Chemical Composition of Seed and Leaf Extracts of *Persea Americana*: A Comparative Study

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ABSTRACT

Persea americana is a plant whose fruit (avocado pear) is consumed as food while the leaves and seeds are employed in traditional medicine. The chemical composition of the aqueous and ethylacetate leaf and seed extracts were analyzed using standard procedures and compared. Proximate analysis revealed significantly (P<0.05) higher levels of carbohydrates, fats and moisture in the seeds and protein, fibre and ash in the leaves. Alkaloid concentration in the deconized water leaf (DWL), deionised water seed (DWS), ethylacetate leaf (EAL) and ethylacetate seed (EAS) extracts was not significantly (P<0.05) different. Cardiac glycosides, tannins, flavonoids and saponins were significantly (P<0.05) different in the extracts with phenols and flavonoids being significantly (P<0.05) higher in the DWL and EAL extracts. The minerals phosphorous, potassium, magnesium, zinc, copper, manganese, sodium, iron and calcium and the vitamins, ascorbic acid, tocopherol, phylloquinone, thiamin, riboflavin, niacin, pyridoxine folic acid and retinol were found in all the extracts at significantly (P<0.05) different concentrations. The leaf extracts had significantly (P<0.05) higher concentration of iron, copper, zinc, sodium, ascorbic acid (Vit C) pyridoxine (Vit B6) Macin (VitB3) and riboflavin (Vit B2) than the seed extracts.

Keywords: *Persea americana*, aqueous and ethyl acetate extracts, chemical composition

INTRODUCTION

*Persea americana* (avocado) is a tree classified as a member of the flowering plant family *Lauraceae*. The wild progenitor of the cultivated avocado comprise three botanical races viz the West Indian; Mexican and Guatemalan race [1]; [2]; [3]. Although it originated from Mexico, it is now cultivated in the United States of America, Asia, parts of Europe and tropical Africa [4]. It has been reported by various authors that the seeds and leaves of the plant are used by traditional health practitioners for treating and managing various ailments in man [5]; [6]; [7]; [8]; [9]; [10]; [11]; [12]. The apparent benefits derived from these plant parts maybe due to the presence of some bioactive compounds in them. These bioactive compounds include nutrients like vitamins and minerals which elicit medicinal and pharmacologic actions in medicinal plants [13]. This work compared the chemical composition of the leaf and seed extracts of *Persea americana* with the view of ascertaining the part likely to be more beneficial based on the chemical composition.

MATERIALS AND METHODS

**Chemicals, Reagents and Equipments**

All chemicals and reagents use for this work were of analytical grade and products of Radox USA, May and Baker England and Marck Germany. All equipment were in good working condition.

**Sample Preparation**

The *Persea americana* seeds were grated and air dried. The leaves were also air dried. The dried samples were separately
ground into fine powder and stored in air-tight receptacles.

**Preparation of Deionised Water and Ethylacetate Extracts**

Five hundred grams each of seed powder were soaked in 1000ml ethylacetate and 1250ml deionized water respectively. The same quantity (500g) of leaf powder was also soaked in 1400ml of ethylacetate and 1750ml deionized water respectively. They were allowed to soak for 48 hours with intermittent shaking. The suspensions were filtered using muslin cloth and concentrated using rotary evaporator. The extracts were then stored in air-tight receptacles.

**Proximate Analysis**

**Moisture Content Determination by AOAC(1990) Method.**

The ground sample of *Persea americana* leaf and seed (2.0g each) were weighed into dishes and dried in an oven at 105°C. They were weighed at 30min interval till the samples attained constant weights. The moisture content was then calculated using the following relationship:

\[
\% \text{ moisture} = \frac{w_1 - w_2}{w_3} \times 100
\]

Where
- \( w_1 \) = weight of sample and dish before drying
- \( w_2 \) = weight of sample and dish after drying
- \( w_3 \) = weight of sample

Samples (2.0g each) were separately weighed into platinum crucibles. The crucibles were placed into a muffle furnace and heated at 600°C until whitish – gray ash were obtained. The dishes were allowed to cool in a desiccator after which they were weighed. The ash content was calculated using the following relationship.

\[
\% \text{ Ash} = \frac{\text{weight of ash}}{\text{Weight of sample}} \times 100
\]

**Fat Content Determination by AOAC (1990) Method.**

Petroleum ether (150ml) was introduced into a soxhlet extractor flask. Sample (2.0g) was weighed and placed in the thimble of the extractor. The apparatus was assembled and the sample extracted for eight hours (8hrs). The solvent was recovered using a rotary evaporator and the fat content was calculated using the following relationship.

\[
\% \text{ Fat} = \frac{w_2 - w_1}{w_3} \times 100
\]

Where
- \( w_1 \) = weight of flask and extract
- \( w_2 \) = weight of flask
- \( w_3 \) = weight of sample

**Crude Fibre Determination by AOAC (1990) Method.**

Sample (2.0g) was de-fatted by extracting with petroleum ether. The defatted sample was air-dried and transferred into a conical flask. Precisely 200ml 0.25N H₂SO₄ was added and boiled gently for 30 min. Thereafter, the solution was filtered and the residue washed with boiling water to remove acid. The residue was returned to the flask and 200ml 0.3N NaOH was added and boiled for 30min. The solution was filtered under suction and washed thoroughly with boiling water. The insoluble matter was dried to a constant weight at 100°C and thereafter incinerated in a muffle furnace at 600°C. The crude fiber was calculated using the following relationship.

\[
\% \text{ Crude fat} = \frac{w_1 - w_2}{w_3} \times 100
\]

Where
- \( w_1 \) = dry weight of sample before ashing
- \( w_2 \) = weight of residue after ashing
- \( w_3 \) = Weight of sample

**Protein Content Determination (Kjeldahl method) by AOAC (1990) Method.**

Sample (1.0g) was weighed and transferred to a kjeldahl digestion flask followed by 8.0g of catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) and 20.0ml concentrated sulphuric acid. The flask was gently heated in an inclined position until the initial frothing stopped. A loose stopper was used to cover the flask and heated strongly to boil for one hour after the liquid had become clear. The digest was cooled and 400ml ammonia-free water was used to wash it into a distillation flask, followed by a large piece of granulated zinc as anti-bump. To the receiving flask, 50.0ml 2% boric acid solution was added followed by
screened methyl red indicator (0.016% methyl red and 0.083% bromocresol green in alcohol). The diluted digest was made alkaline by the addition of 75ml 50% sodium hydroxide solution. The distillation apparatus was set up with the delivery tube dipping below the boric acid solution. After collecting 300ml of the distillate, it was titrated with 0.05M sulphuric acid. The percentage nitrogen and protein were calculated using the relationship.

\[ \%N = \frac{(V_s - V_b) \times F \times C \times M(N)}{m \times 1000} \]
\[ \% \text{Protein} = \%N \times \text{PF} \]

Where \( V_s \) = titre of sample, \( V_b \) = titre of blank

\[ F = \text{molar reaction factor (H}_2\text{SO}_4 = 2) \]
\[ C = \text{concentration of titrant (mol/L}, \text{PF} = 6.25 \]
\[ 1000 = \text{conversion factor (ml in L)} \]
\[ M(N) = \text{molecular weight of nitrogen (14.0007g/mol)} \]

Carbohydrate Content Determination by the method of Banigo and Akpapuna (1999)

This was determined by difference as follows,

\[ \% \text{Carbohydrate} = 100 - \% (\text{Ash + moisture} + \text{protein} + \text{fibre}) \]

Phytochemical Analysis of the Deionised Water and Ethylacetate Extracts of Persea americana

Tannins Determination by the Method of Van-Buren and Robinson (1981)

Sample extract (0.5g) was weighed into a 100ml bottle followed by 50ml distilled water. It was then shaken for 1hour using a mechanical shaker. Thereafter, the solution was filtered into a 50ml volumetric flask and made up to mark. The filtrate (5.0ml) was pipetted into a test tube followed by 2.0ml 0.1MFeCl\(_3\) in 0.1NHCl and 0.008M potassium ferrocyanide. The absorbence was measured at 120nm within 10min and tannins concentration calculated using the following formula.

\[ \text{Tannins (mg/100ml)} = \frac{\text{Absorbance of sample} - \text{Absorbance of blank}}{\text{Absorbance of standard} - \text{Absorbance of blank}} \times \frac{\text{Concentration of standard}}{\text{Standard}} \]

Saponin determination by the Method of Obadoni and Ochuko (2001) with slight Modifications.

The extract (5.0g) was weighed into a conical flask followed by 25ml of 20% aqueous ethanol. It was heated in a water bath at 55°C for 4hours with continuous stirring. Thereafter, the solution was filtered and the residue re-extracted with 25ml 20% ethanol. The pooled extracts were reduced to 20ml in a water bath at 90°C. The concentrated extract was transferred into a 250ml separatory funnel and 10ml diethyl ether was added and shaken vigorously. The aqueous layer was recovered and the process repeated. 30ml of n-butanol was added to the recovered solution and washed twice with 5ml 5% aqueous sodium chloride. The resulting solution was heated in water bath to evaporate. After evaporation, the extract was dried to constant weight in an oven. Saponin content was then calculated.

\[ \% \text{Saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100 \]
**Flavonoid Content Determination by using the Method of Park et al. (2008).**

Into a 10ml test tube, 0.03ml extract was added followed by 3.4ml 30% methanol, 0.15ml 0.5M NaNO$_2$ and 0.15ml 0.3M AlCl$_3$$\cdot$$6$H$_2$O and mixed. After 5min, 1.0ml 1M NaOH was added. The solution was mixed properly and the absorbance measured against the reagent blank at 506nm. The standard curve for flavonoid was made using rutin standard solution (0 to 100mg/L) using the same procedure. Total flavonoid was expressed as milligram of rutin equivalent per g of dried fraction [14].

**Phenolic Content Determination by the Method of Kim et al. (2003).**

Sample extract (1.0ml) was pipetted into a test tube followed by 1.0ml Folin-Ciocalteu’s phenol reagent and allowed to stand for 5min. Thereafter, 10.0ml 7% Na$_2$CO$_3$ solution was added followed by 13.0ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90min at 23°C after which the absorbance was read at 750nm. Total phenolic content was determined by extrapolation from a standard curve prepared with gallic acid. The total phenolic content was expressed as mg of gallic acid equivalent (GAE) per g of dried sample [15].

**Cardiac Glycoside determination by the Method Described by Tofighi et al. (2016)**

Samples (10.0ml) were dispensed into separate flasks followed by 10.0ml each of Baljet’s reagent (95ml 1% picric acid + 5ml 10% NaOH), mixed and allowed to stand for 1hour. Thereafter, the mixtures were diluted with 20ml each of distilled water [16]. Their absorbance were measured at 495nm against a reagent blank. The glycoside concentration was read from a standard curve of securidaside (12.5-100mg/l).

**Steroid Estimation by Method of Madhu et al. (2016)**

Sample extract solution (1.0ml) was measured and introduced into a 10ml volumetric flask. This was followed by 2ml 4N sulphuric acid and 2ml 0.5% w/v ferric chloride. Thereafter, 0.5ml 0.5% w/v potassium ferricyanide was added. The mixture was heated in a water bath at 70°C for 30 minutes with intermittent shaking and diluted to the mark with distilled water. The absorbance was measured at 780nm against the reagent blank [17].

**Mineral Analysis**

Mineral content of the extracts were determined using atomic absorption spectrophotometer. Deionized water (250ml) each was dispensed into five dry beakers. Different volumes of stock solution of calcium (0.0, 0.5, 1.0, 1.5 and 2.0) were pipetted into the beakers respectively. The AAS was set up as prescribed by the operating manual and the various standards and samples were separately aspirated into the flame and readings taken. This was repeated for all the minerals assessed using the appropriate hollow cathode tube. The concentration of the minerals were extrapolated from the standard reading.

**Vitamin Analysis**

**Determination of Vitamin A by the method of Rutkowski and Grzegorczyzyk, (2007)**

Sample (1.0ml) was introduced into a test tube with stopper followed by 1.0ml 1M KOH in 90% ethanol. The mixture was vigorously shaken for 1min. The tube was then heated in a water bath at 60°C for 20min, cooled in cold water and 1.0ml xylene added [18]. The tube was again shaken vigorously for 1min. Thereafter, the solution was centrifuged at 1500g for 10min and the upper layer collected. The absorbance ($A_1$) of the extract obtained was measured at 335nm against xylene. The extract in the second test tube was irradiated with uv light for 30min after which the absorbance was measured ($A_2$). The concentration of vitamin A ($C_x$) in $\mu$M was calculated using the formula.

$$C_x = (A_1 - A_2) \times 22.23$$

Where

22.23 - is multiplier received on the basis of absorption coefficient of 1% solution of vitamin A (as retinol) in xylene at 335nm in a cuvette of 1cm thickness.
Determination of Vitamin E the method of Rutkowski and Grzegorcyzyk (2007)
Sample (0.5ml) was pipetted into a centrifuge tube (1) with stopper, followed by 0.5ml anhydrous ethanol and shaken vigorously for 1min. Xylene (3.0ml) was added and again shaken vigorously for another 1min and centrifuged at 1500g for 10min. Simultaneously, 0.25ml solution of bathophenanthroline was measured into a second centrifuge tube (II). 1.5ml of the extract (upper layer of tube 1) was transferred to tube II and mixed. 0.25ml FeCl$_3$ solution was added followed by 0.25ml H$_3$PO$_4$ solution and mixed. Standard $\alpha$-tocopherol was prepared with 0.5ml deinonized water and carried through the same process [19]. The absorbance of test Ax and standard As were measured at 539nm against a reagent blank. Vitamin E (µM) was calculated using the following relationship.

$$C_x = \frac{Ax}{As} \times Cs$$

Cx = Concentration of Vitamin E.
Cs = concentration of standard
Ax = Absorbance of extract
As = Absorbance of standard.

Determination of Vitamin C by the method of Rutkowski and Grzegorcyzyk (2007)
Sample (1.0ml) was measured into a centrifuge tube followed by 1.0ml phosphotungstate reagent mixed thoroughly and allowed to stand at room temperature for 30min.. Thereafter the tube was centrifuged at 700g for 10min and the supernatant collected [20]. Standard vitamin C solution (56.8µM L-ascorbic acid in 50M oxalic acid) was prepared and treated as above without centrifugation. The absorbance of both test and standard were measured at 700nm against a phosphotungstate reagent and 50M oxalic acid (1,1) blank. Vitamin C was calculated using the relationship

$$C_x = \frac{Ax}{As} \times Cs$$

Determination of Vitamin B$_1$ (Thiamine) by modified method of AOAC (2005)
Sample (1.0g) was dissolved in 50ml alcoholic sodium hydroxide solution and filtered into a 100ml flask. The filtrate (10ml) was pipetted into a beaker and 10ml potassium dichromate added for colour development [21]. The absorbance was read against a reagent blank at 560nm. Thiamine concentration was calculated using the formular.

$$\text{Vit B}_1 (mg) = \frac{\text{Abs} \times \text{Df} \times \text{vol of cuvette}}{E}$$

Determination of Vitamin B$_2$ (Riboflavin) modified method of AOAC (2005)
Sample (2.5g) was extracted with 50ml 50% hydrogen peroxide and allowed to stand for 30min; after which 1ml 40% sodium sulphate was added and made up to 25ml. The absorbance was taken at 510nm and vitamin B$_2$ concentration calculated using the formular

$$\text{Vitamin B}_2 (mg) = \frac{\text{Abs} \times \text{Df} \times \text{volume of cuvette}}{E}$$

Where Abs = absorbance, Df = dilution factor, E = extinction coefficient.

Determination of Vitamin B$_3$ (Niacin) modified method of AOAC (2005)
Sample (2.5g) was introduced into 50ml sulphuric acid in a flask and shaken for 30 minutes. This was followed by the addition of 3 drops of ammonia solution after which the mixture was filtered. Filtrate (10ml) was introduced into a flask and 5.0ml potassium cyanide was added. The mixture was acidified with 0.02M H$_2$SO$_4$. The absorbance was read at 470nm and vitamin B$_3$ concentration calculated using the following formular.
Vitamin B\textsubscript{3} (mg) = \frac{\text{Abs x Df x volume of curette}}{E}

### Determination of Vitamin B\textsubscript{6} (Pyridoxine) modified method of AOAC (2005)

Sample (1.0g) was extracted with 500ml distilled water for one hour and filtered. Into 1.0ml of the filtrate in a test tube was added 2ml distilled water, 0.4ml 50% sodium acetate, 0.1ml diazotized reagent and 0.2ml 5.5% sodium carbonate and mixed thoroughly. The absorbance of the solution was read at 540nm. Vitamin B\textsubscript{6} concentration was calculated using the following relationship.

Vitamin B\textsubscript{6} (mg) = \frac{\text{Abs x Df x Volume of curette}}{E}

### Determination of Vitamin B\textsubscript{9} (Folic acid) by modified method of AOAC (2005)

Sample (1.0g) was introduced into a beaker followed by 100ml of distilled water and heated slightly. The mixture was shaken thoroughly and filtered after cooling. The absorbance was read at 325nm and vitamin B\textsubscript{9} concentration calculated thus -

Vitamin B\textsubscript{9} (mg) = \frac{\text{Abs x Df x Volume of curette}}{E}

### Determination of Vitamin K

Sample (1.0g) was dissolved in 10ml distilled water and filtered. To 1.0ml of filtrate 2ml of distilled water was added followed by 1.5ml 0.04% hypochloric acid. The mixture was heated in boiling water for 45 minutes and cooled. It was then diluted with 10ml 1.3 ammonium hydroxide and the absorbance measured at 635nm. Vitamin K concentration was calculated using the following relationship.

Vitamin K (mg) = \frac{\text{Abs x Df x Volume of curette}}{E}

### STATISTICAL ANALYSIS

Data generated were expressed as mean ± standard deviation. The different among groups were determined using SPSS for windows software programme (version 20). Group comparison were done employing analysis of variance (ANOVA) test P-values less than 0.05 (P<0.05) were regarded as significant.

### RESULTS

The concentration of saponins was significantly (P<0.05) different in all the extracts. Ethylacetate seed extract (EAS) had a significantly (P<0.05) higher concentration of saponins followed by aqueous seed extract (DWS). The concentration of cardiac glycosides in the DWL and EAS extracts was not significantly (P>0.05) different, while the concentration in DWS and EAL extracts were significantly (P<0.05) different from the other extracts [22]. Tannins concentration in the DWS, EAL and EAS extracts were significantly (P<0.05) different while DWL and DWS extracts were not significantly (P>0.05) different. EAL extract had a significantly (P<0.05) higher concentration of tannins than the other extracts. The concentration of
phenols in the DWL extract was significantly (P<0.05) higher than the concentration in the other extracts. The DWS and EAS extracts had phenol levels that were not significantly (P>0.05) different. The concentration of flavonoids was significantly (P<0.05) different in all the extracts with the DWL extract being significantly (P<0.05) higher followed by EAL extract. Steroids concentration was significantly (P<0.05) higher in the EAL extract when compared with the other extracts. (Figure 2)

Mineral Content of the Aqueous (DW) and Ethylacetate (EA) Seed and Leaf Extracts of Persea americana
The results showed that all the extracts had the minerals phosphorus (P), potassium (K), magnesium (Mg), zinc (Zn), copper (Cu), Manganese (Mn), iron (Fe), sodium (Na) and calcium (Ca). The concentrations of P, K, Mg, Cu, Mn, Fe and Na were significantly (P<0.05) higher in the DWL extract than the other extracts. The concentrations of P and Mn in DWS and EAS extracts were not significantly (P>0.05) different while the concentrations of K in all the extracts were significantly (P<0.05) different. Mg concentration in all the extracts was also significantly (P<0.05) different [23]. The concentrations of Zn, Fe and Na were not significantly (P>0.05) different in the DWS and EAS extracts. (Table 1).

Vitamin Contents of the Aqueous (DW) and Ethylacetate (EA) Leaf and Seed Extracts of Persea americana
All the extracts had varied concentrations of Vitamin C (ascorbic acid), E (tocopherols), B1 (riboflavin), B3 (niacin), B6 (Pyridoxine), B1 (Thiamin), B9 (folic acid), A (retinol), and K (Phylloquinone). All the extracts had significantly different (P<0.05) levels of vitamin C and A while the concentration of vitamins E, B2, B3, B6, and K in DWS and EAL were not significantly (P>0.05) different. DWL extract had a significantly (P<0.05) higher concentration of vitamin C, B2, B3, B6, than all the other extracts. The EAS extract had a significantly (P<0.05) higher concentration of vitamin E than the other extracts. (Table 2).
Figure 1, Proximate composition of *Persea americana* (avocado) leaves and seeds.

The data are presented as mean ± SD. Bars in a group with the same letter are not significantly different (P<0.05).

Figure 2, Phytochemical Content (mg/100g) of aqueous (DW) and ethylacetate (EA) leaf and seed extracts of *Persea americana*.

DWL = aqueous leaf and DWS = aqueous seed extracts. EAL = ethylacetate leaf and EAS = ethylacetate seed extracts. The figures are presented as mean ± SD. Bars with the same letter are not significantly different (P<0.05).
Proximate analysis of the leaves and seeds of *Persea americana* revealed the presence of the nutrients, protein, fat, moisture, fibre, ash and carbohydrate in both plant parts. However, the seeds had significantly (P<0.05) higher concentration of fats, moisture and carbohydrate while the leaves were significantly (P<0.05) higher in ash, fibre and protein (figure 1). The presence of fibre and ash in both plant parts may be a contributory factor to their beneficial use in treating some ailments such as hypertension. The effect of dietary fibre on hypertension relates to its action on cholesterol and...
LDL-cholesterol. Studies show that consumption of dietary fibre decreases serum total cholesterol and LDL-Cholesterol levels [24]. The presence of the photochemical alkaloids, saponins, cardiac, glycosides, tannins, phenols, flavonoids and steroids in all the seed and leaf extracts of *Persea americana* again may in part contribute to their beneficial use in treating some diseases that are triggered by reactive oxygen species (ROS) generated by oxidative stress. Flavonoids and tannins are known to have antioxidant activity [25]; [26]; [27]. The mineral and vitamin analysis revealed the presence of all the minerals and vitamins in all the extracts with the leaf extracts being higher in most of the vitamins and minerals. This suggests that the leaf may be more beneficial than the seed for use in treating disease where they have been found to be useful.

REFERENCES


