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PROXIMATE, ELEMENTAL COMPOSITION AND ANTIMICROBIAL ANALYSIS OFAVOCADO (*Persea americana*) SEED

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ABSTRACT

The seed of Persea americana (Avocado Seed) was analyzed for its Proximate, Element Composition and Anti-Microbial Properties in accordance with the Association of Analytic Chemistry method, Atomic Absorption Spectrometric method and Standard Method f Antimicrobial determination respectively. The Proximate analysis indicated the presence of Cruc fibre (6.56%), Protein (1.4%), Fat (26.72%), Ash (3.4%), Moisture Content (8.3%) and Carbohydra (53.09%) while the Elemental analysis showed the presence of Cadmium (0.013ppm), Calciu (6.645ppm), Nickel (1.0733ppm), Sodium (31.724ppm) and Iron (1.387ppm). The result showed th Cadmium fell within the recommended standard (0.01ppm). The Efficacy of Antimicrob activities of Aqueous and n-hexane extract was carried out against different fungi (Candic albican and Aspergillus niger), and bacteria (Escherichia coli, Proteus, Streptococcus pyoger and Staphylococcus aureus). The results obtained from both extracts showed no significant An microbial activities against the tested organisms. Nevertheless, Avocado seed which is normo discarded as a waste is a rich source of nutrients.

Keywords: *Persea Americana, Nutritional values, Elemental Composition and Anti-microbial properties.*

INTRODUCTION

Avocado is the common name for the herbaceous plants of genus *Persea*. Also commonly known as alligator pear or aguacate, it is scientifically known as *Persea americana* of the family Lauraceal. The word avocado comes from the Aztec word "ahuacati" which is translated in Spaniard as agucate, meaning "testicle" due to its shape
(Padma and Sunil 2015). It is a tropical fruit that originated from America. Avocado is classified as an evergreen, although some varieties lose their leaves for a short time before flowering. The fruit is a berry, consisting of a single large seed, surrounded by a buttery pulp. It contains 3-30% oil (Florida varieties range from 3-15%). The

seed is oblate, round, conocal or ovoid, 5 – 6.4cm long. They are hard and heavy, ivory in colour but enclosed in two brown, thin, papery seed coats often adhering to the flesh cavity, while the seed slips out readily. (Orwa *et al*., 2009).

The avocado leaf, stem fruit and peel have biological activities scientifically proven (Miranda et al. 1997; Adeyemi et al., 2002). The pulp of avocado has been reported to have beneficial cardiovascular health effect. Avocado oil is used for dermatological applications and its unsaponifiable portion is reported to have beneficial effects against osteoarthritis. Currently, the seed represents an under-utilized resource and a waste issue for avocado processors (Haas 1951, Dabas *et al.,* 2013; Ogbuagu and Okoye, 2020).

Table 1: Scientific classification of *Persea amaericana* (Daurte *et al*., 2016).

Rank	**Scientific name**	**Common name**
Kingdom	Plantae	Plant
Subkingdom	Tracheobionta	Vascular plant
Supervision	Spermatophyta	Seed plant
Division	Magnoliophyta	Flowering plant
Class	Magnoliopsida	Dicotyledons
Subclass	Magnoliidae	
Order	Laurales	
Family	Lauracease	Laurel family
Genus	Persea	Bay
Species	Pearsea Americana	avocado

There is ethno-pharmacological information on the use of seeds for the treatment of health related conditions, especially in South American countries where avocados are endemic and currently grown on a large scale. Current research has shown that avocado seed may improve hypercholesterolemia and be useful in the treatment of hypertension, inflammatory condition and diabetes. The avocado seed are rich in phenolic compounds and these play a role in the pubative health effects. Avocado seed oils have also been reported to be used in healing of skin eruptions (Swisher, 1988; Ogbuagu and Okoye, 2020).

Historically, extracts of avocado seed were also used as ink for writing and research

has explored the potential colourant properties of polyphenol oxidase – produced coloured avocado seed extract (Dabas, 2013; Ogbuagu and Okoye, 2020).

Phytochemical studies of the avocado seed allowed the identification of several classes of active compounds such as flavonoid, anthocyanins condensed tannins, alkaloids and triterpenoids in methanolic extract while sterols and triterpenes were detected in the hexane extract (Leite *et al.,* 2009).

The use of avocado in traditional herbal medicine can be attributed to its pharmacological activity (Padma and Sunil, 2015). Seed and leaf extracts have been used for variety of medical application including treatment of diarrhoea, dysentery and as an antibiotic (Lahav and Whiley 2002). It contains nutrients which are beneficial in the synthesis of skin protein called collagen (Ding *et al.,* 2007). The fruit is considered one of the most potent anti-oxidant fruit in the world because of its high content of mono saturated fats (Lin *et al.*, 2009), thus, people consuming a special avocado based diets showed lower cholesterol levels. It is noteworthy that enthnopharmcology of Aztec and Maya cultures used decots of avocado seeds as a potent agent to treat mycotic and parasitic infection (Oberlies *et al*., 1998). Avocado seeds preparations are traditionally used as anti-inflammatory. Some lipids isolated from the avocado fruits have shown selective activity against human prostate Aden carcinoma (Oberlies *et al.,* 1998). Avocado fruits have a skin healing effects which may be due to the positive influence on fatty acid (Carranza *et al.,* 1995). The powdered seed is believed to cure dandruff. An ointment made of the pulverised seed is used as a facial rubefacient. Oil extracted from the seed has been applied on skin eruptions.

MATERIALS AND METHODS

Materials: The *Persea americana* fruits were obtained at various locations from Nnaba village in Awka-Etiti, Anambra State, Nigeria. It was identified by the herbarium curator of the Botany Department, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. All reagents used were of analytical grade.

Sample Preparation: The fruit was left to ripe and the edible part consumed. The seed were manually cut into pieces, after which it was sundried for seven days and then pulverized to powdered form. Only the undamaged seed were chosen.

Moisture Content Determination: A crucible was washed and dried in the oven at 105^{o}c for 30 minutes. It was cooled in the dessicator and weighed empty. 2.291g of the sample was weighed into crucible and left in the oven at 105^{o}c for 24 hours (to achieve maximum moisture content). This was later removed, cooled in the dessicator and weighed.

% moisture content =

$$\frac{W_1 - W_2}{W_3} \times 100$$

Where; w_1 = weight of crucible + sample before drying

w_2 = weight of petri dish and sample after drying

w_3 = weight of sample.

Crude Protein Determination: This involved the digestion of sample with hot concentrated sulphuric acid in the presence of a metallic catalyst (selenium powder). Then the organic nitrogen in the sample was reduced to ammonium and was retained in the solution as ammonium sulphate. This was then made alkaline, and then distilled to release the ammonia. The ammonia was trapped in dilute acid and then titrated.

0.5g of the sample was gently weighed into a 30ml kjehdal flask and then the flasks were stoppered and shaken. 0.5g of the kjedahl catalyst mixture and the mixture was digested using a heating mantle. Digestion was continued until a clear Solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. The digested sample was made up to 100ml with distilled water to avoid caking and then 50ml was transferred to the kjadahl distillation apparatus. A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue was placed under a condenser of the digested sample in the apparatus and distillation so that the tap was about 20cm inside the solution. 5ml of 40% sodium hydroxide was added to the digested sample in the apparatus and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.

Calculation:

% Nitrogen = titre value x 0.01 x 14 x4

% Protein = % Nitrogen x 6.25

Ash Content: Ash is the inorganic residue obtained by burning off the organic matter of feedstuff at 500°C. 2g of the wet sample was weighed into a washed, dried and weighed platinum and placed in a muffle furnace at 500°C for 3hours or until whitish grey ash is obtained. The sample was cooled in a dessicator after burning and then weighed.

Calculations:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where; W_1= weight of empty platinum crucible

W_2 = weight of platinum crucible + sample before burning

W_3 = weight of platinum + ash

Crude Fibre Determination: 2.014g of the sample was weighed and was defatted with 1.26g of NaOH that was dissolved in 100ml of distilled water. The solution was boiled for 30minutes and was filtered through a linen on a fluted funnel. The residue was transferred to a beaker containing 1.26g of sulphuric acid and was made up to 100ml with distilled water and boiled again for 30 minutes. The solution was filtered and the residue was transferred into a crucible which was then

dried in an oven for 1hr at 100°C after which was cooled and weighed

% crude fibre =

$$\frac{\text{weight of fibre}}{\text{Weight of sample}} \times 100$$

Crude Fat (Ether Extract): The ether extract of a feed represents the fat and oil in the feed and is carried out by continuously extracting a food with non-polar organic solvent such as petroleum ether for 1 hour or more, using the soxhlet extractor.

250ml clean boiling flasks was dried in oven at 110°C for 30 minutes and was allowed to cool and weighed. 300ml of anhydrous diethyl ether of boiling point of 40-60^0 c is placed in the flask. 2.002g of the sample was weighed into a thimble and the thimble is plugged tightly with cotton wool. The thimble with the content is placed into the extractor; the ether in the flask is then heated. As the ether vapor reaches the condenser through the side arm of the extractor, it condenses to liquid and drops back into the sample in thimble, the ether soluble substances are dissolved and are carried into solution through the siphon tube back into the flask. The extraction continues for at least 4 hours. The thimble is removed and most of the solvent is distilled from the flask into the extractor. The flask is then disconnected and placed in an oven at 105°C for 1 hour, cooled in dessicator and weighed.

% of fat =

$$\frac{\text{Weight of fat}}{\text{Weight of samples}} \times 100$$

Mineral Composition Analysis: The minerals were determined using a solution that was obtained by dry ashing the sample at 550°C and dissolving it in distilled water that contains 10ml of concentrated hydrochloric acid in a volumetric flask. Na, Ca, Fe, Cd and Ni contents were determined using Varian AA240 Atomic Absorption Spectrometer according to the method of APHA 1995 (American Public Health Association).

Antimicrobial Analysis:

Preparation of media: 36g of Nutrient Agar was weighed and dissolved in 100ml of water which was then boiled and autoclaved for 30 minutes. The media was allowed to cool to about 40°C and was poured into the petri-dish and then allowed to set on the bench.

Culturing of Media: The media was bored at the centre using core borer; the wire loop was flamed and used to transfer organisms from the stock culture into the media by streaking. 2 drops each of n-hexane and aqueous extract of avocado seed were transferred into the incubator for 24hours (bacterial) and 48 hours for fungi after which the result was read.

RESULTS AND DISCUSIION

Proximate Analysis Results: The Proximate analysis on the seed of *Persea Americana* as seen in table 2 above showed that the crude fibre content is 6.56% which tallies with the study carried out by (Dreher and Davenport, 2013) and this shows that its

ingestion will help to reduce blood cholesterol level, the risk of bowel cancer and gall stones and it can also serve as a good supplement for weight loss. The relatively high fat content 26.72% signifies that the seed is a good source of oil, however this is higher than the value 21.63% as reported by (Ogbuagu and Okoye, 2020); this could be as a result of difference in plant species and varying time of drying. The moisture content 8.83% which tallies with the study performed by (Arukwe *et al,* 2012) but is below the stipulated moisture content of 13-15% and this could be an added advantage to the shelf life of the seed, given that values exceeding 15% might lead to the seed developing undesirable moulds and fungus. The total ash content of 3.4%, which

Table 2: Result of the Proximate Analysis

Parameter	Composition
Moisture Content	8.83%
Ash Content	3.4%
Crude Fiber	6.56%
Fat Content	26.72%
Carbohydrate	53.09%
Crude protein	1.4%

tallies with the study carried out by (Talabi *et al,* 2016) showed that *Persea Americana* seed contains less inorganic compounds, while the protein content of 1.4% signifies a very low protein content. The carbohydrate content of 53.09% indicates that it is a good source of starch in food and can be used as flour for baking e.g. biscuit (Mahawan *et al*, 2015).

Elemental Analysis Result: The highest concentrated metal is sodium (31.725ppm) as shown in table 3, while the lowest is cadmium (0.013ppm). The value obtained for sodium tallies with the study carried out by (Morais *et al*, 2017), which is higher than the WHO recommended standard, as well as iron (1.387ppm). The concentrations of calcium (6.645ppm), cadmium (0.013ppm) and Nickel (1.073) all fell within the WHO recommended standard.

Table 3: Mineral (Elemental) Composition of Avocado Seed

Parameters	Concentration (ppm)	WHO Recomended Standard (ppm)
Calcium	6.645	≤10
Sodium	31.725	≤5
Cadmium	0.013	≤.01
Iron	1.387	≤1.0
Nickel	1.073	≤.03

Antimicrobial Analysis Results: From the result of the analysis (table 4), it was found that the hexane and aqueous extract of *Persea americana* seed does not inhibit the growth and hence does not have any antimicrobial activity for the selected organism.

Table 4: Antimicrobial activities of n-hexane and aqueous extract

Test organism	Hexane extract	Aqueous extract
Candida	-	-
Aspergillus Nnniger	-	-
E.coli	-	-
Proteus	-	-
Streptococuspyogene	-	-
Staphylococus aureus	-	-

CONCLUSION

Based on the results of the analysis, as summarized in tables 2, 3 and 4, the following conclusions can be drawn.

Avocado seed contains a diverse number of nutrients and elemental in varying proportions. The high carbohydrate content of seed makes it a good source of energy food; the relatively high fat content could increases the amount of HDL – Cholestrol (good cholesterol) in the blood and liver lipids and therefore could be a good source of cooking oil and in the production of margarine.

Sodium controls the body water balance and plays a role in muscle contraction but its high content in avocado seed poses the threat of increasing blood volume in the body when consumed, which in turn raises blood pressure. The calcium content could help in blood clotting, muscle contraction and in metabolic processes in certain enzymes. Calcium is also capable of assuming a corrective role if some inorganic elements such as sodium and potassium are excess in the body. Iron facilitates the oxidation of carbohydrates, fats and proteins and therefore contributes significantly to the prevention of anemia. Iron also plays an important role in the oxidative processes of respiration in living organisms and also in the normal functioning of the central nervous system. The slightly higher content of iron however, can cause liver toxicity and increase the risk of liver failure or hepatocellullar carannions in humans. Nickel and cadmium are present in the right proportions and although they are regarded as essential metals which the body needs in very small quantities, they are also very toxic but shouldn't pose any significant threat on consuming the seed.

The n-hexane and aqueous extract of the seed did not show any anti-microbial activity for the selected organisms as shown in table 4. However, it is a potential source of nutrients and medicinal compounds and could find many uses in clinical application and food processing.

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