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PROXIMATE, PHYTOCHEMICAL, MINERAL COMPOSITION AND ANTIOXIDANT EFFECTS OF METHANOL EXTRACT *OF Persea americana* (Avacado pear) SEED

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ABSTRACT

The proximate, phytochemical, mineral, and antioxidant composition of *Persea americana* seed methanol extract was examined in this work. Standard procedures were used for both proximate, mineral composition and quantitative evaluations. Lipid peroxidation, reducing power, and 2,2-diphenyl-1-picryhydrazyl tests were used to measure the antioxidant activity. Using absorption spectroscopy techniques, antioxidant vitamins A, E, and C as well as enzymes (superoxide dismutase, peroxidase, and catalase) were evaluated. Based on the proximate analysis, the P. americana seeds have higher levels of protein (21.00 ± 0.88 %), fat (25.73 ± 2.01 %), and carbohydrates (32.05 ± 1.21 %), but they have lower levels of moisture (8.98±0.10 %) and ash (3.28±0.16 %). A quantitative phytochemical study found that there were phenols (3.51±0.42 mg/100 g),saponins (0.05±0.38 mg/100 g), alkaloid (0.70±0.37mg/100 g), beta-carotene (32.24±0.55mg/100 g), lycopene (6.84±0.52 mg/100 g), flavonoids (34.14±3.13 mg/100 g), phytate (0.00±0.03 mg/100 g), cardiac glycosides (0.37±0.01 %), total phytosterol (21.25±0.12 mg/100 g), stigmasterol (13.16±0.15 mg/100 g) and campesterol (6.16±0.12 mg/100 g). The mineral composition showed that iron and manganese had the lowest quantities, while potassium, calcium, and magnesium had the highest concentrations. The analyzed samples' potential therapeutic benefits in phytomedicine may be attributed to their respective chemical compositions. Out of all the antioxidant vitamins tested, vitamin E had the highest amount (267.73 ± 0.07 mg/100g), followed by vitamin A (7.60 ± 0.01 mg/100 g) and vitamin C (3.88 ± 0.03 mg/100 g). As extract concentration increased, there was a substantial ($p<0.05$) rise in the DPPH scavenging effect and suppression of lipid peroxidation activity. The ability of the extract to reduce power was evidenced by an increase in the spectroscopic absorbance value as the extract concentration in the reaction mixture increased.

Keywords: *Persea americana* seeds, Phytochemical composition, Proximate, Minerals, Antioxidant enzymes, Vitamins

INTRODUCTION

A plant native to Central America (Mexico, Guatemala, the Antilles), *Persea americana* Mill. (Lauraceae) has demonstrated simple adaption to various tropical climates, including Africa. The fruit, commonly referred to as an avocado pear, has a thick, pale yellow pulp that is rich in vegetable oils and valued for its sensory qualities, as well as olive-green skin. (Joao *et al*., 2009). In ethnomedicine, *Persea americana* seeds have been utilized as a powerful cure for a variety of purported medical ailments, including menstrual and muscle pains, hypertension, diabetes mellitus, anemia, sleeplessness, hyperlipidemia, diarrhea, dysentery, and stomach and peptic ulcers. (Okorie, et al., 2023; Adeboye *et al.,* 1999). According to Kopp (1966), Persea is an evergreen tree in the Lauraceae family of laurel trees. The most well-known species in the genus is Persea americana, or avocado, which is grown extensively in subtropical areas for its enormous, edible fruit. Avocado seed treatments are known to provide local anesthetic effects that lessen muscle pain; avocado seeds have long been used to treat parasite infections and mycoses. (Ramos *et al.*, 2004). At high doses of the seed extract, P. americana has been shown to lower blood pressure in normotensive and hypertensive rat models, as evidenced by reductions in total cholesterol, LDLC (low-density lipoprotein cholesterol), and triacylglycerol in the plasma, kidney, liver, and heart (Anaka et al., 2009). Imafidon and Amaechina, 2010). Due to their ability to compete with cholesterol for absorption sites, phytosterols have been proven to be useful in treating hypercholesterolemia, or excessive cholesterol. As a result, less cholesterol is absorbed (Hirota et al., 2003). Although phytosterols are more soluble in bile salt micelles than cholesterol, it is also thought that this suppression of cholesterol absorption contributes to the cholesterol-lowering impact of phytosterols. The most common phytosterols are campasterol and stigmasterol which occur in higher plants in both *in vivo* and *in vitro* tissue culture (Hirota *et al*., 2003). In addition, (Joao *et al*., 2009) and (Arukwe *et al.*, 2012) have shown that avocado seeds contain bioactive compounds such 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"s disease and ulcer (Deepti *et al.*, 2013). Due to the high concentration of phenolic components, such as flavonoids and ascorbic acid, the seed of P. americana has been shown to have better antioxidant qualities than its pulp (Bertling et al., 2007). This provided as the foundation for a study on the

antioxidant characteristics of avocado seeds that concentrated on the examination of natural compounds that might be extracted and used for food and medicine purposes.

MATERIALS AND METHODS

Obtaining and identifying samples

In February 2022, avocado and pear samples were bought from the new market in the Enugu East Local Government Area of Enugu State, Nigeria. Mrs. B. Aziagba, a taxonomist at Nnamdi Azikwe University in Awka, Anambra State's Department of Botany, identified the pear samples. A voucher specimen bearing the number BN246/A/016 was placed in the department's herbarium

Getting the sample extract prepared

After their separation from the fruit, the seeds were cleaned, cut, and dried for ten days at room temperature before being processed into a fine powder with a hand grinder. Until further examination, the powdered material was kept in an airtight container. As stated by (Kumar et al., 2010), cold maceration was used to remove the material. For 24 hours, the 10 g powdered material was dissolved in 100 ml of 100 % methanol. Whatman paper No. 4 was used to filter the mixture, and the filtrate was concentrated over a water bath at 40 ^{0}C. For additional analysis, the concentrated extract was weighed, dissolved in methanol at a concentration of 100 mg/ml, and kept at 4 ^{0}C.

Proximate Analysis

Standard AOAC, 1999 procedures were used to evaluate the moisture content, ash content, crude fiber, protein, and fat content; however, a difference of 100 was used to establish the total carbohydrate content 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 was carried out using the methods of (Trease and Evans, 1989) and (Harborne, 1973)

Determining Phytochemical Constituents quantitatively

Through titration, the contents of phytate and oxalate were ascertained, utilizing the techniques of Young and Greaves (1940) and Osagie (1998), respectively. Terpene was ascertained using the procedure described by Narayan et al. (2016). Weighing and calculating the proportion of the saponin and alkaloids content was done (Harborne, 1973). According to Pearson's (1974) instructions, the tannin concentration was determined and represented as a percentage using the Folins Dennis
titration method. Using the method of (Barros et al., 2007), the sample's contents of beta carotene and lycopene, total phenol, and flavonoids were ascertained. The sample's phytosterol content was ascertained using the methodology described in (Larissa et al., 2013).

Mineral content determination

Using the Varian AA240 Atomic Absorption Spectrophotometer in accordance with the APHA (1995) technique, mineral content was determined.

Determination of Antioxidant Activity

Scavenging action of DPPH

The methanolic extract of P. americana seeds was tested for its ability to scavenge free radicals using the stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH). The procedure of (Ebrahimzadem et al., 2009) was used to assail this.

Using thiobarbituric acid (TBA) in lipid peroxidation inhibition

This was ascertained using the (Barros et al., 2008) approach, which computed the inhibition ratio (%) using the formula below:

Inhibition ratio (%) = [(A-B)/A] × 100 %.

Decreasing the assay's power

The reduction power was calculated using the (Oyaizu, 1986) approach. The idea behind this approach is that the reaction mixture's absorbance will rise. The benchmark was BHA (Barros et al., 2007).

Assay of Antioxidant Enzymes

Superoxide Dismutase (SOD) Activity Determination

The ability of superoxide dismutase to prevent adrenaline from auto-oxidizing was measured by measuring an increase in absorbance at 480 nm, as reported by Sun and Zigma in 1978

Calculating the Catalase Activity

Using a UV recording spectrophotometer, the sample's catalase activity was measured by measuring the drop in absorbance at 240 nm caused by the breakdown of H_2O_2, in accordance with the Beers and Sizer method as reported by Usoh et al. (2005).

Peroxidase

The procedure suggested by Reddy et al. (1995) was used to measure peroxidase activity. Peroxidase changes H_2O_2 into H_2O and O_2 when a hydrogen donor, such as pyrogallol or dianisidine, is present

Non-enzymatic Antioxidant Assay

The absorption spectroscopic approach of (Rutkowski et al., 2005 and 2006) was used to analyze vitamins A and E. The amount of ascorbic acid in P. Americana seeds was calculated using (Klein and Perry, 1982).

RESULTS AND DISCUSSION

An assessment of the proximate, phytochemical, mineral, and antioxidant properties of Persea americana seeds was conducted.

Proximate Analysis

Figure 1 depicts the approximate composition of Persea americana seeds. With a comparatively low moisture level of 8.98 %, which may indicate a long shelf life (Chikezie et al., 2008), the dry matter content came out to be 91.02 %. This suggests a food substance that is high in nutrients and may be used in a variety of ways, including as a feed supplement (Onuegbu et al., 2016). The sample's 3.28 percent ash level may have something to do with its mineral composition. These minerals, which are primarily chemical components, have a variety of uses in the body that contribute to better health (Olusanya, 2008). Generally, fats have many functions aside from 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). The fat content of the seed (32.05 %) is relatively high, in agreement with the results of (Ifesan et al., 2015), and may be an indication that Persea americana could be a good source of oil. According to this study, the *P. americana* seed has a

higher crude fiber content (8.35%) than the African pear seed (3.17 %) (Onuegbu et al., 2016). Sufficient consumption of dietary fiber modifies the environment in the colon, protecting against heart disease, diabetes, hypertension, constipation, and colorectal disorders as well as decreasing blood cholesterol (Ishida et al., 2000). By boosting faecal bulk, it mitigates the effects of elevated colonic bile acid concentrations brought on by a high-fat diet (Dillard and German, 1990). When the protein content (21.00 %) in this study is compared to previous studies, the results are consistent with the findings of Ifesan et al. (2015). In addition to being a component of diet, protein also has a relative effect on bodily systems. As chemical substances, they create blood proteins, strengthen the immune system, replenish and repair damaged cells, and generate globular and structural materials that support the body (Olusanya, 2008). The results of previous research, such as those in (Onuegbu et al., 2016) and (Ifesan et al., 2015), are similar to the findings of this study regarding proximate composition, where the carbohydrate content is higher. The sample's carbohydrate content and energy production are connected (Olusanya, 2008). The presence of carbohydrates in the sample may indicate that it contains the potential to generate energy upon ingestion, which would power the body's tissues and cells.

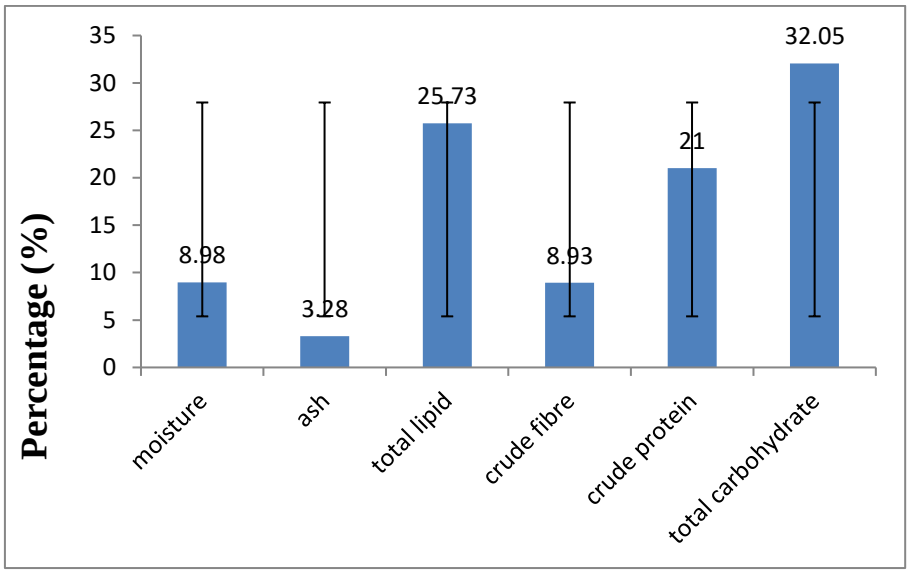

Figure 1: Bar Chart of Proximate composition of *Persea americana* seed displaying Error Bars for the selected parameters with one standard deviation.

Phytochemical Composition

Important compounds called phytochemicals are almost exclusively present in plants, albeit in varying amounts and different sections (Umerie et al., 2022, Duke, 1992). P. americana seed phytochemical profile (fig. 2), expressed as a concentration (mg/100g), demonstrated that they have high concentrations of terpenoids (221.67), flavonoids (34.14), and phenols (3.51). Many studies have been conducted on phenols as potential disease preventives (Duke, 1992). The phenols found in the *P. americana* seeds examined in this study may also have additional functions, such as anti-inflammatory, anticoagulant, antioxidant, immune-stimulating, anticarcinogenic, and antiaging (Arukwe et al., 2012). The findings for tannin (249.39) and flavonoid concentrations matched those of other studies (Nwaoguikpe and Braide, 2011). Strong water-soluble super antioxidants and scavengers of free radicals are flavonoids. They have a potent anticancer effect, stop oxidative cell damage, and guard against carcinogenesis at all stages (Salah, 1995). They are the most prevalent and widely dispersed classes of plant phenolic compounds in the gastrointestinal system, and they reduce the risk of inflammation and heart disease (Imafidon and Amaechina, 2011). Alkaloids are important secondary metabolites of plants with medicinal use. Because of their bactericidal and analgesic properties, isolated pure alkaloids and their synthesized derivatives are used as fundamental therapeutic agents (Stray, 1988). The seed extract's use in ethnomedicine may be explained by the presence of alkaloids (0.7) in it (Ramos et al., 2004). The free astringency and bitter taste of P. Americana may be attributed to its tannin concentration (Okwu, 2004). They are used in the treatment of injuries because they speed up the healing process for wounds and irritated mucous membranes (Okwu and Okwu, 2004). According to Lieber (1994), tannins can have the ability to bind to proteins irreversibly by creating insoluble complexes that prevent intestinal enzymes from breaking down the proteins and affecting their bioavailability. Since the seed sample's saponin content (0.05) was moderate, it was unable to negatively impact animal growth (Arukwe et al., 2012). Saponins are generally characterized by their ability to produce foams in aqueous solutions, their hemolytic activity, and their ability to bind cholesterol (Sodipo and Akiniyi, 2000). There won't be much of an adverse effect because the oxalate level (118.67) in the seed sample was below the lethal dosage of soluble oxalate ingestion (3–5 g) (Antia et al., 2006). Avocado raw seed has less phytic acid than the 25 mg/100 g daily allowance amount (Hurrel et al., 1992), therefore it won't have a significant anti-nutritional impact. Phytic acid is considered anti-nutritional because of its capacity to chelate divalent minerals, including calcium, iron, zinc, and copper, making them inaccessible to the body. The phytic acid content of avocado seeds was significantly ($p < 0.05$) reduced by the processing methods of soaking and boiling (Justina et al., 2016). The primary source of phosphorus in many seeds is phytic acid; according to Muhammad et al. (2007), phytic acid accounts for 60–90% of phosphorus in seeds, which lowers the avocado's overall phosphorus content. Further on the analysis, avocado seeds were found to be rich in beta carotene (32.24) and lycopene (6.84). Lycopene is a good antioxidant and a precursor for beta carotene while beta carotene serves as a food colorant (responsible for the yellowish colour of the seeds) and a

precursor for vitamin A (Susan, 1998). According to Niizuma et al. (2006), retinoic acid receptor-induced cell cycle arrest, apoptosis, or both have been demonstrated to initiate growth suppression in mammary cancer cells. Lycopene inhibits several number of inflammatory processes at the cellular level. Also, Figure 2 revealed that the seed sample contain a good amount of total phytosterols (21.25), stigmasterols (13.16) and campesterols (6.16). The majority of plant sterols have known functions. Despite their structural similarities to cholesterol, plant sterols like sitosterol, stigmasterol, and campesterol work by inhibiting the intestinal cells' ability to absorb cholesterol. This is one method used to lower cholesterol levels (Evans, 2005). Because phytosterols are more soluble in bile salt micelles than cholesterol, they are thought to limit the absorption of cholesterol, which contributes to their ability to decrease cholesterol (Hirota et al., 2003).

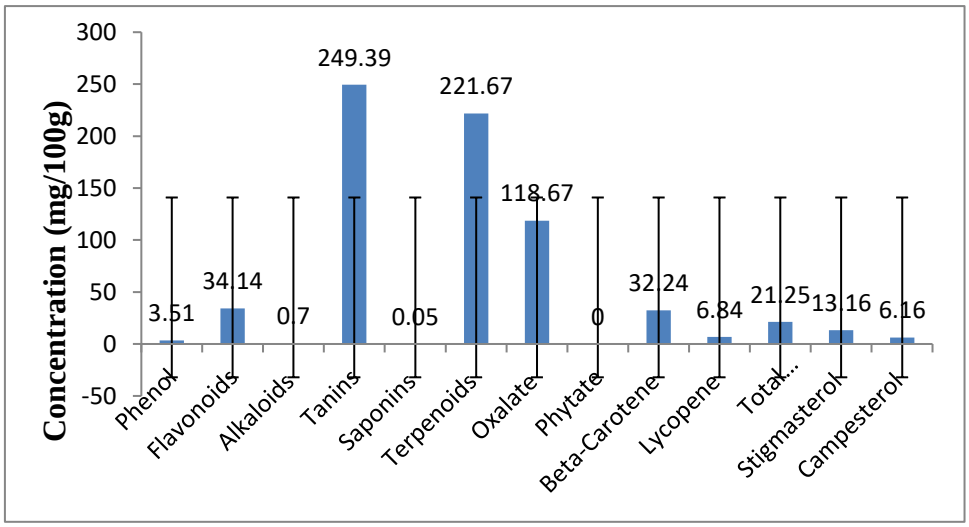

Figure 2: Bar Chart of Phytochemical composition of *Persea americana* seed displaying Error Bars for the selected parameters with one standard deviation

Mineral Content

The mineral composition (mg/100 g) result from Figure 3, clearly indicates that *P. americana* seeds are good sources of macro minerals like phosphorus (28.16), potassium (40.65), calcium (13.89) and magnesium (24.03), except sodium (0.34) 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 of evaluation. For the development of bones and teeth, blood clots, cyclic AMP and other second messengers, bodily processes, etc., phosphorus, calcium, and magnesium are necessary (Olusanya, 2008). Potassium is essential for maintaining electrolyte balance and managing elevated pressure. This may also be connected to traditional medicine's usage of P. americana seeds to treat high blood pressure (Arukwe et al., 2012). Since increasing blood levels of sodium salt have been linked to high blood pressure in the body (Olusanya, 2008), the low sodium level suggests that the seed sample cannot imperil blood pressure. However, in a circumstance where the potassium content is higher, this may not be achievable. Trace elements as molybdenum (0.37), selenium (0.39), 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). The most common trace element in this study, zinc (0.71), is necessary for the synthesis of insulin and, as such, can be useful in the treatment of diabetes (Ajayi and Adesanwo, 2009). According to Olusanya (2008), zinc aids in wound healing, iron (0.2) is known to make hemoglobin, and manganese (0.13) and copper (0.23) help the body absorb iron. Through its roles as a cofactor for glutathione peroxidase, an enhancer of alpha-tocopherol activities, and a supporter of DNA repair mechanisms, selenium plays a significant part in the antioxidant system. According to Passmore and Eastwood (1986), copper is necessary for the synthesis of hemoglobin, healthy bone growth, and the preservation of myelin in the neurological system.

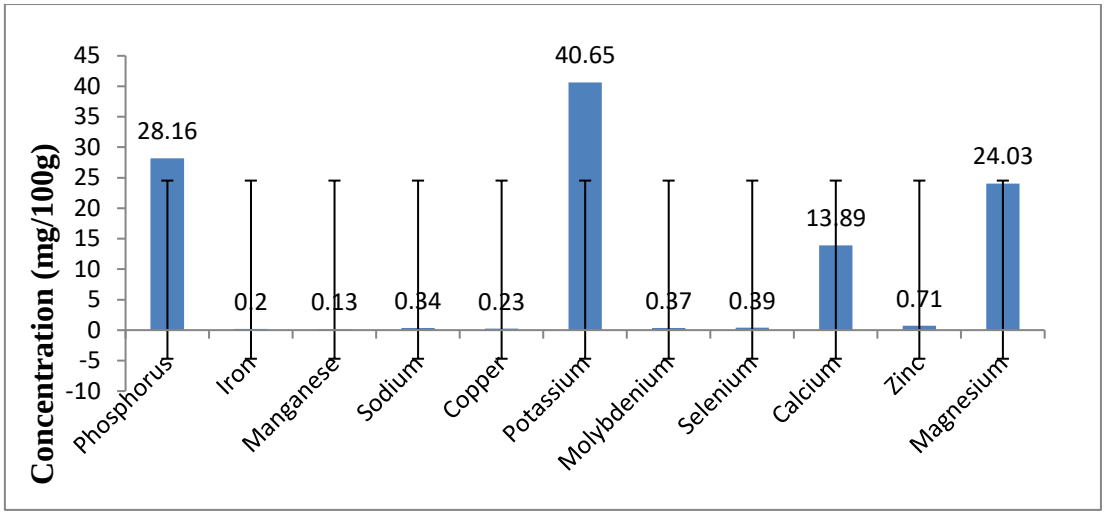

Figure 4: Bar Chart of Mineral composition of *Persea americana* seed displaying Error Bars for the selected parameters with one standard deviation

Non-enzymatic Antioxidant Assay

This study's (fig. 4) observation showed that the methanol extract of P. americana seed's ability to scavenge radicals on DPPH radicals increased with concentration. With an EC50 value of 610 µg/ml, the methanol extract of P. americana seeds showed moderate RSA values. The presence of flavonoids and phenolic compounds in the Persea americana seed methanol extract may be the cause of this extract's notable scavenging capacity. According to Muhammad et al. (2007), the primary source of phenolic compounds' antioxidant action is their redox characteristics, which can be crucial in absorbing and neutralizing free radicals, quenching singlet and triple oxygen, or breaking down peroxides.

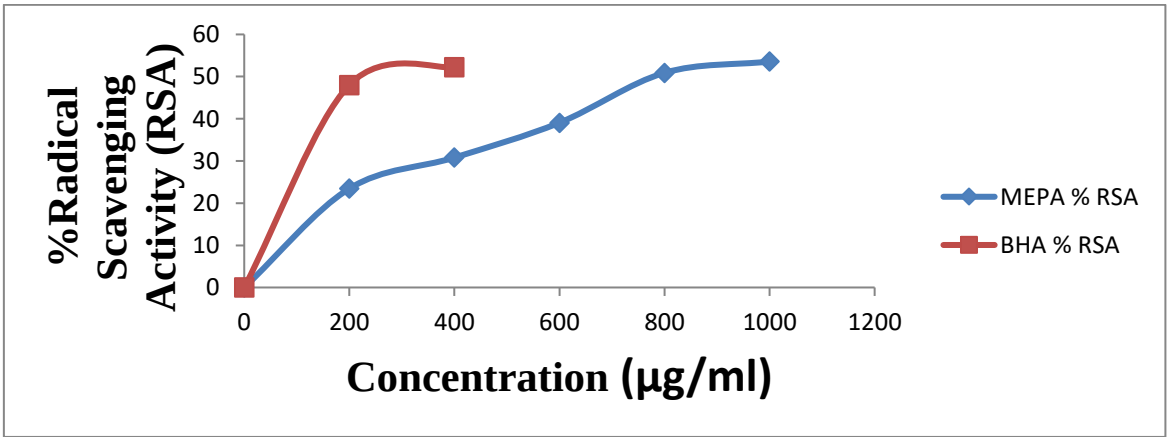

Figure 4: Radical scavenging activity (%) of methanol extract of *P. americana* seed on DPPH radicals

It was discovered that the methanol extract effectively inhibited the production of lipid peroxides via membrane lipid peroxidation of goat brain employing TBA reactive agent. As seen in Figure 5, the extract was able to prevent the development of lipid peroxide, and the inhibition ratio (IR) for the assay's inhibition of lipid peroxidation increased with increasing concentration. The *P. americana* seed methanol extract showed strong percentage inhibition ratio values, with an EC50 value of 640.51 µg/ml. Fe^{3+} to Fe^{2+} transition was observed to occur in the presence of an extract sample for reductive ability assessments (Oyaizu, 1986).

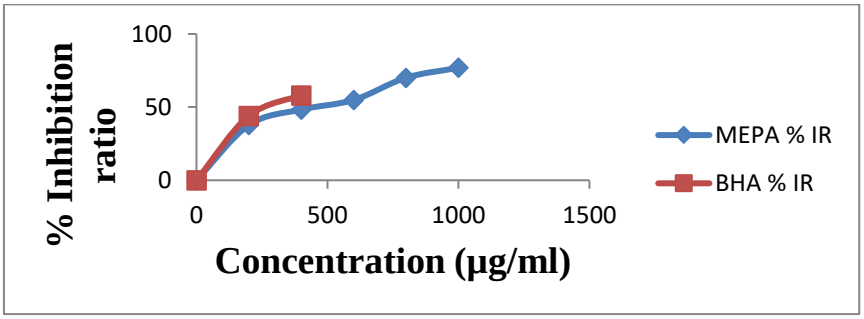

Figure 5: Inhibition of lipid peroxidation activity (%) of methanol extract of *P. americana* seed

Reducttones, which have been demonstrated to exhibit antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990), are typically linked to the reducing characteristics (Duh et al., 1999). The P. americana methanol extract has a great reduction power since its concentration grew gradually (Figure 6). The significant reducing power that the extract demonstrated may be a sign of the active species' capacity to donate hydrogen (Okorie et al. 2023; Shimada et al., 1992). The lowering power EC50 value was greater than the RSA and lipid peroxidation inhibition EC50 values.

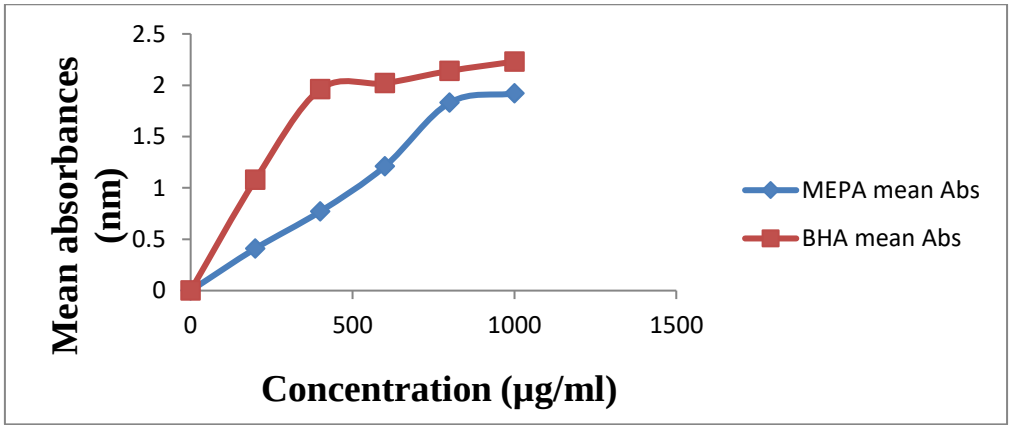

Figure 6: Reducing Power activity (%) of methanol extract *P. americana* seed

The seed sample also included a significant level of vitamin E (267.73), according to the results of the vitamin assay (mg/100g) displayed in Figure 7. The value of vitamin C (3.88) found in Persea americana raw seed is within the range seen in its pulp (Morton and Dowling, 1987). Vitamin C is a heat-labile, water-soluble antioxidant vitamin. When compared to citrus fruits, avocado seeds may be a poor source of ascorbic acid due to their low ascorbic acid value (Adeboye et al., 1999). The seed sample's reasonable vitamin A (β-carotene equivalent) content (7.60) could be attributed to the good amount of beta-carotene present. Retinoic acids (RAs) and carotenoids are both potent antioxidants that can control transcription factors (El-Agamey et al., 2009). Tumor necrosis factor-α, interleukin (IL)-6, and NF-κB activation are all inhibited by β-carotene. Additionally, carotenoids influence cell apoptosis. According to Laruri et al. (1996), antioxidants including ascorbic acid, carotenoids, and tocopherol have been linked to the protection of diseases like cancer, coronary heart disease, and obesity that are linked to nutrition. The dry substance of the seed contains the majority of the oil-soluble vitamins A and E. Because of this, the water used to prepare them does not contain them (Justina et al., 2016).

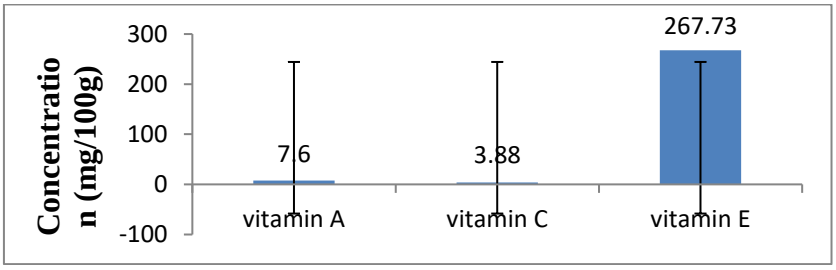

Figure 7: Bar Chart of Vitamins in *P. americana* seed displaying Error Bars for the selected parameters with one standard deviation

Enzymatic Antioxidant Assay

Furthermore, the results as illustrated in Figure 8 demonstrate the activities of catalase (CATs), superoxide dismutase (SODs), and peroxidase in the seed extract of P. americana. The historical application of P. Americana seed extract for the treatment of ulcers, diabetes, hypertension, and cancer may be explained by the existence of these enzymes (Deepti et al., 2013). Very effectively, CATs and peroxidases eliminate H2O2 (Scandalios, 1993), while SODs scavenge the superoxide anion from cells. The most effective antioxidant enzymes are SODs and CATs. Together, they prevent cellular damage by converting hydrogen peroxide (H2O2) and the potentially harmful superoxide radical (O2-) to water (H2O) and molecular oxygen (O2). The hydroxyl radical (OH-), which can react indiscriminately with all macromolecules, is the most damaging and highly reactive oxidant; nevertheless, the joint action of SOD and CAT reduces its generation (Scandalios, 1993).

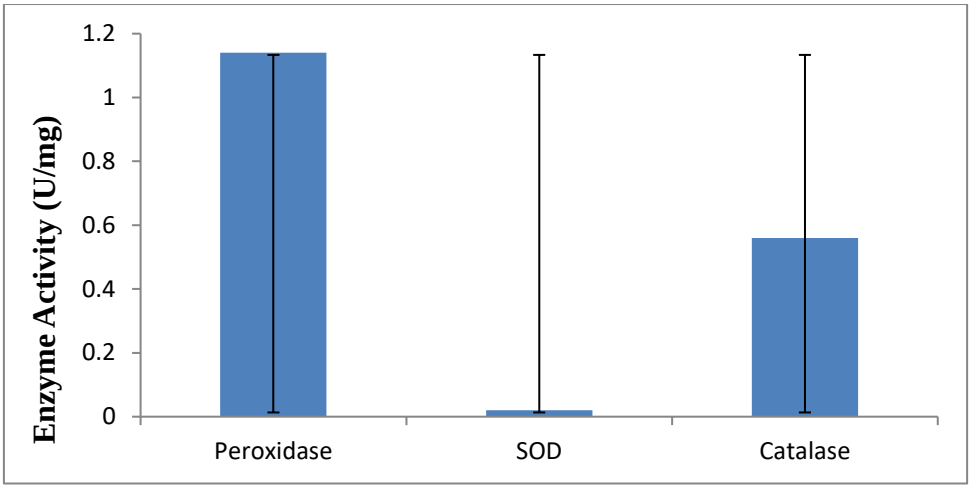

Figure 8: Bar Chart of Enzymatic activity of *P. americana* seed displaying Error Bars for the selected parameters with one standard deviation

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. The appreciable antioxidant activities exhibited by *Perseaamericana* seed extract can be attributed to the presence of antioxidant vitamins (A, E and C) as well as the enzymatic activities of peroxidase and catalase. The study also demonstrated high DPPH scavenging and lipid peroxidation activities relative to standard vitamin C. The seeds could be potential sources of natural antioxidants that could have great importance as therapeutic agents and in preventing or slowing down the process of ageing and age-associated oxidative stress-related diseases These possible dietary and drug functions of these seeds have generated background for phyto-medicinal usage of avocado pear seeds thereby suggesting that these seeds have possible usage in medicine and food industries.

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