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Comparative Study of Antioxidant Activity and Mineral Composition of Methanol Extract of Seeds of Ripe and Unripe Avocado Pear (*Persea americana,* Mill.)

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Abstract
Avocado pear has been used for many years as a rich source of nutritional and medicinal material in oriental medicine and several health benefits have also been attributed to its seeds. This study was designed to investigate the mineral composition and antioxidant activities of the seeds of ripe and unripe avocado pear. The concentrations of sodium (Na), potassium (K), calcium (Ca), zinc (Zn), iron (Fe), manganese (Mn), and magnesium (Mg) were investigated. The antioxidant activity was investigated using DPPH, superoxide, and hydrogen peroxide scavenging assays. Mineral analysis showed that the seeds of ripe avocado pear contain higher values of Na (96.1mg/kg), Ca (2353.1mg/kg), Mg (392.2mg/kg), Fe (420.65mg/kg) and Mn (890mg/kg) compared to the unripe seeds (Na:84.25mg/kg; Ca:1566.5mg/kg; Mg:365.15mg/kg; Fe:299.7mg/kg; and Mn:10.15mg/kg). The concentrations of potassium (4483mg/kg) and zinc (113.35mg/kg) were, however, higher in the unripe seeds than in ripe seeds (K: 3982.5mg/kg and Zn: 81.3mg/kg). Antioxidant activity assays showed that seeds from ripe avocado pears exhibited higher free radical scavenging ability with lower IC_{50} values relative to the unripe seeds. The findings of this study suggest that the seeds from both ripe and unripe avocado pears contain significant antioxidant potentials which could offer great health benefits. The result further suggests that seeds from unripe avocado pears, being rich in potassium compared to sodium, could be used in the management of hypertension.

Keywords: Antioxidant, Mineral, Avocado, Free radical, Seed

Introduction
Several important biochemical processes are known to generate free radical species, which are by nature, toxic to a number of biological compounds, and hence to living organisms. Excessive generation of these harmful chemical species have been linked to various disease conditions such as cancer, liver disease, kidney disease, asthma, diabetes, cardiovascular diseases, hypertension e.t.c. (1). Living organisms are constantly exposed to exogenous oxidizing chemical agents from their environment. These chemical agents undergo various reactions within the cells to generate reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet}$), hydroxyl ($HO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals, or reactive nitrogen species (RNS), namely peroxynitrite anion ($ONOO^{\bullet}$) and nitric oxide ($NO^{\bullet}$) radical, among others. There are also non-free radical species such as hydrogen peroxide (H_2O_2), nitric oxide (NO) and hypochlorous acid (HClO) which are potentially harmful to biological systems (2).
Medicinal plants have continued to attract attention in the global search for effective agents in the treatment and management of several disease conditions affecting humans (3). Many important drugs currently used in medicine are direct or indirect products of plants. Many plants have been shown to contain high level of bioactive compounds such as alkaloids, phenolic compounds, flavonoids, steroids, tannins, among others which have beneficial effects to humans (4).
Persea americana, Mill., also known as avocado, is a tropical plant native to Central America. It belongs to the *Lauraceae* family. It derived its name 'avocado' from the Aztec word 'abucatl' and it is alternatively called alligator pear or butter pear. It has been traditionally cultivated for food and medicinal purposes due to its high nutritive content and therapeutic properties (5). Several medicinal effects, which includes hypotensive, hypoglycaemic, anti-viral, analgesic and anti-inflammatory, have been attributed to avocado seeds (6). A study conducted by (7) reported that avocado seeds contain high amount of phenolic compounds and are able to exhibit cytotoxic effects against T47D cell lines. Another study conducted by (8) reported the anti-diabetic effects of aqueous extract of the seeds of avocado pear. In this work therefore, we have reported a comparative study on the mineral composition and antioxidant properties of the seeds of ripe and unripe avocado pear in order to justify their various therapeutic and nutritive uses.

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Materials and Methods

Chemicals/Reagents

Methanol was purchased from Guangdong Guanghua Chemical Factory Co. Ltd. (Shantou Guangdong, China), 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was purchased from Sigma Chemical Co, St. Louis, MO, USA. All other chemicals and reagents used in this study were of standard analytical grade.

Collection of Plant/Preparation of Extracts

Avocado fruits (ripe and unripe) were purchased from New Benin market, Benin City. The fruits were identified in the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. The avocado fruits (ripe and unripe) were cut open to obtain the seeds. The seeds were then cuts into small pieces and kept under shade until they were dried. This was followed by pulverizing the dried seeds into powdery form. Five hundred grams (500g) of each of the powdery dried avocado seeds samples (ripe and unripe) was extracted in 2500ml of methanol for 48hr with occasional stirring. This was then filtered using Whattman No 1 filter paper. The filtrate was recovered and freeze dried, and the dried extract was kept under refrigeration until use.

Mineral Analysis

The mineral composition of the two samples was carried out by the dry ash extraction method described by (9), and (10). Sodium and potassium concentrations were determined using flame photometer. Calcium and Magnesium concentrations were determined by the versanate EDTA complexometric titration method, while Manganese, Zinc, and Iron were determined according to the method of (11) using Atomic absorption spectrophotometer.

DPPH Radical Scavenging Assay

The free radical scavenging ability of the extract against DPPH (1, 1-diphenyl-2-picryhydrazyl) was carried out according to the slightly modified method of (12). To 2.5ml of the extracts (ripe and unripe) was added 3ml of methanol and 0.5ml of DPPH. The mixture was shaken and left to stand in the dark at room temperature for 30min. The absorbance of the resulting mixture was read at 517nm. The same procedure was repeated using a control sample (DPPH without extracts). Ascorbic acid was used as the standard antioxidant. The scavenging ability of the extracts was calculated as:

$$\text{DPPH radical scavenging activity (\%)} = \frac{(Abs\ of\ control - Abs\ of\ sample)}{Abs\ of\ control} \times 100$$

Superoxide Radical Scavenging Assay

Superoxide radical scavenging activity of each extract was determined according to the nitroblue tetrazolium (NBT) reduction method of (13). One millilitre of nitroblue tetrazolium solution (1M NBT in 100mM phosphate buffer, pH 7.4), 1ml of NADH solution (1M NADH in 100mM phosphate buffer, pH 7.4), and 0.1ml of the extracts (ripe and unripe) (0.5-1.5mg/ml) were mixed. The reaction was started by adding 100µl of phenazine methosulphate (PMS) solution (60µM PMS in 100mM phosphate buffer, pH 7.4) into the mixture. The reaction mixture was incubated at 25°C for 5min, and the absorbance at 560nm was measured against blank samples, containing all other reagents except the PMS. The positive and negative controls were subjected to the same procedures as the sample. Ascorbic acid was used as the positive control. The percentage inhibition of superoxide anion radical was calculated as:

$$\text{Superoxide anion radical scavenging activity (\%)} = \frac{(Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control} \times 100$$

Hydrogen Peroxide Scavenging Assay

The method of (14) was employed for the hydrogen peroxide scavenging assays of the avocado seeds extracts. A solution of hydrogen peroxide (2mM) was prepared in 50mM phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically at 230nm using the molar extinction coefficient for H_2O_2 of $81 mol^{-1} cm^{-1}$. Samples of the various extracts (0.5-1.5mg/ml) were transferred into the test tubes and their volumes were made up to 0.4ml with 50mM phosphate buffer (pH 7.4). After addition of 0.6ml of hydrogen peroxide solution, tubes were vortexed and absorbance was read at 230nm after 10min against a blank (50mM phosphate buffer without hydrogen peroxide was used as blank). Ascorbic acid was used as the standard antioxidant. The percentage inhibition of hydrogen peroxide was calculated as:

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{(Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control} \times 100$$

Statistical Analysis

All the data are expressed as means ± SEM. Students t-test was used to determine the significance difference in all the data. The differences were considered significance at $p<0.05$

Results and Discussion

When the various functions and health benefits of mineral elements in the body of organisms are considered, it becomes imperative to evaluate the mineral contents of plant materials in order to ascertain their nutritive value. Fig. 1 below shows the presence of sodium and potassium in the two samples investigated. Potassium is the predominant element, with a concentration far higher than that of sodium in both the ripe and unripe samples. High sodium content in the body has been associated with high blood pressure (15). Potassium is important for

electrolyte balance, control of high blood pressure, etc. This could explain why foods that are rich in potassium but deficient in sodium are used to manage high blood pressure. The result of this present study shows that the seeds of unripe avocado pear contain a higher concentration of potassium and a lower level of sodium compared to seeds of ripe avocado pear. This suggests that the seeds of the unripe could be more effective in managing high blood pressure in traditional medicine. The two samples investigated contain appreciated amounts of calcium, magnesium, iron, manganese, and zinc. These elements are very important to health. Calcium is tightly linked to many of the roles that vitamin D plays in the body, and hence is very important in bone formation and formation of blood clot (16).

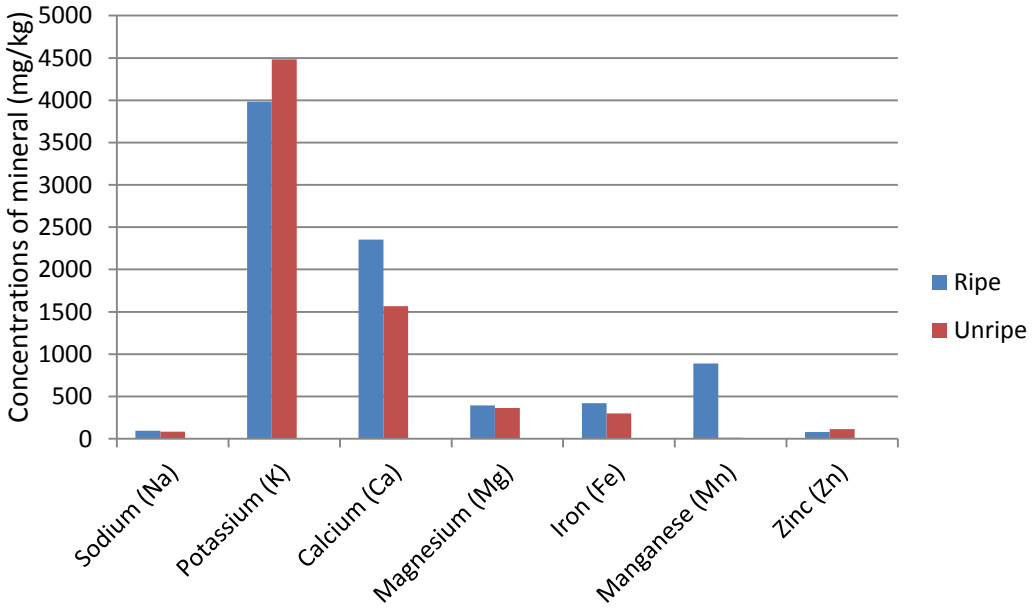

Fig. 1: Mineral composition of the seeds of ripe and unripe avocado pear.

Magnesium acts as a cofactor for many cellular enzymes required in energy metabolism, and it may help support normal vascular tone and insulin sensitivity. Zinc plays important role in fast healing of wounds, while iron is required for haem formation. Manganese helps in the absorption of iron in the body, and in the synthesis of collagen and prevention of osteoporosis (15, 16). In this study, the seeds of unripe avocado pear contain higher amount of zinc compared to the seeds of ripe. This suggests that the seeds of unripe avocado could be useful in wound healing.

One of the quick methods to evaluate antioxidant activity of any extract is the scavenging activity against DPPH, a stable free radical widely used in *in vitro* antioxidant assays (17). In this study, the antioxidant activities of two samples of avocado seeds were evaluated as shown in table 1 below. The IC_{50} was calculated for each of the sample, and it was discovered that the scavenging effect of the extracts increased with the concentration of the extracts.

Table 1: Antioxidant activity of seeds of ripe and unripe avocado pear against DPPH radical

	Concentration of extract(mg/ml)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Ripe avocado seeds	0.5	0.287 ±0.005	52.87 ±0.002	0.5
	1.0	0.139 ±0.001	77.18 ±0.004	
	1.5	0.128 ±0.003	78.98 ±0.001	
Unripe avocado seeds	0.5	0.324 ±0.001	46.80 ±0.006	1.6
	1.0	0.299 ±0.002	50.90 ±0.004	
	1.5	0.247 ±0.007	59.44 ±0.001	

Values are represented as Mean ± SEM.

The extract from ripe avocado seeds exhibited the highest scavenging activity, and hence is posses a better protective ability against the damaging effects of free radicals in the body. This result is consistent with the report

of (18) who recorded a high antioxidant activity of the seeds of avocado pear against the free radical activity of DPPH.

Table 2: Antioxidant activity of the seeds of ripe and unripe avocado pear against superoxide anion

	Concentration of extract (mg/ml)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Ripe avocado seeds	0.5	0.294 ±0.009	45.25 ±0.005	1.5
	1.0	0.237 ±0.010	55.87 ±0.032	
	1.5	0.191 ±0.002	64.43 ±0.028	
Unripe avocado seeds	0.5	0.407 ±0.003	24.21 ±0.017	3.4
	1.0	0.325 ±0.020	39.48 ±0.101	
	1.5	0.299 ±0.002	44.32 ±0.034	

Values are represented as Mean ± SEM

The result of the antioxidant activity of the seeds of ripe and unripe avocado pear, as seen in table 2, indicates that both the ripe and unripe seeds of avocado posses potent antioxidant effect against superoxide anions, with the ripe sample exhibiting the highest scavenging effect.

Hydrogen peroxide is non-reactive, but at high concentrations, is toxic to living cells. It is converted into a free radical called hydroxyl radical which can easily cross cell membranes and react with most biomolecules causing tissue damage, cancer, and cell death (15). The removal of this radical is necessary in protecting life, and it is for this reason that the scavenging effect of the seeds of ripe and unripe avocado pear against this free radical was evaluated. The result as shown in table 3 indicates that the extracts from both seeds possess antioxidant effect against hydrogen peroxide with the extract from seeds of ripe avocado having the higher scavenging effect as indicated by its lower IC_{50}.

Table 3: Free radical scavenging activity of the seeds of ripe and unripe avocado pear against hydrogen peroxide

	Concentration of extract (mg/ml)	Absorbance	% Inhibition	IC_{50} (mg/ml)
Ripe avocado seeds	0.5	0.135 ±0.023	40.53 ±0.010	1.5
	1.0	0.087 ±0.041	61.67 ±0.078	
	1.5	0.053 ±0.027	76.65 ±0.028	
Unripe avocado seeds	0.5	0.182 ±0.020	19.82 ±0.071	2.2
	1.0	0.113 ±0.001	50.22 ±0.024	
	1.5	0.079 ±0.021	65.20 ±0.107	

Values are represented as Mean ± SEM.

The benefits of the avocado pear seeds are immense ranging from its nutritional potentials which is reflected in its mineral composition to its ability to scavenging free radicals. However, the findings of this study suggest that the seeds of unripe avocado pear may be efficacious in the management of hypertension since it contains a higher amount of potassium compared to the seeds of ripe avocado pear. The two samples may be useful as good sources of antioxidant compounds.

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