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Comparative Study of the Proximate, Mineral and Phytochemical Compositions of Avocado (*Persea americana*) Pulp and Seed

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Abstract: The proximate, mineral and phytochemical compositions of avocado pear (*Persea americana*) seed and pulp were studied using standard methods. Qualitative analysis of the phytochemicals revealed the presence of flavonoids, saponin, tannins and phenols in both the seed and pulp of *P. americana* . Alkaloid was only present in the seed. Quantitatively, the concentration of saponins, flavoids, tannins, phenols and alkaloids in the seed were higher than in the pulp. The proximate contents (%) of the avocado seed, in decreasing order were carbohydrate (48.51± 0.56) > protein (18.08 ± 0.58) > moisture content (14.64 ± 0.38) > fat (13.31 ± 0.05), fibre (4.61 ± 0.13) > ash (0.85 ± 0.12) while the pulp had moisture content (54.27 ± 0.49%) > fat (30.85 ± 0.43%) > carbohydrate (9.33 ± 0.66%) > protein (2.92 ± 0.59%) > ash (1.38 ± 0.17%) > fibre (4.61 ± 0.13%). The concentration of minerals in the seed of *P. americana* were significantly higher ($p<0.01$) than in the pulp except for zinc and sodium. The *P. americana* seed contains higher substantial nutrients that could meet the needs and requirements of the body, thus good for human and animal consumption.

Keywords: *Persea americana*, Phytochemical screening, Proximate analysis, Mineral analysis

1. INTRODUCTION

Avocado (*P. americana*) is a plant in the Lauraceae family that produces avocado pear. It's a tropical fruit that originated in America. Avocados are considered evergreen, though some kinds lose their leaves before flowering. The fruit is a berry composed of a single big seed surrounded by a buttery pulp [1]. Its pulp is high in lipids and oils, protein, and fiber. Avocado pear, with its high amount of digestible oil and low sugar content, is a great source of energy and a crucial component of diabetic diets [2]. Roughly 65% of the pear is pulp, 20% is seed, and 15% is skin. It is high in protein, vitamins, and vital minerals that are necessary for excellent health. In conventional medicine, the fruits are used to treat hypertension and other associated conditions [3]. The fruit's high mono-saturated lipid content makes it one of the world's most powerful antioxidants; consequently, individuals who ate a diet focused on avocados had reduced cholesterol [4].

Various avocado pear sections were utilized as antimicrobials and for other uses in traditional medicine. Ancient societies and traditional medicine have long utilized avocados, including their pulp and waste, as infusions with antibacterial, anti-inflammatory, antioxidative, and

anticarcinogenic qualities. Numerous categories of bioactive molecules, or phytochemicals, such as polyphenols, carotenoids, and tocopherols, are responsible for these characteristics [5]. In addition to the fruits, the seeds—which are typically thrown away after consumption—have been shown to be high in vital minerals like potassium and to have antioxidant properties. They may also help treat hypertension and other health issues brought on by free radicals [6]. There have been reports of the seed's anti-oxidative, antihypertensive, fungicidal, and hypolipidemic biological effects [7]. The avocado pear seeds are underutilized since they are discarded as agro-food wastes. They cause an ecological problem in that being a by- product, they are discarded when processing the pulp [8]. Exploring the possible dietary and therapeutic potentials of these underutilized agro-food wastes will reduce the possible environmental waste burden [5]. This study on the avocado seed and pulp was aimed to determine its enhanced utilization for dietary and industrial uses, value added products and to reduce agro-food wastes which will in addition reduce the possible environmental waste burden by investigating the mineral, proximate and phytochemical properties. As agro-food waste, the avocado pear seeds are not being used to their full potential. Due to their disposal as a by-product during pulp manufacturing, they pose an ecological hazard [8]. By investigating the potential nutritional and medicinal benefits of these underutilized agro-food wastes, the potential environmental waste burden can be decreased [5]. The purpose of this study was to investigate the mineral, proximate, and phytochemical properties of avocado seed and pulp in order to determine its enhanced utilization for dietary and industrial uses, value-added products, and to reduce agro-food wastes, which will also reduce the potential environmental waste burden.

2. MATERIALS AND METHODS

2.1. Materials

Materials used in the study included manual grater (Binatone), domestic blender (Binatone), beakers, whatman (No. l) filter paper (Sigma). conical flasks, funnels, water bath, Bunsen burner, plastic bottles, mechanical shaker, weighing balance, microplate reader (Thermo Fisher), spectrophotometer (Pye Unicam), desiccator (Infitek), Kjeldahl Analyzer (Infitek), Soxhlet extractor (Infitek), muffle furnace (Infitek), volumetric flask and crucible. Reagents used included distilled water ammonium hydroxide (Merck), ethanol (Merck), sodium chloride (Merck), sulphuric acid(Merck) n-butanol, diethyl ether (Merck), ferric chloride (Merck), Folin-Ciocalteu reagent (CCI), sodium carbonate (Merck), dilute ammonia (Merck), bromocresol blue (Sigma,), methylene blue (Sigma), sodium hydroxide (Merck), ammonium sulphate (Merck), potassium hydroxide (Merck) and concentrated hydrochloric acid (Merck).

2.2. Collection and Preparation of Sample

The Avocado pear fruits were bought in a local market in Benin City, Edo State, Nigeria. The fruits were identified and physically certified as that of Persea americana Mill (Lauraceae) in the Department of Biological Sciences, Igbinedion University, Okada, Edo State. The fleshy cover was removed to deseed the fruits. Clean tap water was used to wash the seeds. A manual grater was used to break up the pulp and seeds into smaller bits, and the seeds were then allowed to dry in the sun for three days. A home blender was then used to grind the sun-dried seeds into a powder. The several assays were then conducted using portions of the grated pulp and powdered seed sample.

2.3. Phytochemical Analysis

Qualitative and Quantitative phytochemical screening for the presence of alkaloids, saponins, flavonoids, tannins and phenols were carried out.

2.3.1. Determination of Alkaloid

Alkaloid was identified using the Harbome [9] approach, which was explained by Akusu et al. [8]. After measuring five grams (5 g) of the sample into a 250 ml beaker, 100 ml of 20% ethanol/acetic acid was added, and the mixture was left to stand for four hours. After four hours, the mixture was agitated and filtered using Whatman (No. l) filter paper. For twenty minutes, the filtrate was thickened. Drop by drop, 20 cm3 of concentrated ammonium hydroxide (NH4OH) was added to the extract until the precipitation was finished. After the entire solution was given time to settle, the precipitate was filtered out and weighed. Three separate analyses were performed on each sample. The amount of alkaloid in the sample was determined by calculating the difference in weights.

2.3.2. Determination of Saponin

Saponin was identified using [8]'s approach. A conical flask was filled with 5 g of the sample and 25 ml of 20% aqueous ethanol. Over a water bath, the sample was heated to approximately 55 °C for one hour while being constantly stirred. Following the transfer of the liquid into a 250 ml separator funnel, 5 ml of diethyl ether was added and forcefully shaken. Recovery of the aqueous layer occurred. First, 2.5 ml of 5% aqueous sodium chloride was added, followed by 15 ml of n-butanol. In order to evaporate the resultant solution to a consistent weight, it was heated. Every sample underwent triplicate analysis.

2.3.3. Determination of Flavonoid

A few drops of 0.1% ferric chloride were added to filtered, boiled avocado water to test for the presence of flavonoids. When 5 ml of diluted ammonia solution, 5 ml of filtrate, and 1 ml of sulfuric acid were combined, the liquid turned yellow, indicating the presence of flavonoids [10].

2.3.4. Determination of Tannin

A 50 ml plastic bottle filled with 50 ml of distilled water was pipetted with 10 milliliters (10 ml) of the sample. This is shaken on a mechanical shaker for an hour. After filtering the solution, 5 milliliters of the filtrate were combined with 2 milliliters of FeCl3 in 0.1 NH4Cl. At 120 nm, the absorbance was measured [11].

2.3.5. Determination of Total Phenolic Content

Total phenolic content was determined calorimetrically using Folin-Ciocalteau reagent as described by Akusu et al. [8]. The total phenolic content of avocado seed extract was determined by diluting Folin-Ciocalteu reagent and 15% sodium carbonate. The absorbance at 765 nm using a microplate reader was then measured. In short, a 0.25 ml methanolic solution extract, 0.25 ml Folin-Ciocalteu reagent diluted with distilled water (1:1), 0.5 ml sodium carbonate are mixed, and 4 ml water was added. The mixture was incubated at room temperature for 30 minutes, and then the absorbance at 725 nm is measured using a spectrophotometer.

2.4. Proximate Analyses

The proximate contents (moisture content, protein, ash, crude fibre, fat and carbohydrate) of the samples were determined.

2.4.1. Determination of Moisture Content

A crucible was washed and dried in the oven at 105oC for 30 minutes. It was cooled in the desiccator and weighed empty. 2.291g of the sample was weighed into crucible and left in the oven at 105oC for 24 hours (to achieve maximum moisture content). This was later removed, cooled in the desiccator and weighed [4].

2.4.2. Determination of Crude Protein

With modifications made by Ogbuogu et al. [4], Kjeldahl's technique was used to determine crude protein. The sample was digested using hot, concentrated sulfuric acid while selenium powder, which served as a metallic catalyst, was present. The sample's organic nitrogen was converted to ammonium, which was then stored as ammonium sulphate in the solution. The ammonia was then extracted by distillation after this was rendered alkaline. The ammonia was titrated after being caught in diluted acid. A 30ml Kjeldahl flask was filled with 0.5g of the sample, which was then carefully weighed before being shaken and sealed. A heating mantle was utilized to digest 0.5g of the Kjeldahl catalyst combination. The process was carried out until a transparent solution was obtained. After 30 minutes, the transparent solution was left to stand and cool. To prevent caking, 100 milliliters of the digested sample were mixed with distilled water, and 50 milliliters were then moved to the Kjeldahl distillation device. A condenser of the digested sample was placed beneath a 100 ml receiver flask with 5 ml of 2% boric acid and an indicator mixture that contained 5 drops of bromocresol blue and 1 drop of methylene blue. The tap was positioned approximately 20 cm inside the solution. The digested sample was placed in the apparatus with 5 ml of 40% sodium hydroxide, and distillation started immediately until 50 drops reached the receiver flask. 0.01N hydrochloric acid was then used to titrate the sample to a pink colour.

$$\%\, Nitrogen = titre\ value \times 0.01 \times 14 \times 4$$

$$\%\, Protein = \%\, Nitrogen \times 6.25$$

2.4.3. Determination of Crude Fibre

Weende's method was used to determine crude fiber [12]. The technique relies on the solubilization (digestion) of non-cellulosic substances using solutions of potassium hydroxide and sulfuric acid. Crude fiber, which is measured by weight difference, is the amount of dried residue that is lost upon igniting after the sample has been digested.

2.4.4. Determination of Fat Content

Continuous solvent extraction was used to measure the fat content [4]. A sample's ether extract, which is made by continuously extracting it with a non-polar organic solvent, like petroleum ether, for an hour using a Soxhlet extractor, indicates the fat and oil in the sample. After being cleaned, 250 ml boiling flasks were dried for 30 minutes at 110 °C in the oven, allowed to cool, and then weighed. Anhydrous diethyl ether with a boiling point between 40 and 600 C is then added to the flask in an amount of 300 milliliters. After weighing two grams of the sample, it was placed in a thimble and securely sealed with cotton wool. The extractor is then used to heat the ether in the flask once the thimble containing the contents has been placed inside. The ether vapor

condenses to liquid as it passes through the extractor's side arm and returns to the sample in a thimble. After being dissolved, the ether-soluble materials are returned to the flask via the siphon tube. At least four hours are spent on the extraction process. The majority of the solvent is distilled from the flask into the extractor when the thimble is taken out. After that, the flask is unplugged, heated to 105°C for an hour, cooled in a desiccator, and weighed. Weight of fat divided by sample weight multiplied by 100 is the percentage fat.

2.4.5. Determination of Ash

Total ash content was determined by furnace 'incineration method [12]. Ash is the weight of residue obtained after burning a weighed quantity of avocado in an open crucible at 750°C in a muffle furnace till a constant weight is achieved.

$$\%\ Ash\ in\ Avocado = \frac{\text{weight of residue ash formed}}{\text{weight of avocado initially taken}} \times 100$$

2.5. Mineral Composition Analyses

The samples' mineral compositions were identified. One gram of the sample was weighed in a crucible and then dried in a muffle furnace at 550 degrees Celsius for three hours. In a volumetric flask, distilled water containing 10 milliliters of strong hydrochloric acid was then used to dissolve the washed samples. Next, a 100 ml volumetric flask was filtered using Whatman no. 1 filter paper. While iron, zinc, selenium, and copper were calculated using a Unicam atomic absorption spectrophotometer based on the methods of Nwaokobia et al. [12], calcium, sodium, and magnesium were analyzed using the Versanate EDTA complexometric titration method [13].

2.6. Statistical Analysis

All the analyses were carried out in triplicate. Data obtained were subjected to Analysis of Variance (ANOVA); differences between means were evaluated using Tukey's multiple comparison tests with 95% confidence level. Results were expressed as mean ± standard deviation, SD of duplicate samples. Statistical Package for the Social Sciences (SPSS) version 16 software was used for easy analysis of data.

3. RESULTS AND DISCUSSION

The qualitative analysis of the phytochemical compounds present in the seed and pulp of *P. americana* samples. Alkaloids, saponins, flavonoids, tannins and phenols were present in the seed while alkaloid was absent in the pulp (Table 1).

Table 1. Qualitative Analysis of the Phytochemical Composition of *P. americana* Seed and Pulp Samples

Parameters	Seed	Pulp
Alkaloids	+	-
Saponin	++	+
Flavonoids	++	+
Tannins	+++	++
Phenols	++	+

Values are means ± standard deviations of duplicate determinations. Key: + = mild; ++ = moderate; +++ = high; - = not detected

The qualitative phytochemical analysis of *P. americana* samples showed the presence of alkaloids, saponins, flavonoids, tannins and phenols in the seed and pulp, with the absence of

alkaloids in the pulp [21]. High amount of tannins were detected in the seed of *P. americana* with moderate amounts of flavonoids, phenols and saponins.

Table 2. Quantitative Analysis of the Phytochemical Composition of *P. americana* Seed and Pulp Samples

Parameters	Seed	Pulp
Alkaloids (%)	1.02 ± 0.21	0.00 ± 0.00
Saponin (%)	2.01 ± 0.09	0.25 ± 0.21
Flavonoids (mg/100g)	69.89 ± 0.05	42.44 ± 0.77
Tannins (mg/100g)	402.75 ± 0.11	117.58 ± 0.98
Phenols (mg/100g)	171.13 ± 0.20	78.71 ± 0.21

Values are means ± standard deviations of duplicate determinations

Table 2 showed that these phytochemicals were present more in the seed than the pulp samples and this study corroborates with the results reported by Nwaokobia et al. [13]. They found phenolics, flavonoids, saponins, tannins and alkaloids present more in the avocado seed extract. Alkaloids had concentration of 1.02 ± 0.21% present in the seed only. Alkaloids are known to be used to cure of malaria, diabetes, and hypertension and can be used as tranquilizer [13]. The concentration of flavonoids in the pulp was 42.44 ± 0.77 mg/100g, whereas the concentration in the seed was 69.89 ± 0.05 mg/100g. This is consistent with a study by Umeaku et al. [2021] that found that avocado seeds have higher levels of flavonoids and total phenols than pulp. Plants manufacture flavonoids in reaction to microbial attack, which makes them potent antimicrobial agents. In addition, flavonoids have antiviral, anticancer, antiallergic, anti-inflammatory, antioxidant, and antiplatelet properties that are good for the body [10]. The concentration of saponin was higher in the avocado seed (2.01 ± 0.09%) than in the pulp (0.25 ± 0.21%). The pulp and seed had lower saponin contents than those reported by Nwaokobia et al. [13], which were 0.15±0.02 mg/100g and 18.91±2.01 mg/100g, respectively. Saponins are consumed in many common foods and beverages including: oats, peanuts, tea and beer [8]. Blood cholesterol, cancer, bone health, and immune system stimulation are just a few of the health advantages that saponins offer [10]. Both the pulp (117.58 ± 0.98 mg/100g) and the seed (402.75 ± 0.11 mg/100g) had higher tannin contents in our investigation than those reported by Ejiofor et al. [15]. In experimental animals, tannin has been implicated in reductions in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility, according to research by Ejiofor et al. [15]. Tannin-containing plants are used to heal mildly wounded skin, mouth and throat swellings, and general diarrhea. In humans, tannins have been shown to improve appetite, reduce blood pressure, and lessen respiratory issues [14]. The pulp and seed samples had total phenol levels of 78.71 ± 0.21 mg/100g and 171.13 ± 0.20 mg/100g, respectively. As naturally occurring oxidants, phenols have certain positive health effects. It can lower the risk of inflammatory disorders and stop lipid oxidation [16]. Avocado pulp and seed include tannins, flavonoids, and phenolic compounds, which indicate that they are good sources of functional and bioactive components for food compositions that would improve their antioxidant capabilities [8].

Table 3. Proximate Composition of *P. americana* Seed and Pulp Samples

Parameter (%)	Seed	Pulp
Moisture	14.64 ± 0.38	54.27 ± 0.49
Ash	0.85 ± 0.12	1.38 ± 0.17
Fat	13.31 ± 0.05	30.85 ± 0.43
Fibre	4.61 ± 0.13	1.24 ± 0.19
Protein	18.08 ± 0.58	2.92 ± 0.59
Carbohydrate	48.51 ± 0.56	9.34 ± 0.66

Values are means ± standard deviations of duplicate determinations

Table 3 showed the proximate compositions of the avocado seed and pulp samples. There was significant difference between the proximate composition values at $p< 0.01$. The pulp had higher moisture (54.27 ± 0.49%), ash (1.38 ± 0.17%) and fat (30.85 ± 0.43%) than the seed with moisture (14.64 ± 0.38%), ash (0.85 ± 0.12%) and fat (13.31 ± 0.05%) which agreed with the study of Nwaokobia et al. [13]. The fibre, protein and carbohydrate contents of the seed at 4.61 ± 0.13%, 18.08 ± 0.58% and 48.51 ± 0.56% were higher than the pulp at 1.24 ±0.19%, 2.92 ± 0.59 and 9.34 ± 0.66% respectively. The ash content measures the mineral content present in a plant. The ash content of *P. americana* in this study were lower than values recorded by Egbuonu et al. [5]. The quantity of ash possessed by the samples in this study shows that the pulp and seed can hinder the growth of micro-organism [13]. The moisture content reported in the present study indicated that the pulp is rich in moisture. Moisture content helps in maintaining the protoplasmic content of cells [15]. The lower moisture content of the seed indicated that the seed probably has a good keeping quality and agrees with the study of Ogbuogu et al. [4]. In this investigation, the ash content of *P. americana* was lower than what Egbuonu et al. [5] had found. This study's samples' amount of ash indicates that the pulp and seed can prevent microorganisms from growing [13]. The present study's moisture content revealed that the pulp had a high moisture content. The protoplasmic content of cells is maintained by moisture content [15]. According to Ogbuogu et al.'s study, the seed's decreased moisture content suggests that it likely has a good keeping quality [4].

The crude fibre value of the seed at 4.61 ± 0.13% from this study result is comparable with the results of Nwaokobia et al. [12]. The value for the avocado pulp (1.24 ± 0.19%) was comparable with the result of 1.81 ± 0.01% from the study of Okibe et al. [17]. Diets low in crude fiber are undesirable because they may lead to constipation and have been linked to colon disorders including piles. As a result, the avocado pear may offer health advantages and be a strong source of dietary fiber [5]. In addition to boosting meal size, appetite satisfaction, and motility through the digestive system, fiber may also lower cholesterol levels and prevent plaque formation by enhancing the absorption and re-absorption of bile acids and cholesterol, respectively [5]. The pulp and seed in the sample had protein contents of 2.92 ± 0.59% and 18.08 ± 0.58%, respectively. In comparison to the value of 2.64±0.01% published by Egbuonu et al. [5], the protein content was 2.92 ± 0.59 percent. Proteins in food have a number of physiological effects, such as forming globular and structural components, repairing and replacing damaged cells, and strengthening the immune system [17]. The results for the avocado seed (12.71±3.41%) and pulp (8.45±4.12%) reported for *P. americana* by Nwaokobia et al. were lower than the carbohydrate content of the avocado seed (48.51 ± 0.56%) and pulp (9.34 ± 0.66%). [13] At 49.03±0.02%, the avocado seed's carbohydrate content was similar to that of the research conducted by Ejiofor et al. [15]. Avocado pear seeds may be a greater carbohydrate source

with a higher energy value, and the carbohydrates found in the examined samples suggest that the samples may provide energy to power the body's cells and tissues when consumed [17].

Table 4. Mineral Composition of *P. americana* Seed and Pulp Samples

Parameter (mg/kg)	Seed	Pulp
Zinc (Zn)	5.23 ± 0.08	8.06 ± 0.48
Calcium (Ca)	37.82 ± 0.34	23.57 ± 0.40
Copper (Cu)	1.47 ± 0.20	0.82 ± 0.04
Iron (Fe)	20.95 ± 0.31	13.7 ± 0.23
Sodium (Na)	0.05 ± 0.00	0.08 ± 0.00
Magnesium (Mg)	0.82 ± 0.01	0.64 ± 0.01
Selenium (Se)	0.49 ± 0.02	0.43 ± 0.01

Values are means ± standard deviations of duplicate determinations

In Table 4 is shown the mineral composition of *P. americana* seed and pulp samples. Compared to the pulp, the avocado seed's calcium, copper, iron, magnesium, and selenium contents were significantly greater ($p < 0.01$). Persea americana seed and pulp have a high mineral content, including calcium, magnesium, zinc, iron, and copper. It also has trace quantities of selenium and salt. In a variety of metabolic processes, these minerals are essential. Iron and magnesium levels were similar to those found in Okibe et al.'s study [17]. The calcium value in this study was 37.82 ± 0.34, which was less than the value of 434.9 ± 39.5% in the study by Damila et al. [18]. The high 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 [4]. The magnesium composition of the pulp and seed indicate that avocado can be used to cure diabetes, hypertension, depress and osteoporosis. Iron composition of avocado indicates that the fruit can be used to synthesize red blood cells and help prevent iron-deficiency anaemia [4]. Sodium levels in the pulp and seed were found to be 0.08 ± 0.00 and 0.05±0.00, respectively, which is less than the *P. americana* result of 39.44±11.3% that Damila et al. [18] reported. Hypertension has been associated with a high salt content in the body [19]. The high levels of zinc in avocado pulp and seed are similar to the 7.23±0.01 mg/kg found in a study by Nwaokobia et al. [12]. Because it aids in the immune system's ability to combat and eliminate infections, zinc is extremely vital. Zinc is essential for several stages of the immune response and is used by the body to make immune cells [20].

4. CONCLUSION

Excellent nutritional components found in *P. americana* fruit may meet the needs and requirements for both human and animal consumption. The fruit's various constituent fractions contain bioactive substances that can improve the body's cellular and physiological functions. When eaten, the pulp has been shown to provide health benefits. This study has demonstrated the great nutritional value of *P. americana* seed extract, which contains significant levels of iron, zinc, selenium, calcium, and magnesium, as well as fiber, carbohydrates, protein, and fat. Therefore, rather than being thrown away, it would provide livestock with nourishment. Avocado pulp and seed contain phytochemicals, which indicates that they are a valuable source of functional and bioactive components for food formulations that are safe and nutritious for both human and animal use.

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