



Phytochemical Constituents, Nutritional and Anti-Nutritional Composition of *Persea americana* Seeds

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ABSTRACT

Persea americana is a tropical plant commonly found in the South Eastern part of Nigeria. The fruit is mostly eaten as food. The fruits, leaves and seeds are used in folkloric medicine in the management of various ailments. However, there is paucity of information on the nutritional components and phytochemical constituents of the seeds. This study investigated the proximate composition, phytochemicals (qualitative and quantitative), Pro-vitamin A and vitamin C, minerals content, amino acid profile and anti-nutrients of *Persea americana* seeds. Phytochemical, nutritional, vitamin, mineral, amino acid analyses and anti-nutritive factor of *P. americana* seeds were carried out according to standard procedures. Qualitative phytochemical screening of *P. americana* seeds revealed the presence of saponin, cardiac glycosides, terpenoid, flavonoids, alkaloids and tannins. Furthermore, quantitative analysis of phytochemicals showed the presence of phenol (3.22mg/100g), alkaloids (1.86 mg/100g), saponins (0.95 mg/100g) and flavonoids (2.14 mg/100g). The proximate composition of *P. americana* seeds shows moisture (6.98%), ash (4.44%), fat (5.30%), crude fibre (3.31%), crude protein (11.20%) and carbohydrate (68.77%). The result for vitamin reveals the presence of vitamin C (24.1mg) and Pro-vitamin A (12.3IU). Also, mineral analysis showed the presence of sodium, iron, zinc, copper, manganese, calcium, chromium, potassium and lead in varying concentration. Amino acid analysis of the seed showed that the concentration of total amino acid was 54.53g/100g protein while, the total essential amino acid was 26.33g/100g protein. The total non-essential amino acid present in the seed was 28.20g/100g protein with a very high proline concentration (6.50g/100g). The anti-nutrient contents of the seeds revealed the presence of tannin, oxalate, saponins, alkaloids, cyanogenic glycosides and phytate. The results obtained from this study indicated that *P. americana* seeds is rich in nutrients and phytochemicals. This study therefore suggests that the seeds of *P. americana* may be developed in to supplements that may have beneficial effects on the nutrition and health of humans.

KEYWORDS: *Persea americana*, Phytochemicals, Nutrients and Anti-nutrients.

Introduction

Orthodox medicines have been found to cause severe side effects on patients when consumed. The toxic effect of most orthodox drugs ranges from allergy, ulcers to even death in some extreme cases. Hence, the need to seek for alternative drugs which are safe and have little or no side effects. From time immemorial, plants have been used in ethno-medicine and folkloric medicine in the management / treatment of various ailments and diseases. Several studies have shown that plant possess potent chemicals which are beneficial to human health. These phytochemicals are known to possess strong antioxidant, anti-inflammatory, anti-pyretic properties (Zulfiqar *et al.*, 2020). Hence, their use in the management of several disorders and diseases. Plants have also been used in food preservation and in food flavouring. Some studies have shown that phytochemicals from plants may be developed into nutraceuticals, food supplements and food additives with nutritional and health benefits for the management of various ailments and disease. Thus, mitigating side / toxic effects, resulting from the use of modern drugs (Cencic and Chingwaru, 2010).

Avocado fruit is highly nutritional. The fruit is rich in proteins, fats and oils and low in sugar (Maitera *et al.*, 2014). Avocados are well known plants in West Africa and have various applications in ethno-medicine (Pavela *et al.*, 2018). The fruits are edible. The bark, leaves, stem and roots are used as local medicine against diseases

(Jirovetz *et al.*, 2003; Annabelle *et al.*, 2004). The leaves have been reported to possess anti-inflammatory and analgesic properties (Adeyemi *et al.*, 2002). The seeds of *Persea americana* have diverse application in ethno-medicine, some studies have shown it's used in the treatment for diarrhoea, dysentery, toothache, intestinal parasites, skin treatment and also in beautification (Ejiofor *et al.*, 2018). Previous studies in Nigeria have shown that leaf extracts of *Persea americana* possess a catalogue of pharmacological activities, including anti-tussive (reduces cough), anti-convulsant, vasorelaxant, analgesic, anti-inflammatory, antidiabetic, hypoglycaemic, hypotensive and antihypertensive properties (Adeboye *et al.*, 1999; Adeyemi *et al.*, 2002; John and George, 2006; Gomez-Flores *et al.*, 2008). The oil obtained from the seeds has several health benefit e.g. for controlling human weight (Roger, 1999).

In Nigeria, *Persea americana* has been part of our diet, however, people ignorantly throw away the seeds after eating the fleshy part of the fruit as a result of scarcity of information on its nutritional and medicinal potentials. The knowledge of the Phytochemicals and nutritional composition of the seed will be important in assessing it's nutritional and therapeutic potential. Therefore, this study assessed the phytochemical constituents and nutritional composition of the seeds of *P. americana* for its dietary use and potential to be developed into nutraceuticals.

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Materials and methods

The fruits of *Persea americana* were purchased at the mature green stage from orange market in Mararaba, Nasarawa state, Nigeria. The fruits were allowed to ripen at room temperature within 3-4 days, to allow optimum processing quality. The seeds were identified and authenticated at the Department of Botany, Ahmadu Bello University, Zaria, Nigeria. Voucher specimen with herbarium number ABU00992 and was deposited at the University herbarium.

Sample Preparation

The fruits were washed with clean tap water and allowed to dry for 5 minutes. The inner seed of the fruits were removed by cutting with a clean knife and washed with water. The seeds were chopped into pieces and then air dried for two weeks. The dried seeds were pounded and blended to obtain a fine powder which was stored in an airtight plastic container till required for further use. Five hundred grams (500 g) of the powdered seed was extracted with 3000 ml of 80% ethanol for 72 h. The supernatant was carefully decanted and filtered using cheese cloth. The clear supernatant was thereafter freeze-dried to obtain a crude extract which was kept airtight in readiness for reconstitution.

Qualitative and Quantitative Phytochemicals Screening of seed extract

The extract of *Persea americana* seed was subjected to both qualitative and quantitative test to determine the presence and concentration of secondary metabolites in the seed. Qualitative phytochemicals screening of major constituents in the extracts was carried out using standard method as described by Sofowora (1993); Trease and Evans (2002). Quantitative phytochemical screening of saponins was determined by double extraction gravimetric method described by Herborne (1973). The tannins content was determined using methods described by Hagerman and Butler (1978). The Alkaloid content was determined by gravimetric method as described by Herbone (1973). The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method and Determination of total flavonoids content was based on aluminium chloride method (Shilpi and Sarita, 2018).

Proximate Analysis

Proximate analysis of *Persea americana* seeds was carried out using the standard method of (AOAC, 2005).

Results and Discussion

Table 1: Phytoconstituents of *Persea americana* seeds

Phytochemical	Ethanol extract
Saponins	+
Anthraquinone	-
Cardiac glycosides	+
Terpenoids	+
Steroids	-
Flavonoids	+
Alkaloids	+
Tannins	+

Key: + = Detected; - = Not detected

Carbohydrate content was determined by difference. (%Carbohydrate) = [100-(%Protein + %Moisture+ %Ash+% Fibre% + %Crude Lipid)] (Eyeson and Ankrah, 1975).

Mineral Content Analysis

The mineral content (Iron, Copper, Zinc, Manganese, lead and Chromium) of *Persea americana* seed was determined using computer control thermo fisher scientific ICE 3000 series Atomic Absorption Spectrometry (AAS). Briefly, 2g of the sample was weighed and digested using concentrated nitric acid and Hydrochloric acid in ratio of 1:3, 12 mL of Aqua regia until the sample dissolve completely and the final volume was made up to 100 cm³ with deionized water. The sample was then transferred into atomic absorption spectrometer (AAS) for elemental analysis. Sodium, calcium and potassium were analyzed using digital flame Photometer (Dipietro *et al.*, 1988).

Amino Acids Analysis

The amino acids present in *Persea americana* seed was quantitatively measured by the method described by Siddan *et al.* (2020), using Applied Biosystems PTH Amino Acid Analyzer.

Determination of Vitamins

Pro-vitamin A was determined according to the method of Stuetz *et al.* (2019) and Vitamin C content was determined according to the method of Barros *et al.* (2007).

Determination of Anti-nutrients

The total oxalic acid of the powdered samples was determined by the modified method of Hodgkinson (1970). Phytate content was estimated by the titration method of Sudarmadji and Markakis (1977). Tannin content was determined by the spectrophotometric method of Griffiths and Jones (1977). Saponin content was determined by the method of Makkar and Becker (1996) and alkaloid content was estimated by the distillation and tritrimetric method of Talabi *et al.* (2016). The phytate content of *Persea americana* seed was determined according to method described by Lucas and Markaka, (1975). The percentage phytic acid content was calculated using the method of Russel (1980) Maga (1982). Cyanogenic glycoside was determined using the method of Onwuka, (2005).

Table 2: Quantitative phytochemical composition of *Persea americana* seeds

Phytochemical	Concentration (mg/100g)
Phenol	3.22
Alkaloids	1.86
Saponins	0.95
Flavonoids	2.14

Table 3: Proximate composition of *Persea americana* seed

Parameters	Percentage Composition (%)
Moisture	6.98 ± 0.12
Crude fibre	3.31 ± 0.21
Fat	5.30 ± 0.21
Ash	4.44 ± 0.07
Crude protein	11.20 ± 0.55
Carbohydrate	68.78 ± 0.91

Results are expressed as means ± standard deviation (n = 2)

Table 4: Mineral composition of *Persea americana* seeds

Mineral	Amount (µg/g)
Chromium	0.0002
Lead	0.0002
Zinc	0.002
Calcium	0.003
Iron	0.005
Sodium	0.03
Potassium	0.60
Copper	0.0006
Manganese	0.001

Table 5: Vitamins A and C content of *Persea americana*

Vitamin	Concentration
Pro-vitamin A (IU) 100g	12.3
Vitamin C (mg/100g)	24.1

Table 6: Amino acid profile of *Persea americana* seeds

Amino acids	Concentration (g/100g)
Lysine	3.18
Histidine	1.60
Arginine	4.30
Aspartic acid	4.90
Threonine	2.47
Serine	3.08
Glutamic Acid	6.05
Proline	6.50
Glycine	3.06
Alanine	1.89
Cystine	0.66
Valine	3.40
Methionine	0.77
Leucine	4.43
Tyrosine	2.06
Phenylalanine	3.37
Isoleucine	2.16
Tryptophan	0.65

Table 7: Classification of Amino acid composition of *Persea americana* seeds

Classification	Result
TAA	54.53
TEAA	26.33
% TEAA	48.29
TNEAA	28.20
% TNEAA	51.71

TAA = Total amino acid, TEAA = Total essential amino acid, TNEAA = Total non-essential amino acid

Table 8: Anti-nutrients composition of *Persea americana* seeds

Anti-nutrients	Concentration (%)
Oxalate	0.29 ± 0.01
Saponins	3.90 ± 0.14
Alkaloids	0.63 ± 0.04
Tannins (mg / 100g)	18.48 ± 0.33
Cyanogenic glycoside (mg / 100g)	10.13 ± 0.46
Phytate	0.71 ± 0.02

Values are mean ± standard deviation (n=2).

Phytochemicals are important chemicals found in plants at different parts and at different concentrations. Table 1 and 2 revealed the presence of secondary metabolites such as Alkaloids, Tannins, Flavonoids, Terpenoids, Phenol, Saponins and Cardiac glycoside; while Steroids and Anthraquinones were not detected. These secondary metabolites have been reported to be of pharmacological importance.

Alkaloids are one of the largest groups of phytochemicals in plants with amazing effects on humans (Akinpelu and Onakoya, 2006). Alkaloids can act as anti-malaria, anticancer, antiasthma and antibacterial. Tannins noted for astringency and bitter taste, hasten the healing of wounds and inflamed mucus membrane (Okwu and Okwu, 2004). Flavonoids are potent water soluble antioxidants and free radical scavengers. They prevent oxidative cell damage and have strong anti-cancer activity and protect against all stages of carcinogenesis (Arukwe *et al.*, 2012). Cardiac glycosides have been adapted for the treatment of cognitive heart failure and cardiac arrhythmia. Tannins have been reported to hasten the healing of wounds and inflamed mucus membrane (Okwu and Okwu, 2004). Saponins have been reported to be widely used in pharmacological industry as adjuvant to enhance absorption of other drugs by increasing the solubility or by interfering in the mechanism of absorption (Ajayi *et al.*, 2017). Terpenoids are known to exhibit anti-inflammatory, anti-cancer and antimalaria activities (Mahato and Sen, 1997; Huang *et al.*, 2012). Phenols were highest in *Persea americana* seed and phenols have been extensively researched as disease preventives (Duke, 1992). Phenols detected in seeds of this study could further indicate their ability to act as anti-inflammatory, anti-clotting, anti-oxidants, immune enhancers.

The proximate composition of *Persea americana* seed presented in Table 3 shows that the crude protein value is 11.20 ± 0.55 slightly lower than the value as reported by (Ejiofor *et al.*, 2018), but was found to be quite higher than values reported by (Egbonu *et al.*, 2018). Aside contributing to diets, the relative impacts of proteins in the body cannot be over emphasized. As chemical compounds, they participate in the repair and replacement of worn out tissues and boost immune system (Olusanya, 2008). The values of the fat content of current study was higher than values reported by (Egbonu *et al.*, 2018) and is lower than values reported by (Arukwe *et al.*, 2012) and (Ejiofor *et al.*, 2018) for *Persea americana* seeds. Generally fats have many functions such as insulation and conservation of body temperature.

The ash content is 4.44 ± 0.07 which is higher compared to 2.40 ± 0.19 and 3.82 ± 0.10 reported by Arukwe *et al.*

(2012) and Egbonu *et al.* (2018) respectively. The ash content is a proxy index of mineral content. Minerals play numerous functions towards the improvement of health (Olusanya, 2008; Onwuka, 2005). The Moisture content reported in this study is 6.98 ± 0.12 which is lower than (9.92 ± 0.01) reported by Arukwe *et al.* (2012). This could indicate a comparatively higher storability (shelf life). The Crude fibre content of the *Persea americana* is higher compared with the value reported by Egbonu *et al.*, (2018). Fibre improves fecal bulk, appetite, satisfaction and motility through the digestive system and by improving the absorption and reabsorption of cholesterol and bile acids respectively could also lower cholesterol level and prevent the formation of plaque (Okwu and Emenike, 2007; Edem *et al.*, 2009). Mineral elements in plants become important when their health benefits are considered in the body of organism. Most of these minerals occur as chemical compounds in solution form hence, they are able to diffuse to different part of plant (Frossard *et al.*, 2000).

The result in Table 4 shows that Potassium is the most abundant micronutrient in the seeds of *Persea americana*. A high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risk. Calcium which forms component of bones and teeth is necessary for blood clotting and muscle contraction. Sodium is a mineral that the body needs to function properly. It is also one of the electrolyte minerals that conduct electricity when dissolved. It works closely with the other electrolyte minerals potassium and chloride. Iron is essential for metabolism, growth, immune function, preventing anemia and involved in oxygen binding to haemoglobin heme by playing a role in blood formation (Musallam and Taher, 2018).

Zinc is an essential element which functions as an integral part of numerous enzymes, as a stabilizer of molecular structure of sub-cellular constituents and membrane (Haase and Rink, 2009). Zinc participates in the synthesis and degradation of carbohydrates, proteins, lipids and nucleic acids and has been shown to play an essential role in poly nucleotide transcription and translation and thus in gene expression (Koide *et al.*, 2006). Manganese is another microelement essential for human nutrition. It acts as an activator for many enzymes (Prashanth *et al.*, 2015). Copper is necessary for synthesis of hemoglobin. Low level of Lead and Chromium when compared to the WHO safe limit for Chromium (0.003 ppm) and Lead (0.01 and 0.1 ppm) could be an indication that the investigated samples are free of toxic metals and this makes the seed safe from lead poisoning.

Vitamin A which apart from disease resistance delays ageing, presides over the health of the eye, skin, nails and hair (Adeolu and Enesi, 2013). Its deficiency can lead to visual impairment and increase susceptibility to infectious diseases. Vitamin C helps in health of gums, bones and joints and purifies in the blood (Kumar and Joshiba, 2019). It is one of many antioxidants that block some of the damaged caused by free radicals. Free radicals are made when the body breaks down foods or when exposed to tobacco smoke or radiation. Free radicals have been implicated in the pathogenesis of cancer, cardiovascular diseases and arthritis (Sen *et al.*, 2010).

The amino acid profile (g/100g protein) of *Persea americana* seeds revealed that Proline and Glumatic acid have the highest values. Leucine was the most concentrated essential amino acid (EAA) in the sample with 4.43g/100g. Both Histidine and Arginine are particularly essential for children (FAO/WHO/UNU, 1985). Methionine is needed for the synthesis of Choline which in turn forms lecithins and other phospholipids in the body. Phenylalanine is the precursors of some hormones and the pigment melanin in hair, eyes and tanned skin. Result showed that the sample contained all essential amino acids at moderate concentration. This indicates that the sample contained nutritional useful essential amino acids and is also an indication that the seeds could be useful as supplements when essential amino acids are required.

The classification of amino acids composition in the sample was shown in Table 7. The total amino acid (TAA) was 54.33g/100g which is lower to the value reported for canarium seeds (83.01g/100g) (Ayoade *et al.*, 2017). The total essential amino acids was (26.33g/100g protein) at 48.29% of total while total nonessential amino acids concentration was (28.20g/100g protein) at 51.71%. The percentage ratio of essential amino acid (EAA) to the total amino acid (TAA) in the sample is 48.29%. This value is well above the 39% considered adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985).

Anti-nutritional composition results as presented in Table 8 also shows presence of phytate, tannin, oxalate, saponins, alkaloids and cyanogenic glycosides. The tannin content is higher than the value reported (6.98±0.04) by (Ejiofor *et al.*, 2018). Tannin has been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals (Aroche *et al.*, 2018). Therefore, foods rich in tannins are considered to be of low nutritional value. Oxalate content of Avocado seed (0.29±0.01) is lower than the value (4.07±0.00) reported by (Justina *et al.*, 2016). Oxalate binds with calcium to form calcium-oxalate crystals which are deposited as urinary calcium (stones) that are associated with blockage of renal tubules (Blood and Radostits, 1989). The phytate content of avocado seed is higher than the value (3.18±0.16) reported by (Ejiofor *et al.*, 2018). Phytate forms stable complexes with Cu²⁺, Zn²⁺, Co²⁺, Mn²⁺, Fe²⁺ and Ca²⁺ (Demir *et al.*, 2017). Phytate and oxalate can bind micronutrients which form complexes that prevent the bioavailability of these micronutrients most especially the micronutrients of public health importance.

The knowledge of the cyanogenic glycosides content of food is vital because cyanide being an effective cytochrome oxidase inhibitor interferes with aerobic respiratory system (Umoh, 2013). Alkaloids are considered to be anti-nutrients because of their action on the nervous system, disrupting or inappropriately augmenting electrochemical transmission (Gemed and Ratta, 2014). Saponins levels in the seed were low and thus could not produce adverse effects on the growth of animals. Proper food processing would reduce these anti-nutrients. However, the anti-nutrient parameters tannin, phytate and oxalate reported in the present study were lower than the lethal doses (30 mg/kg, 50-60 mg/kg and 2-5mg/kg) as reported by (Inuwa *et al.*, 2011). The value of cyanogenic glycosides is lower than the lethal dose (50-60 mg/kg) as reported by (Bolhius, 1954). It can be concluded that the levels of oxalate, phytate, tannin and cyanogenic glycosides values of avocado seed are considered low and would be unsafe for animal consumption if properly processed as most of the values are below the lethal doses of these phytochemicals.

Conclusion

This study has provided information on the nutritional composition and the phytochemical constituents of *P. americana* seeds. The Phytochemical screening suggests that *P. americana* seeds contain bioactive components that can lead to the discovery of new therapeutic agents. The study further reveals that *P. americana* seeds contain substantial nutrients that could meet the needs and requirements of the body for protein, minerals and the general wellbeing. We therefore suggest that the seeds may be useful in the development of dietary supplements for the management and treatment of diseases associated with nutrient deficiencies.

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