Mensah and Golomeke, 2015; Mensah et al., 2015). The non-edible parts of avocado pear (skin and seed) have been studied in order to ascertain their potential use as cheap sources of bioactive compounds for the food, pharmaceutical and dermo-cosmetic industries (Ana et al., 2013). Although the seed represents about

100 to 300 g/kg of the fruit which is a considerable percentage (Bora et al., 2001), scientific research on the nutritional compositions, phyto-chemistry and biological effects of avocado seeds is still at the nascent stage.

However, studies have shown that the seeds from avocado pear contain oil with considerable nutritional values. The oil content of the fruit depends upon its ecological origin and on the cultivar. According to Mason (1981), the oil content of an avocado pear varies from species to species. For example, Biale and Young (1971) reported the oil content of the Guatemalan and Mexican cultivars to varies from 10 to 13% and 15 to 25%, respectively while in the fruits from Carrebean, a low lipid content of 2.5 to 5% has been reported (Hatton et al., 1964). Depending on the amount of oil they contain, the taste and texture of avocado pear may vary greatly. Some works have been published on the physicochemical properties and the fatty acids composition of seed oil obtained from some important avocado cultivars (Mazliak, 1971; Tango et al., 1972; and Itoh et al., 1975). The fruit oil has been shown to have several health benefits some of which include but not limited to; serving as a high source of nutrients in the production of cosmetics, enhancing the skin's good looking condition (Le poole, 1995) and in controlling human weight especially used for obese patients for weight loss (Roger, 1999).

In this study, the oil from the seed of avocado pear was extracted and characterized for its physiochemical properties as well as antioxidant potential in order to establish its edibility, industrial as well as medicinal usability.

MATERIAL AND METHODS

Sample collection and pre-treatment

Mature and healthy fruits of avocado pear were obtained from Ilishan market in Ogun state Nigeria and kept at room temperature for 4days in order for them to ripe. The fruits were washed with distilled water, cut open with a knife and the seeds as well as the seed coverings were manually removed. The seeds were then chopped into smaller sizes, oven dried at 50ºC for 48 hours and then pulverized with the use of laboratory blender (LEXUS MG-2053 OPTIMA). The pulverized samples were packaged in waterproof polyethylene bags and stored at 4ºC until required for analysis.

Extraction of Avocado Seed oil

Solvent extraction was carried out on 100 g of pulverized sample with soxhlet apparatus for a period of 8 hours using n-hexane as the extraction solvent. The extraction solvent was removed *in vacuo* using rotary evaporator (Eyela N-1001) at 40^{o}C to recover the seed oil. The oil was placed on a water bath at 50^{o}C for 2hours to ensure complete removal of residual solvent after which it was stored in a glass bottle and the analysis was carried out on the freshly extracted seed oils.

Determination of physiochemical properties of oil

The oil extracted from avocado pear seed was analyzed for its physiochemical properties by using various standard methods. The color and state of the oil at room temperature were noted by visual inspection while ester value was obtained by finding the difference between the saponification value and the acid value (Duduyemi et al., 2013). Every analysis was carried out in triplicates; mean and standard deviation were calculated.

Oil yield (%)

The % oil yield of the seed was determined and calculated by using Eq. 1.

$$\text{Percentage yield (\%)} = \left\{\frac{Oil\ weight}{Seed\ weight}\right\}.100 \qquad \text{(Eq. 1)}$$

Specific gravity

The specific gravity of the oil was determined gravimetrically by employing the weight ratio of the oil to the equivalent amount of water according to the following formula;

$$\text{Specific gravity} = \frac{W_2}{W_1} \qquad \text{(Eq. 2)}$$

Where, W_2 and W_1 are the weights of oil and equivalent amount of water respectively.

Acid value

The acid value of the oil was determined using the method described by AOAC (1990). Ethanol was boiled on a water bath for a few minutes to remove dissolved gases. The boiled ethanol was neutralized by adding a few drops of phenolphthalein and about 10 ml 0.1N potassium hydroxide until a pale pink color was obtained. 6 g of oil was weighed into a 250 mL conical flask and 50 ml of hot previously neutralized ethanol was added. The mixture was then brought to a boil on a water bath and the hot mixture was titrated with 0.1N potassium hydroxide solution until the pink color (stable for few minutes) returned. The acid value was calculated from the relation shown in Eq. 3.

$$\text{Acid value (mgKOH/g)} = \frac{V\,.\,N\,.\,56.1}{W} \qquad \text{(Eq. 3)}$$

Where, V is titre value (mL), N is normality of KOH = 0.1N and 56.1 = molar mass of KOH and W is weight of sample.

Free fatty acid (%)

The percentage free fatty acid (as oleic acid) was obtained by multiplying the acid value with the factor 0.503. Thus, percentage FFA (as oleic acid) = 0.503 × acid value (Akubugwo et al., 2008)

Saponification value

AOAC, (1990) method was used to determine the saponification value of the oil. 2 g of oil was weighed into a conical flask and 25 ml of 0.5 N alcoholic KOH were added. A blank was also prepared by taking 25 mL of alcoholic KOH in a similar flask. Reflux condensers were fitted to both flasks and the contents were heated in a water bath for one hour, swirling the flask from time to time. The flasks were then allowed to cool a little and the condensers washed down with a little distilled water. The excess KOH was titrated with 0.46 N HCl acid using phenolphthalein as indicator. The saponification value was calculated using Eq. 4.

$$\text{Saponification value (mgKOH/g)} = \frac{(a-b).\,F\,.\,28.05}{W} \qquad \text{(Eq. 4)}$$

Where, b = titre value of blank (mL), a = titre value of sample (mL), F = factor of 0.46 N HCl = 1 (in this case) and 28.05 = mg of KOH equivalent to 1 ml of 0.46 N HCl and W is weight of sample.

Peroxide value

Peroxide value of the oil was assayed as described by the (ISO, 1975) method. 2 g of oil sample was weighed into a 500 mL conical flask and 10 ml of chloroform was added to dissolve the sample. This was followed by addition of 15 ml of acetic acid and 1ml of freshly prepared saturated potassium iodide solution. The flask was immediately closed, stirred for about 1minute and kept at room temperature away from light for exactly 5minutes. About 75 ml of distilled water was added to the content of the flask and then shaken vigorously. Few drops of starch solution were added as indicator. The liberated iodine was titrated against 0.01N sodium thiosulphate solution. The same procedure was carried out for blank and the peroxide value (PV) expressed in milliequivalent of active oxygen per kilogram of sample was calculated using Eq. 5.

$$\text{Peroxide value } (\text{mg}O_2/\text{Kg}) = \frac{(V_1 - V_0)\,.\,T\,.\,1000}{M} \qquad \text{(Eq. 5)}$$

Where, V_0 is the volume of the sodium thiosulphate solution used for blank, V_1 is the volume of the sodium thiosulphate solution used for determination of sample, T is the normality of the sodium thiosulphate used, and M is the mass of the test sample in gram.

Iodine value

Iodine value of the oil was assayed according to the titration method of Pearson (1970). 2 g of oil sample was weighed into a dry 250ml glass stopper bottle and 10ml of carbon tetrachloride was added to the oil. About 20ml of Wij's solution was then added and allowed to stand in the dark for 30 min. 15 mL of 10% Potassium Iodide and 100 mL of water were added and the resulting mixture was then titrated with 0.1M Sodium thiosulphate solution using starch as indicator just before the end point. A blank determination was carried out alongside the oil samples. Iodine value was calculated thus:

$$\text{Iodine value (Wij's)} = \frac{(V_2 - V_1)\,.\,1.269}{W} \qquad \text{(Eq. 6)}$$

Where, V_2 = titer value for blank, V_1 = titer value for sample and 1.269 = Concentration conversion coefficient and W is weight of sample (g).

Assays of antioxidant activities

Flavonoid content

Total flavonoid content of the oil sample was determined by aluminum trichloride spectrophotometric method reported by Dewanto et al. (2002) using quercetin as standard. The method was based on formation of a flavonoid-aluminum complex. 1 mL aliquot of oil sample in methanol (1 mg/mL) or standard at various concentrations (2, 4, 6, 8 and 10 µg/mL) was diluted with distilled water (4 mL) in a 10 ml volumetric flask. Then, 5% $NaN0_2$ solution (0.3 ml) was added to the volumetric flask. After 5 min, 10% $AlCl_3$ (0.3 mL) was added and at 6 min, 1M NaOH (2 mL) was added. Water (2.4 mL) was then added to the reaction flask and mixed thoroughly. Absorbance of the reaction mixture was read at 510 nm. Total Flavonoid Content was estimated from quercetin calibration curve (R^2=0.9982) and results expressed as mg of Quercetin Equivalent per gram oil sample (mgQE g^{-1}). The analysis was carried out in triplicates.

Phenolic content

The total phenolic content of the oil was assayed by the method of Singleton and Rossi, 1965. The assay is based on the reduction of Folin-Ciocalteu reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds present in the sample. 1 ml aliquot of oil sample (1 mg/mL) in methanol was diluted with distilled water (9 mL) in a standard volumetric flask. 1ml of Gallic acid standards of various concentrations (2, 4, 6, 8 and 10µg/ml) in methanol was similarly prepared. Folin-Ciocalteu's reagent (1 mL) was added to the mixture and vortexed. After 5 mins, 10 mL of sodium carbonate solution (7%) was added to the mixture, and then incubated for 90mins at room temperature. After incubation, the absorbance against the reagent blank was determined at 760 nm. A reagent blank was prepared using distilled water instead of the oil solution. The amount of total phenolic component in the oil was estimated from gallic acid calibration curve (R^2=0.9984) and expressed as mg of Gallic Acid Equivalent per gram oil sample (mg GAE g^{-1}). The analysis was carried out in triplicates.

Free radical scavenging activity

The antioxidant activity of the oil sample was evaluated spectrophotometrically through free radical scavenging effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical by the method of Mensor et al., (2001). 2.5 mL of oil solution of various concentrations (2, 4, 6, 8 and10mg/mL) prepared in methanol was added to 1.0 mL of methanolic solution of DPPH (0.3mM) and kept in the dark at room temperature for 30 mins. The same procedure was carried out on Gallic acid at various concentrations (2, 4, 6, 8 and 10µg/ml). The absorbance of the resulting mixture was read at 518 nm and converted to percentage inhibition using Eq. 7.

$$\text{Inhibition of DPPH (\%)} = \frac{(A_c - A_s)\,.\,100}{A_c} \qquad \text{(Eq. 7)}$$

Where, A_c is absorbance of control and A_s is the absorbance of sample. The analysis was carried out in triplicate for each concentration. Methanol (2.5 ml) plus 1 ml of 0.3mM DPPH was used as the control. The IC50 value representing the concentration of the compounds that caused 50% inhibition of radical formation was obtained by interpolation from linear regression analysis (Stoilova et al., 2007).

Reducing power

Table 1: Physiochemical properties of Persea Americana Seed oil

Parameters	Values/Quality
Color	Brownish-red
Odor	Fruity
State at room temperature	Liquid
% yield	8.10±0.07
Specific gravity	0.91±0.02
Acid value (mgKOH/g)	4.51±0.08
% free fatty acid(as oleic acid)	2.26±0.08
Peroxide value (mgO_2/Kg)	2.40±0.57
Ester value (mgKOH/g)	31.26±0.03
Saponification value (mgKOH/g)	35.76±0.07
Iodine value (wijs)	23.5±0.07

Data are expressed as mean ± standard error of three replicates

The total reducing power of the oil was assayed according to the method of Yen & Duh, 1993 as described by Premanath & Lakshmidevi (2010). 1 ml of various concentration of the oil sample (2, 4, 6, 8 and10mg/ml) was mixed with phosphate buffer (500µL 20mM, pH 6.6) and 1% potassium ferricyanide (500µL). It was incubated at 50^0C for 20mins; after which 500µL of 10% Trichloroacetic acid was added, and the mixture centrifuged at 2500 rpm for 10 min. The supernatant was mixed with distilled water (1.5 ml) and 0.1% ferric chloride (300µL) and the absorbance was read at 700 nm. Gallic acid solution of various concentrations (2, 4, 6, 8 and 10µg/ml) were analyzed likewise. Increase in the absorbance of the reactions mixture indicated increase in the reducing power. The sample concentration providing 0.5 of absorbance (IC_{50}) was calculated from the graph of absorbance at 700 nm against sample concentration (Barros et al., 2007). The analysis was carried out in triplicates.

RESULTS AND DISCUSSION

The physiochemical properties of avocado seed oil assayed in this study are presented in Table 1 shown below. The seed oil is a liquid at room temperature with a brownish-red color similar to that reported for *Persea Gratesima* (Akubugwo et al., 2008). The oil also has a strong characteristic fruity smell. The table shows a percentage oil yield of 8.10 ± 0.07 which is higher than 7.50±3.01% reported by Bwai et al. (2013) for breadfruit seed and much higher than 0.70±0.17 % reported for *Diospyros mespiliformis* seeds (Chivandi et al., 2009). The oil yield obtained in this study is however slightly lower than 9.27±0.02% and 9.47±0.00% reported for unripe and ripe seeds of *Persea americana* respectively (Oluwole et al., 2013).

According to Bwade et al. (2013), the oil content of agricultural products such as fruits and seeds determines largely whether or not oil can be industrially processed from them. According to FAO as reported by Akinoso and Raji (2010), seeds that contain oil yield greater than 17% are considered as oil seeds. The avocado pear seed is therefore not recommended for the purpose of edible oil generation and biodiesel production due to the very low oil yield. However, variation in oil yield may be due to the differences in species of plant, cultivation climate, ripening stage, the harvesting time of the seeds and the extraction method (Marfo et al., 1986) as well as solvent used. Results obtained in this study revealed a specific gravity of 0.91±0.02 for Persea americana seed oil. This is comparable with 0.89±0.02, 0.90±0.02 and 0.914 reported for *Pentaclethra Macrophylla*, *Persea Gratesima* and *Citrullus Colocynthis* L. *seed* oils respectively (Akubugwo et al., 2008 and Mirjana and Ksenija, 2005) and slightly higher than 0.86 reported for *Citrullus vulgaris* seed oil (Zaharaddeen *et al.*, 2014). The result implied that avocado seed oil is less dense than water and could therefore be useful in cream production as this will make the oil spread easily on the skin. Acid value is used to measure the extent to which glyceride in the oil has been decomposed by lipase and other actions such as light and heat. The lower the acid value of oil, the fewer free fatty acids it contains which makes it less susceptible to rancidity. According to Coenen (1976), the lower the acid content, the more appealing the oil. Acid value of 4.51±0.08 mgKOH/g was obtained for the avocado seed oil assayed in this study. This value is lower than the acid value (16.80mgKOH/g) reported for avocado pear oil (Akpabio et al., 2011). This value is also lower than the acid value (11.44±0.44 mgKOH/g) reported for unripe pear seed oil and (17.82±0.22 mgKOH/g) reported for ripe pear seed oil (Oluwole et al., 2013) but on the high side when compared to 1.79mgKOH/g reported for Shea butter oil (Asuquo et al., 2010). The low acid value obtained for avocado seed oil in this study therefore suggests that the oil is edible and less susceptible to rancidity. The percentage free fatty acid (FFA) value of oil is a crucial parameter in determining the quality of oil because the lower the FFA, the higher the quality of the oil especially in terms of its edibility. The percentage free fatty acid of 2.26±0.08 obtained for avocado seed oil in this study is low in comparison with 4.88 ± 0.03, 2.45±0.2, 2.38±0.18 and 38.85±3.34 reported for *Citrullus vulgaris*, soursop, watermelon and breadfruit seed oils respectively (Zaharaddeen et al., 2014; Navaratne and

Table 2: Phenolic content, Flavonoid content and antioxidant activities of Persea Americana seed oil and Gallic acid

Sample	Flavonoid (mgQE/g)	Phenolic (mgGAE/g)	DPPH (%inhibition)	IC_{50} (mg/ml)	Total reducing power (Absorbance)	IC_{50} (mg/ml)
P.A seed oil	80.00±1.41	8.27±0.06	51.54±0.25	4.68±0.02	0.82±0.01	0.92±0.01
Gallic acid	-	-	73.60± 0.03	0.00382± 0.01	0.85±0.01	0.001±0.02

Data are expressed as the average of three determinations ± S.E.

Subasinghe, 2014 and Bwai *et al.,* 2013). This value is however higher than that reported for Carica papaya seed oil (1.27 ± 0.04%) as well as Telfairia *occidentalis* (1.74±0.49) (Cassia et al., 2011 and Muibat et al., 2011). Low FFA content of the oil is also indicative of low susceptibility to enzymatic hydrolysis and could be an advantage over oils with high free fatty acids value which can become off-flavor during storage (Bailey, 1954).

Peroxide value is a measure of the content of hydro-peroxides in oil (McGinely, 1991) which are the primary reaction product formed in the initial stages of oxidation of oil and therefore gives an indication of the likely occurrence of the process of lipid peroxidation (Onwuka, 2005).The peroxide value (2.40±0.57mgO_2/Kg) reported in this work is low in comparison with Baobab seed oil (10.15), peanut oil (5.20) and palm oil (16.08) (Birnin-Yauri and Garba, 2011). It is also lower than that reported for Carica papaya seed oils (5.37 ± 0.13) (Cassia et al., 2011). The low peroxide is also indicative of low susceptibility of the oil to oxidative rancidity. Avocado seed oil had saponification values of 35.76±0.07 which is low in comparison with 231.6 ± 1.40 previously reported by Pushkar et al (2001). This value is also lower than 188.75 ±3.36 reported for *Lavandula bipinnata* seed (Hosakatte et al., 2014) and 179.04 ± 1.60 reported for Telfairia *occidentalis* (Muibat et al., 2011). The relatively low saponification value of this oil may imply its poor suitability for the production of soaps and detergents.

Ester value represents the number of milligrams of potassium hydroxide required to saponify the esters present in 1g of the oil. It is obtained as the difference between the saponification value and the acid value. Ester value of 31.26±0.03mgKOH/g was obtained for the avocado seed oil. This is lower than that of rubber seed oil (191.93mgKOH/g) (Asuquo, 2008) and African pear (128.48mgKOH/g) (Ikhuoria and Maliki, 2001).

Iodine value suggests degree of unsaturation present in oil. Higher iodine value is attributed to high unsaturation. When compared with 119.54 ± 0.002 reported for *Cussonia bateri* seeds (Nwokonkwo, 2013), and 44.079 previously reported for African pear oils (Ikhuoria and Maliki, 2001), the iodine value (23.5±0.07) obtained for avocado seed oil in this work is low. This implies that the oil has relatively low degree of unsaturation and can thus be used as plasticizers and lubricants (Ikhuoria and Maliki, 2001).

Avocado seed oil showed significant antioxidant potential as evident in the results obtained for the flavonoid content, phenolic content, DPPH scavenging activity and total reducing power of the oil (Table 2). Gallic acid was used as positive control in this test. The seed oil showed average flavonoid content of 80.00±1.41mgQE/g and average phenolic content of 8.27±0.06mgGAE/g which implies that the oil has more flavonoids than phenols. However, several works by Pietta (1998); Shahidi et al. *(*1992); Das and Pereira (1990); De Gaulejac et al. (1999); and Hatano et al. (1989) have reported positive correlation between antioxidant activities of various plants and their phenolics and flavonoid contents. Hence, the possession of flavonoid and phenolic compounds implies that avocado seed oil does not only have nutritional and industrial values, but may also possess some medicinal potential.

In order to further substantiate its possible antioxidant potential, the seed oil was subjected to assays of DPPH free radical scavenging and total reducing power assay. The seed oil showed an average of 51.54±0.25% inhibition of DPPH with an IC_{50} of 4.68±0.02mg/ml. This is relatively low when compared with Gallic acid which showed an average of 73.60± 0.03% inhibition of DPPH and IC_{50} of 0.00382± 0.01mg/ml. Additionally, in the total reducing power assay, the oil showed an average absorbance of 0.82±0.01 and IC_{50} of 0.92±0.01mg/ml which is also low in comparison with Gallic acid which exhibited an average absorbance of 0.85±0.01 and an IC_{50} of 0.001±0.02mg/ml. These results however showed that avocado seed oil possesses a significant antioxidant potential.

CONCLUSION

The results of present study showed that oil yield of *Persea Americana* seed was low (< 10%). The extracted oil showed a promising antioxidant activity. Results showed that the oil can be employed for nutritional and industrial purposes and because of its possession of bioactive compounds; it may also be useful for pharmaceutical formulations. Future studies are recommended to evaluate the oil component responsible for bioactivity.

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