Original Research Article

PROXIMATE, PHYTOCHEMICAL AND MINERAL COMPOSITION OF METHANOL EXTRACT *OF Persea americana* SEED

ABSTRACT

This study investigated the proximate composition and evaluated the phytochemicals present in methanol extract of *Persea americana* seed. The seed samples were collected, dried, ground into powder and extracted in methanol by cold maceration. Proximate analysis were carried out using the standard methods of association of official analytical chemists (AOAC) while quantitative phytochemical analysis was done using standard methods. Mineral composition assays were carried out using the standard methods of American public health association (APHA). Proximate analysis revealed that the seeds of *P. americana* contain more of carbohydrate (32.05 ± 1.21%), fat (25.73 ± 2.01%) and protein (21.00 ± 0.88%) with less amounts of moisture (8.98±0.10%), and ash (3.28±0.16%). Quantitative phytochemical analysis, revealed the presence of phenols (3.51±0.42mg/100g), saponins (0.05±0.38mg/100g), alkaloid (0.70±0.37mg/100g), beta-carotene (32.24±0.55mg/100g), lycopene (6.84±0.52mg/100g), flavonoids (34.14±3.13mg/100g), phytate (0.00±0.03mg/100g), cardiac glycosides (0.37±0.01%), total phytosterol (21.25±0.12mg/100g), stigmasterol (13.16±0.15mg/100g) and campesterol (6.16±0.12mg/100g). Mineral contents of the sample revealed that potassium, calcium, magnesium have highest concentrations while manganese and iron have the lowest. The chemical compositions of the investigated samples might be responsible for their medicinal values in phytomedicine. This study shows that *Persea americana* seeds are adequate in lowering cholesterol level probably due to the presence of phytosterols.

Keywords: *Persea americana* seeds, phytochemical composition, proximate and minerals analysis.

INTRODUCTION

Persea americana Mill. (Lauraceae), a plant from Central America (Mexico, Guatemala, Antilles), has shown easy adaptation to other tropical regions including Africa (Joao *et al*., 2009). The fruit is

popularly known as avocado pear and have an olive-green peel and thick pale yellow pulp that is rich in vegetable oils and appreciated for its sensory attributes (Joao *et al*., 2009). Avocado pear pulp is consumed in a similar way as the African pear fruit pulp (*Dacryodes edulis*). However, despite the nutritional potentials of African pear fruit seed (Ajayi and Adesanwo, 2009), it is still discarded after the edible pulp is eaten (Onuegbu *et al.*, 2016). The avocado seed represents 13–18% of the fruit, and it is a byproduct generally not utilized (Ortiz *et al*., 2004). Normally, the seed is discarded during the processing of the pulp like the African pear fruit seed. Discarding of the seed may cause severe ecological problems (Ortiz *et al*., 2004); like increased numbers of insects and rodents and economic losses as result of the high cost of transporting these byproducts to disposal areas (Ferrari *et al.*, 2004).

The seed of *P. americana* has been reported to lower blood pressure (Anaka *et al*., 2009) with reduction in the total cholesterol, LDLC (low density lipoproteins cholesterol) and triacylglycerol in the plasma, kidney, liver and heart of normotensive and hypertensive rat models at high doses of the seed extract (Imafidon and Amaechina, 2010). In hypercholesterolemia, a condition of high blood cholesterol, LDLC does not aid transportation of cholesterol out of body tissues but deposits cholesterol in the lumen of arterial walls (Nwaoguikpe and Braide, 2011). Phytosterols have been found effective in treating high cholesterol (hypercholesterolemia) as the plant sterols compete for absorption sites with cholesterol; they thus reduce the amount of cholesterol absorbed (Hirota et al., 2003). The cholesterol-lowering effect of phytosterols is also believed to be caused by an inhibition of cholesterol absorption resulting from the higher solubility of phytosterols than of cholesterol in bile salt micelles. The most common phytosterols are campasterol and stigmasterol which occurs in higher plants both *in vivo* and *in vitro* tissue culture (Hirota *et al.*, 2003).

Meanwhile, (Joao et al., 2009) and (Arukwe et al., 2012) have shown that avocado seed contain bioactive compounds as flavonoids, phenols, alkaloids, saponins and phytosterols that have tremendous health benefits. These compounds have antioxidant properties that help in preventing and treating putative health diseases as cancer, atherosclerosis, diabetes, hypertension, Alzheimer disease and ulcer (Deepti *et al*., 2013).

MATERIALS AND METHODS

Sample collection and identification

Avocado pear samples were purchased from Eke Awka Market in Awka, Anambra State. The pear samples were identified by Mrs. B. Aziagba, a taxonomist in the Department of Botany, Nnamdi Azikwe University Awka, Anambra State.

Preparation of sample extract

The seeds were seperated from the fruit, washed, chopped in bits and then dried at room temperature for ten days and then ground into fine powder using a manual grinder. The powdered sample was stored in an air-tight container until further analysis. The sample extraction was done by cold masceration as described by (Kumar *et al.*, 2010). The powdered sample (10g) was dissolved in 100% methanol (100ml) for 24 hours. The mixture was filtered through whatman paper No. 4 and the filtrate concentrated over a water bath at 40^{o}C. The concentrated extract was weighed and redissolved in methanol at a concentration of 100mg/ml and stored at 4^{o}C for further analysis.

Proximate Analysis

Moisture content, ash content, crude fibre, protein and fat were determined using standard methods of AOAC, 1999 while determination of total carbohydrate content was determined by difference of 100 as described by (Merrill and Watt, 1973).

Qualitative Analysis of Phytochemical Constituents

Qualitative analysis of the phytochemicals (alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids) of *Persea americana* seeds were carried out using the methods of (Trease and Evans, 1989) and (Harborne, 1973) before quantitative analysis were carried out.

Quantitative Determination of Phytochemical Constituents

The phytate and oxalate contents were determined by titration using the methods of (Young and Greaves, 1940) and (Osagie, 1998) respectively. Terpene was determined according to the method of (Narayan *et al.*, 2016). The saponins and alkaloids content were weighed and calculated in percentage (Harborne, 1973). The tannin content was calculated and expressed in percentage

(Follins Dennis titration method) as described by (Pearson, 1974). Beta carotene and lycopene, total phenol and flavonoid contents of the sample were determined using the method of (Barros *et al*., 2007). The phytosterol content of the sample was determined according to the method of (Larissa *et al*., 2013) while mineral content was done using Varian AA240 Atomic Absorption Spectrophotometer according to the method of (APHA,1995).

RESULTS

The seeds of *Persea americana* were investigated to evaluate the proximate, phytochemical and mineral compositions, the results obtained are as follows:

Result for Proximate Analysis

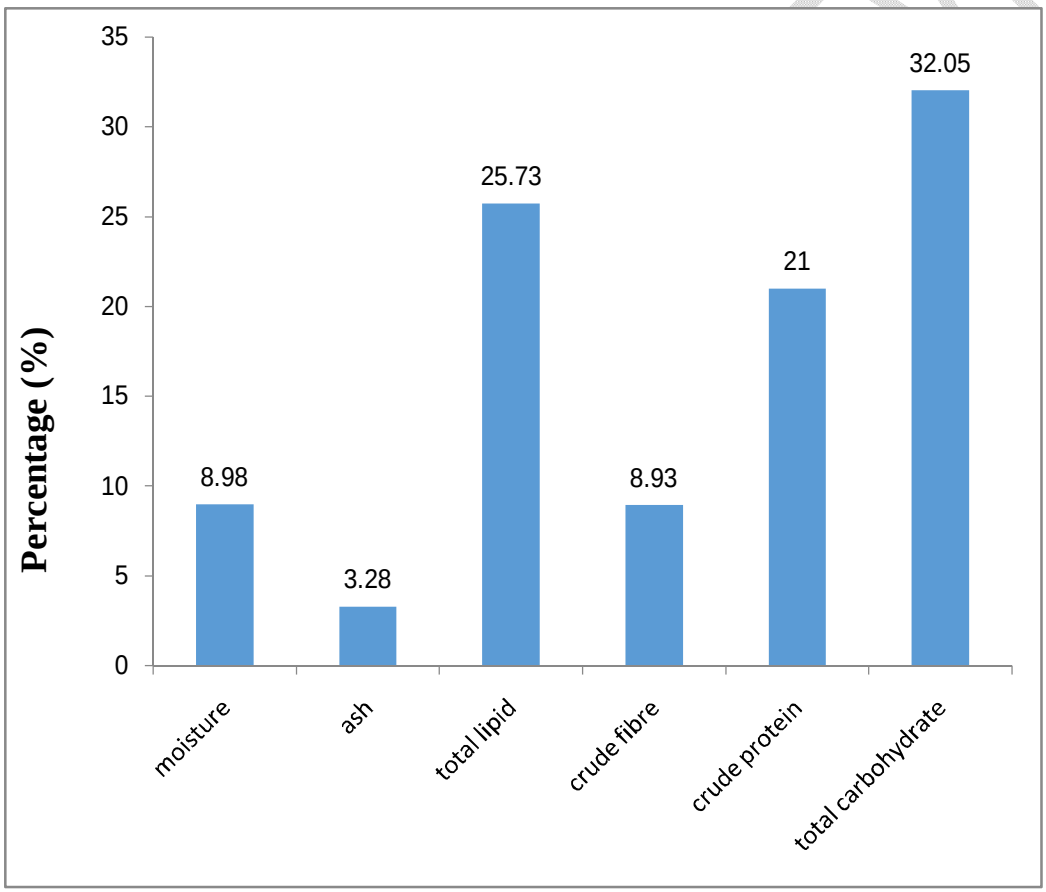

Figure 1: Bar Chart of Proximate composition of *Persea americana* seed. This result shows that *Persea americana* seeds are rich in carbohydrates and lipid but the ash content is relatively low.

Result for Phytochemical Composition

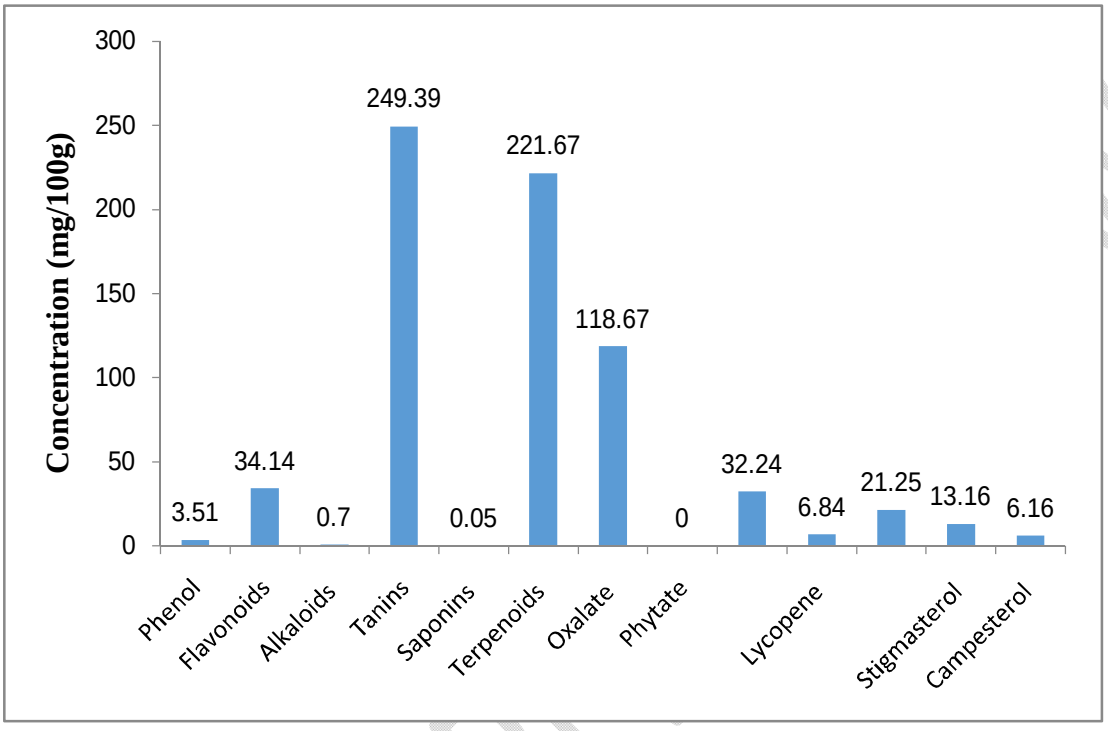

Figure 2: Bar Chart of Phytochemical composition of *Persea americana* seed. This result shows that *Persea americana* seeds are highly packed with tannins, terpenoids but low in phytate, alkaloids and saponins.

Result for Mineral Content

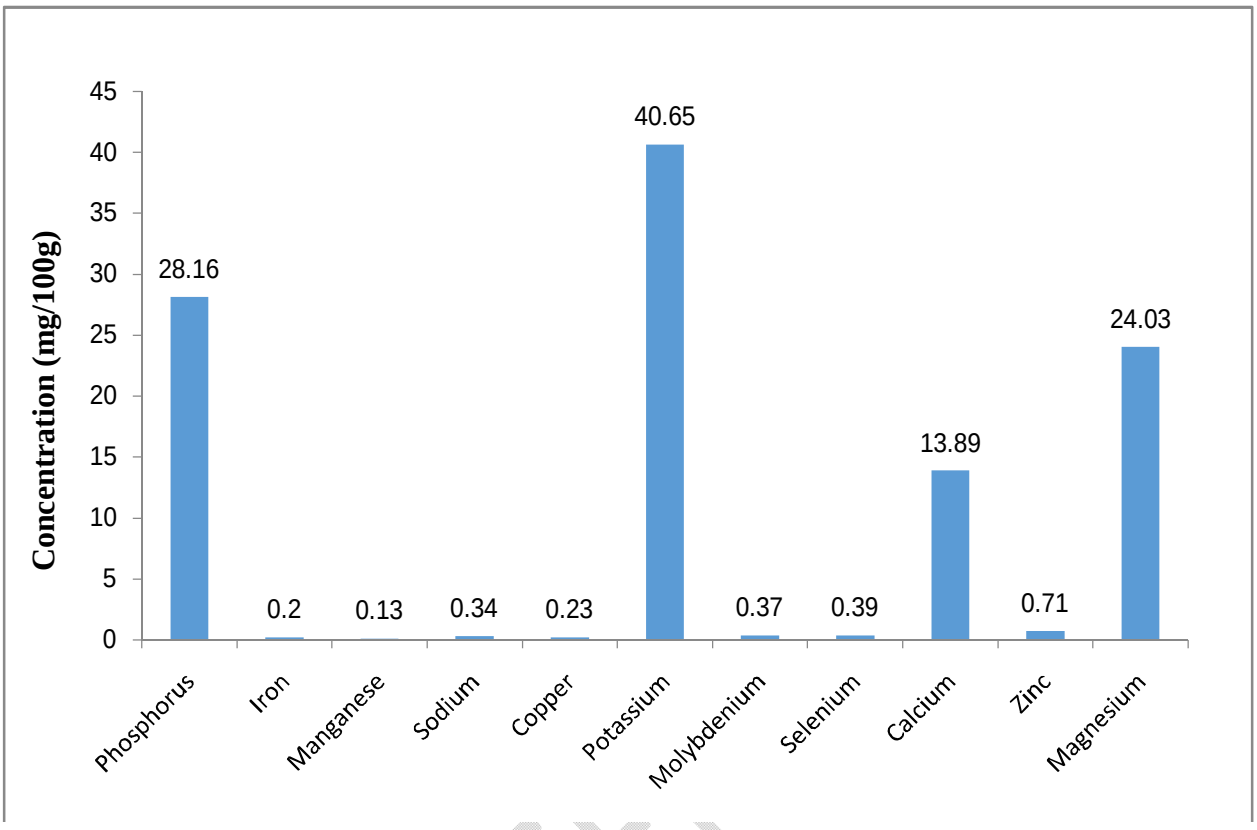

Figure 3: Bar Chart of Mineral composition of *Persea americana* seed. This result shows that *Persea americana* seeds are relatively high with potassium, phosphorus and magnesium but low in manganese, iron, and copper.

DISCUSSION

The seeds of *Persea americana* were investigated to evaluate the proximate, phytochemical and mineral compositions.

Proximate composition of *Persea americana* seeds as shown in Figure 1 revealed that the moisture content was relatively low and could imply long shelf life (Chikezie *et al*., 2008), thus giving a dry matter content of 91.02%. This suggests a nutrient dense food material that can actually be utilized in many ways such as in feed supplementation (Onuegbu *et al*., 2016). Ash content of the sample could be related to the mineral content and these minerals, which are mostly in forms of chemical compounds, play numerous functions towards the improvement of health in the body of organisms (Olusanya, 2008). The fat present in the seed is relatively high, in agreement with the result of

(Ifesan *et al.*, 2015), and may be an indication that *Persea americana* could be a good source of oil. The value falls within the range of values for most oil producing seeds like soybeans (Krogdahl and Bakk-Mckellep, 2002). Generally, fats have many functions aside insulation and conservation of body temperature in organisms, their fatty acid components such as lauric acid and polyunsaturated fatty acids have been reported to improve health (Imafidon and Amaechina, 2010). From this study the crude fibre content in the seed of *P. americana* is high when compared with its content in African pear seed, 3.17%, (Onuegbu et al., 2016). Adequate dietary fibre intake alters the colonic environment in such a way as to protect against colorectal diseases, lowering of serum cholesterol, prevent hypertension, diabetes, constipation and heart diseases (Ishida et al., 2000). It provides protection by increasing faecal bulk, which dilutes the increased colonic bile acid concentrations that occur with a high fat diet (Dillard and German, 1990). In comparing the protein content in this study with other studies, the value agrees with the report of (Ifesan *et a*l., 2015). Aside from contributing to diets, the relative impact of proteins in body system should not be over looked. As chemical compounds, they repair and replace worn out cells, form structural and globular materials that hold the body, form blood proteins and boost immune system (Olusanya, 2008). In this study for proximate composition, the carbohydrate content is highest which is similar to the results of other studies as in (Onuegbu *et al.*, 2016) and (Ifesan *et al*., 2015). Carbohydrate content of the sample is related to energy generation (Olusanya, 2008). Observed carbohydrates in the sample may be an indication that the sample could produce energy to power the cells and tissues of the body on consumption.

Phytochemicals are important chemicals found virtually in plants and they are in different parts and at different concentrations (Duke, 1992). Phytochemical profile of *P. americana seeds* revealed that they are rich in phenols, flavonoids and terpenoids (Figure 2). Phenols have been extensively researched as disease preventives (Duke, 1992). Phenols detected in seeds of *P. americana* investigated in this study could further indicate their ability to act as anti-inflammatory, anticlotting, antioxidants, immune enhancers, anticarcinogenic and antiaging (Arukwe *et al.*, 2012). The results for flavonoid and tannin contents were similar to those of (Nwaoguikpe and Braide, 2011). Flavonoids are potent water-soluble super antioxidants and free radical scavengers. They prevent oxidative cell damage, have strong anticancer activity and protect against all stages of carcinogenesis (Salah, 1995). In the intestinal tract they lower the risk of heart disease,

inflammation and represent the most common and widely distributed groups of plant phenolic compounds (Imafidon and Amaechina, 2011).

Alkaloids are therapeutically significant plant secondary metabolites. Isolated pure form of alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic and bactericidal effects (Stray, 1988). The presence of alkaloids in the seed extract could serve as a reason for its use in ethno medicine (Ramos *et al*., 2004). The content of tannins in *P. americana* may be responsible for their free astringency and bitter taste (Okwu, 2004). They hasten the healing of wounds and inflamed mucous membrane (Okwu and Okwu, 2004) hence their use in treatment of injuries. Tannins are known to bind irreversibly to proteins by forming insoluble complexes with them and thus rendering them indigestible by intestinal enzymes thereby interfering with their bioavailability (Liener, 1994). Saponin level in the seed sample was moderate and thus could not produce adverse effects on the growth of animals (Arukwe *et al*., 2012). Some of the general characteristics of saponins include formation of foams in aqueous solution, haemolytic activity and cholesterol binding properties (Sodipo and Akiniyi, 2000). The oxalate level in the seed sample was below the lethal dosage of soluble oxalate intake (3 – 5 g) (Antia et al., 2006), hence will have little or no adverse effect. The phytic acid content of avocado raw seed is below the recommended daily allowance dose of 25mg/100g (Hurrel *et al.*, 1992) hence will have little anti-nutritional effect. The anti-nutritional nature of phytic acid lies in its ability to chelate divalent minerals such as iron, calcium, copper and zinc, rendering them biologically unavailable. Processing techniques (soaking and boiling) significantly ($p \leq 0.05$) reduced the phytic acid content of avocado seeds (Justina *et al.*, 2016). Phytic acids are the principal form of phosphorus in many seeds; 60–90% of phosphorus in seeds is present as phytic acid (Mohammad et al., 2007) hence lowering the total phosphorus value in avocado.

From this analysis avocado seeds were found to be rich in beta carotene and lycopene. Lycopene is a good antioxidant and a precursor for beta carotene while beta carotene serves as food colorant (responsible for the yellowish colour of the seeds) and a precursor for vitamin A (Susan, 1998). This effect of RA is mediated mainly by retinoic acid receptors and vary among cell types. In mammary carcinoma cells, retinoic acid receptor was shown to trigger growth inhibition by inducing cell cycle arrest, apoptosis, or both (Niizuma et al., 2006). Lycopene is a cellular inhibitor for various inflammatory processes.

Also, Figure 2 revealed that the seed sample contain good amount of total phytosterols, stigmasterols and campesterols. The roles of most plant sterols have been documented. One strategy adopted in the reduction of cholesterol levels involves the use of such plant sterols such as sitosterol, stigmasterol and campesterol, whose mechanism of action despite their structural similarity to cholesterol involves the inhibition of cholesterol absorption by the intestinal cells (Evans, 2005). The cholesterol lowering potential of phytosterols is supposed to be caused by an inhibition of cholesterol absorption resulting from the higher solubility of phytosterols than of cholesterol in bile salt micelles (Hirota *et al.*, 2003).

The mineral composition result (Figure 3) clearly indicates that *P. americana* seeds are good sources of macro minerals like phosphorus, potassium, calcium and magnesium, except sodium which is relatively low. The concentrations of these minerals are not the same as reported in other studies, (Arukwe et al., 2012) and (Olusanya, 2008). The varying composition reported by various studies could vary with season, environment and condition or time evaluation. Phosphorus, calcium and magnesium are required for formation of bones and teeth, formation of blood clot, formation of cyclic AMP and other second messengers, for body mechanisms, etc (Olusanya, 2008). Potassium is necessary for electrolyte balance and control of high pressure. This could also be implicated in the use of *P. americana* seed to treat high blood pressure in traditional medicine (Arukwe et al., 2012). The low sodium level is an indicative that the seed sample can not jeopardize blood pressure, since elevated level of sodium salt in the blood has been associated with high blood pressure in the body (Olusanya, 2008), but this may not be possible in a situation of higher potassium content. Trace elements as molybdenum, selenium, are needed in small quantities by the body; therefore concentrations of these elements found in *P. americana* seeds are of great nutritional importance (Ajayi and Adesanwo, 2009). Zinc, which is the most prevalent trace element in this study, is essential for the production of insulin hence can play a valuable role in the management of diabetes (Ajayi and Adesanwo, 2009). Zinc plays role in wound healing, iron is known for haem formation, manganese and copper aid iron absorption in the body (Olusanya, 2008). Selenium plays an important role in the antioxidant system by acting as cofactor for gluthathione peroxidase, enhancing alpha-tocopherol activities and helping in DNA repairs mechanism. Copper is essential for haemoglobin synthesis, normal bone formation and the maintenance of myelin within the nervous system (Passmore and Eastwood, 1986).

CONCLUSION

P. americana seeds have appreciable nutrients in the form of carbohydrate, crude protein, crude fat and fibre, hence they can be added to our dietary plan (such as homemade smoothies). The appreciable antioxidant activities exhibited by the seed extract are attributed to the presence of phytocompounds such as flavonoids, phenols, lycopene and the like. The total phytosterols present in *P. americana* seed could also serve as preventive measures against cardiovascular diseases since phytosterols exhibit hypocholesterolemia activities. These possible dietary and drug functions of these seeds, have generated background for phyto-medicinal usage of avocado pear seeds thereby demanding further studies to determine these seeds have possible usage in medicine and food industries.

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