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Comparative Assessment of the Proximate, Phytochemical and Free Radical Scavenging Activity of *Persea Americana* Seeds and Leaves

[1]Ude C.M, [2]Eze Steven P.E. and [2]Ogbuanu C.C.

[1]Department of Industrial Chemistry Enugu State University of Science and Technology, Enugu, Nigeria.
[2]Department of Applied Biochemistry Enugu State University of Science and Technology, Enugu, Nigeria.

ABSTRACT

A comparative study of the proximate, phytochemical and free radical scavenging capacity of the leaves and seeds of Persea americana was undertaken to determine which of the plant part was more active. Proximate analysis showed that the leaf was significantly (P<0.05) richer in protein, fibre and ash while the seed had significantly (P<0.05) higher content of carbohydrate, fat and moisture. Phytochemical analysis showed that the seed had significantly (P<0.05) higher levels of saponins while the leaf had significantly (P<0.05) higher content of cardiac glycosides, phenol, flavonoids and steroid. Alkaloid and tannins level were not significantly (P>0.05) different in the seed and leaf. The free radical scavenging activity of the leaf was higher than that of the seed. The higher content of phenols, flavonoids, fibre and ash in the leaf may be responsible for its higher radical scavenging activity thus making it more beneficial than the seed.

Keyword: Phytochemicals, Proximate analysis, radical scavenging, P.americana.

INTRODUCTION

Cells of living organisms are unceasingly exposed to numerous challenges that leads to oxidative stress. Oxidative stress manifests when the biological system is increasingly exposed to oxidents, reduction in the antioxidant capacity of the system or both. It is usually linked or results in the production of reactive oxygen species (ROS) and the inability of the biological system to neutralize or fix the damage produced. Reactive oxygen species are generated endogenously from the mitochondrial electron transport chain, respiratory burst by phagocytes, beta oxidation of fat in peroxisome, auto-oxidation of amino acids, catecholamines, haemoglobin and ischaemia reperfusion injury [1] and exogenously from electromagnetic and cosmic radiation, cigarette smoke, car exhaust, ozone (O_3) and air pollutants [2]; [3]. ROS are connected to the development of many diseases including cancer, arteriosclerosis, hypertension, diabetes and cirrhosis [4]. Oxygen consuming organisms are equipped with enzymic and non-enzymic antioxidant systems which effectively protect against the harmful effects of ROS. However in pathological conditions, the system can be compromised [5]. Thus, antioxidant containing supplements may be required to help ameliorate the effect of ROS. Moreover, the linkage of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) used as preservatives in food, cosmetic and pharmaceutical industries to liver damage and cancer [6] calls for their replacement with natural products that are safe. Plant parts containing antioxidants can be beneficial in containing the effect of oxidative damage in humans and in industries.

Persea Americana (avocado) belongs to the flowering plant family lauraceae. Although it originated from mexico, it is now distributed worldwide [7], [8]. The fruit is widely consumed for its nutritional and medicinal benefit [9], but the other parts are not edible.

However, the seeds and leaves have been reported to be used in herbal medicine for the treatment of a number of diseases which include hypertention, convulsion, diarrhea and diabetes [10], [11]. [12] [13] in their separate studies, showed that the leaf and seed extracts of *P americana* were hypoglycemic. [14] reported the chemical composition of the leaf, fruit and seed of *Persea americana*. Due to the wide spread use of the leaf and seed of *Persea americana* in traditional medicine it is important to ascertain the plant chemical and determine which of the plant part is more beneficial. This study investigated and compared the proximate, phytochemical and radical scavenging activity of the seed and leaf of *P americana*.

MATERIALS AND METHODS

Chemicals

The DPPH (2,2-diphenyl-1-picrylhydrazil), BHA (Butylated hyroxy anisole and other chemicals used were purchased from Sigma-Aldrich, Germany and were of analytical grade.

Sample Preparation

Fresh *Persea americana* fruits and leaves were harvested from the tree in a local farm in Umuaga, Udi Local Governemnt Area, Enugu State Nigeria. The fruits were left to ripen naturally at room temperature after which the seed was separated, grated and air dried with the leaves. The dried seeds and leaves were separately pulverized using a manual grinder. The powdered samples were stored in air tight containers.

Determination of Proximate Composition

Proximate composition was determined using the AOAC (1990) methods

Moisture Content Determination

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

$$\% \text{ Moisture} = \frac{\text{weight of sample before drying} - \text{weight of sample after drying}}{\text{Weight of Sample before drying}} \times 100$$

Ash Content Determination

Samples (2.0g each) were separately weighed into platinum crucibles and placed in a muffle furnace and heated at 600°C until whitish-grey ashes were obtained. The dishes were cooled in a desiccators and weighed. Ash content was calculated using the formula

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of Sample}} \times 100$$

Fat Content Determination

Sample (2.0g) was extracted with 150ml petroleum either using a soxhlet extractor for 8 hours. The solvent was recovered using a rotary evaporator and the fat content calculated using the relationship.

$$\% \text{ Fat} = \frac{\text{Weight of Extract}}{\text{Weight of Sample}} \times 100$$

Crude Fibre Determination

Sample (2.0g) was defatted with petroleum either and air-dried. Into a conical flask was transferred the dried defatted sample followed by 200ml 0.25NH_2SO_4 and boiled for 30 min. The solution was filtered and the residue washed with boiling water to remove acid. The residue was returned to the flask and boiled for 30 min with 200ml 0.3N NaOH. The solution was filtered, washed with boiling water and the insoluble matter dried to constant weight at 100°C and then incinerated in a muffle furnace at 600°C. Crude fibre was calculated using the relationship.

$$\% \text{ Crude fibre} = \frac{\text{Dry weight of residue before ashing} - \text{weight of residue after ashing}}{\text{Weight of sample}} \times 100$$

Protein Content Determination

Sample (1.0g) was digested with 8.0g of catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) and 200ml concentrated sulphuric acid in a Kjeldahl flask. The digest was cooled and 400ml ammonia-free water was used to wash it into a distillationflask followed by the addition of granulated

zinc as anti-bump.To the receiving flask was added 50.0ml 2% boric acid solution and 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 and distilled into the boric acid solution. 300ml of the distillate was collected and titrated with 0.05M sulphuric acid. Percentage nitrogen and protein were calculated using the relationships.

$$\% \text{ Nitrogen} = \frac{(Vs-Vb) \times F \times C \times M(N)}{m \times 1000}$$

Where Vs = titre of sample, Vb = litre of blank
F = Molar reaction-factor (H_2SO_4 = 2),
m = weight of sample
C = Concentration of titrant (mol/L)
M(N) = Molecular weight of nitrogen (14.0007g/mol)
% Protein = % Nitrogen x PF
were PF = 6.25

Carbohydrate content determination as described by Banigo and Akpapuna (1999).

Carbohydrate was determined by difference as follows:
% Carbohydrate = 100 - % (Ash + Moisture + Protein + Fibre)

Determination of Phytochemical Composition

Alkaloid was determined by the method of Harborne (1973). Sample (2.5g) was introduced into 250ml beaker followed by 100ml 10% acetic acid in ethanol, covered and allowed to stand for four hours. It was filtered and the filtrate reduced to 50ml by heating in a water bath. Concentrated ammonium hydroxide was added drop wise until precipitation was completed. The solution was allowed to settle after which the precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue (alkaloid) was dried weighed and calculated.

Tannins was determined by the method of Van-Buren and Robinson (1981). Sample (0.5g) was introduced into a 100ml bottle and 50ml distilled water added. It was shaken for one hour using a mechemical shaker and filtered into a 50ml volumetric flask and made up to mark.Into a test tube was added 5.0ml of the filtrate followed by 2.0ml 0.1M $FeCl_3$ in 0.1N HCl and 0.0008M potassium ferrocyanide. The absorbance was measured of 120mm within 10min and tannins calculated as follows:

$$\text{Tannins (mg/100g)} = \frac{\text{Absorbance of sample} - \text{Absorbance of black}}{\text{Absorbance of Standard} - \text{Absorbance of black}} \times \text{Concentration of standard}$$

Flavonoid concentration was determined by the method of park *et al.* (2008). Sample extract (0.03ml) was added into a 10ml test tube followed by 3.4ml 30% methanol, 0.15ml 0.5M $NaNO_2$ and 0.15ml 0.3M $AlCl_3$. $6H_2O$ and mixed. After 8min, 1.0ml 1M NaOH was added and mixed properly. The absorbance was read against the reagent blank at 506mm. Flavonoid content was read from a standard curve prepared using rutin standard solution (0-100mg/l). Total flavonoid was expressed as milligram fraction equivalent per g of dried fraction.

Phenolic content was determined by the method of kim *et al* (2003). Sample extract (1.0ml) was pipetted into a test tube followed by 1.0ml folin-Ciocalteus phenol reagent and allowed to stand for 50min. Thereafter, 10.0ml 7% Na_2CO_2 solution was added followed by 13.0ml distilled water and mixed thoroughly. The mixture was kept in the dark for 90min at 23^{0}C after which the absorbance was read at 750nm. Total phenolic content was determined by extrapolating from a standard curve prepared with gallic acid. The phenolic content was expressed as mg of gallic acid equivalent per gram of dried sample.

Saponin determination by the method of Obadoni and Ochuko (2001). Sample (5.0g) was weighed into a conical flask followed by 25ml 20% aqueous ethanol. The mixture was heated for few hours in a water bath at 55^{0}C. The solution was filtered and the residue re-extracted with 25ml 20% ethanol and the pooled extract reduced to 20ml in a water bath at 90^{0}C. The concentrated extract was

transferred into a 250ml separating funnel and 10ml diethyl ether added and shaken vigorously. The aqueous layer was recovered and the process repeated. Into the recovered solution was added 30ml n-butanol and washed twice with 5ml 5% aqueous sodium chloride. The solution was heated in a water bath to evaporate and the extract dried to constant weight in an oven.Saponin content was then calculated.

Cardiac glycoside determination by the method of Tofighi *et al* (2016). Sample solution (10.0ml) was dispensed into a flask and 10.0ml Baljet's reagent (95ml 1% picric acid + 5ml 10% NaOH) was added, mixed and allowed to stand for one hour. The solution was diluted with 20ml distilled water and the absorbance read at 495nm against a reagent blank. The glycoside content was read from a standard curve of securidaside.

Steroid determination by the method of Madhu *et al* (2016).Into sample solution (1.0ml) in a 10ml volumetric flask was added 2.0ml 4N sulphuric acid, 20ml 0.5% ferric chloride and 0.5ml 0.5% potassium ferricyanide. The mixture was heated in a water bath at 70^{0}C for 30 minutes with intermittent shaking. The solution was diluted to the 10ml mark and absorbance taken at 780nm against the reagent blank.

Determination of DPPH radical scavenging activity. The DPPH scavenging activity of the samples were determined by the method of Ebrahimzadah *et al* (2009) and Gihasemi *et al* (2009). Different concentrations of *P americana* leaf and seed extracts and BHA (Standard) (0,20,40,60,80,100 mg/ml) were prepared. 1.0ml each of the extracts and standard were introduced into appropriate test tubes and 1.0ml of DPPH (100µm in methanol) added to each tube. The mixture was shaken and kept in the dark for 30mins. The discoloration of the purple colour was measured at 517nm using a spectrophotometer against a methanol blank and DPPH in methanol as positive control. The percentage DPPH scavenging activity (RSA) was calculated using the following formula

$$\% \text{ RSA} = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100$$

Where A_{DPPH} = Absorbance of DPPH solution

A_S = Absorbance of test solution

Statistical analysis

The results of the phytochemical and proximate analysis were expressed as mean ± standard deviation (SD). The experimental data were analyzed using analysis of variance (ANOVA) (Microsoft excel version 20). P-Values less than 0.05 (P<0.05) were regarded as significant.

RESULTS AND DISCUSSION

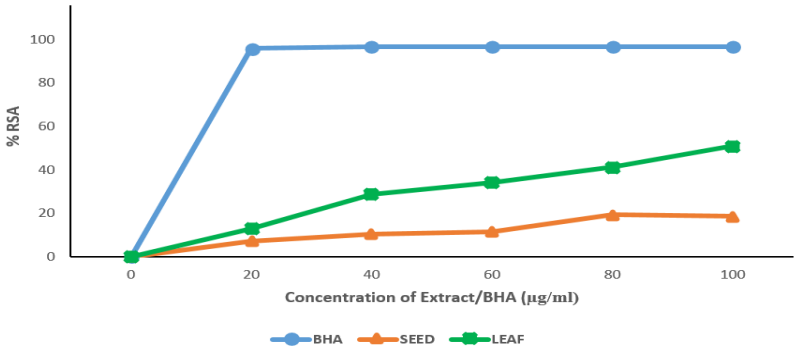

Figure 1. DPPH Scavenging activity of *Persea americana* seeds and leaves.

Table 1. Proximate Composition of *Persea Americana* Seed and Leaf

Component	Leaf	Seed (%)
Carbohydrate	4.16 ± 0.11 [a]	49.43 ± 2.79 [b]
Protein	29.40 ± 2.15 [a]	16.98 ± 2.03 [b]
Fat	3.21 ± 0.14 [a]	14.60 ± 1.26 [b]
Fibre	41.45 ± 1.69 [a]	5.03 ± 0.27 [b]
Ash	17.74 ± 2.43 [a]	6.04 ± 1.02 [b]
Moisture	4.46 ± 0.23 [a]	8.90 ± 0.50 [b]

Data are presented as mean ± SD, n=2. Rows with the same letter are not significantly different.

Table 2 Phytochemical Content (mg/100g) Of the Seed and Leaf Of *Persea Americana*

Phytochemical	Seed	Leaf
Alkaloid	3.87 ± 0.32 [a]	3.54 ± 0.41 [a]
Saponins	22.04 ± 1.82 [a]	5.72 ± 0.37 [a]
Cardiac glycoside	2.54 ± 0.15 [a]	3.68 ± 0.13 [b]
Tannins	3.11 ± 0.14 [a]	4.32 ± 0.20 [b]
Phenols	14.44 ± 2.05 [a]	19.18 ± 1.43 [b]
Flavonoids	4.05 ± 1.31 [a]	13.55 ± 0.71 [b]
Steroids	2.08 ± 0.12 [a]	4.88 ± 0.53 [b]

Data are presented as mean ± SD, n=2. Rows with the same letter are not significantly different.

The seeds and leaves of *Persea americana* are used in traditional medicine to treat a number of diseases which include hypertension and diabetes. This necessitates investigation of the most active of the plant parts and the possible reason for that. Figure 1 is the graph of DPPH radical scavenging activity of *P americana* seed and leaf with BHA as control. Both seed and leaf displayed DPPH scavenging activities that were lower than that of the control. The leaf extract displayed higher radical scavenging activity than the leaves that was dose dependent. The leaf thus appears to have a higher radical scavenging power. Proximate analysis (table 1) revealed the presence of protein, carbohydrate, ash, moisture, fibre and fat in both plant parts. However, the leaves had significantly ($P<0.05$) higher concentration of protein, fibre and ash then the seeds. The higher concentration of fibre in the leaves may partly be responsible for their higher radical scavenging activity. Dietary fibres are said to produce a protective effect by quenching or deleting free radical interchanging ion and counteracting the negative action of free radicals with antioxidant compounds associated with their polysaccharide matrix [15] [16] reported that 50% of total dietary antioxidant mainly polyphenolics are linked to dietary fibre. The higher ash content which is indicative of its higher mineral content may also account for its higher radical scavenging activity. Minerals such as selenium, copper, zinc, ion and manganese are associated with antioxidant systems [17], [18]. Phytochemical analysis revealed the presence of alkaloids, saponins, cardiac glycosides, tannins, phenols, flavonoids and steroids in the seeds and leaves of *P americana.* The leaves had significantly ($P<0.05$) higher concentration of cardiac glycosides, phenols and flavonoids (table 2). Flavonoids and other phenols function as antioxidants and some flavonoids such as luteolin and catechines are better antioxidants than nutrient antioxidants like vitamin C and E [19] [20]. The ability of flavonoids to function as antioxidant is derived from their structure. The position of hydroxyl groups and other features in their chemical structure favour their antioxidant and free radical scavenging activities [21] [22] [23].

CONCLUSION

This study has shown that *Persea americana* seed and leaf possess DPPH radical scavenging activity with the leaf having higher scavenging power. The higher free radical scavenging power of the leaf may be due to the presence of significantly ($P<0.05$) higher concentrations of antioxidant phytochemicals (Flavonoids, and Phenol), fibre and ash (minerals) in the leaf than in the seed.

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