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Phytochemical Analysis and Antioxidant Activity of Avocado Pear Peel (*Persea americana*) Extract

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the phytochemicals and antioxidant activity in *Persea americana* peel it also assessed the efficiency of *Persea americana* peel (Avocado Pear fruit peel) waste in different in vitro activities in order to explore the possibility of utilizing waste as a value-added product in various applications.. Quantitative phytochemical analysis was performed. Quantitative phytochemical screening confirmed the presence of alkaloids (56.5063 ug/ml), flavonoids (245.6875 ug/ml), phenols (15.65 ug/ml), tannins (27.03 ug/ml) phytate (9.68 ug/ml), oxalate (13.65%), saponin (22.73 ug/ml). For antioxidant activity, the peels of fruit sample of *Persea americana* were prepared in ethanol solution. The vitamin content of the peel extract was assayed using calorimetric and titration methods while the antioxidant enzyme assay was determined by spectrophotometric method. The antioxidant enzyme assay was also determined for catalase, Superoxide dismutase (SOD) and glutathione peroxidase. The Vitamins A, C and E were found to be present, with Vitamin A being the highest in concentration. Copper, Zinc, Iron and manganese were also present in the

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peel extract. The result from this study indicates that the peel extract of *Persea americana* possess antioxidant properties and could serve as free radical scavenger or inhibitor.

Keywords: Persea americana; phytochemical screening; anti-oxidant activity.

1. INTRODUCTION

Avocado pear fruits (*Persea americana*) have high nutritional quality and contain high levels of vitamins, minerals, proteins, and fibers, as well as high concentrations of unsaturated fatty acids, beneficial to health. In addition, Avocado pear seed have high contents of bioactive phytochemicals such as phenolic acids, condensed tannins, and flavonoids, including procyanidins, flavonols, hydroxybenzoic, and hydroxycinnamic acids [1]. These bioactive compounds have shown various biological activities such as antioxidant and anti-inflammatory properties. The anti-inflammatory activity of phenolic compounds is largely related to their ability to scavenge oxidative radicals, which is important for cell and oxidative stress regulation [1]. Oxidative Stress that causes tissue damage occurs in cells as a consequence of normal physiological process and environmental interactions [2].

Plant produce have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds; peels etc., i.e., any part of the plant may contain active components. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Plants represent an important source of protective agents, due to their content of polyphenols, vitamins, fiber, phytosterols, and carotenoids. Polyphenols have both antioxidant and prooxidant properties. The antioxidant activity is due to the scavenging effect of free radicals and ensures the protection of intracellular structures against oxidative stress, favouring cell viability. As prooxidants, polyphenols may stimulate apoptosis and inhibit tumour growth. Polyphenols have good effects on degenerative diseases like cancer, cardiovascular diseases, diabetes, and osteoporosis. As for their effect on the cardiovascular system, polyphenols reduce blood pressure, inflammation, and oxidative markers, they prevent endothelial dysfunction, they are antithrombotic, and they act as vasodilators. They also inhibit the proinflammatory activity of cyclooxygenase (COX), lipooxygenase (LOX), and inducible nitric oxide synthase (iNOS) [3],

2. MATERIALS AND METHODS

The fruits of *Persea americana* (Avocado pear) were purchased from new Market in Enugu East L.G.A of Enugu state, Nigeria in December 2021 and was authenticated by a taxonomist, Mr. Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine, A voucher specimen with No PCG/246/A/014 was deposited at the herbarium of the department The fruit was allowed to ripen before it was cut open and its butter removed while the peel was washed with distilled water and allowed to dry at room temperature after which it was pulverized into fine coarse form and taken to the Pharmaceutical and medicinal chemistry laboratory, ESUT for extraction.

2.1 Extraction

The extraction was done by weighing and transferring 1.0 g of the pulverized plant material into a test tube and 15 ml of concentrated ethanol was added. The test tube was allowed to react in a water bath at 60 ^{o}C for 60 minute after which the reaction product contained in the test tube was transferred to a separation funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 1.0 ml of hot water and 3.0 ml of hexane, which was all transferred to the funnel. The extract were combined and washed three times with 10 ml of 10 %v/v aqueous ethanol solution. The extract solution was dried with anhydrous sodium sulfate and the solvent evaporated. The sample was solubilized in 100 µl of hexane of which 200 µl was transferred to a vial until required for analysis.

2.2 Quantitative Phytochemical Screening

The quantitative phytochemical content of *Persea americana* peel was done using BUCK M910 GC equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m x 250 µm x 0.15µm) was used. The injector temperature was up to 280 °C with splitless injection of 2µl of the sample, a linear velocity of 30 cms^{-1}and the carrier gas used was Helium 5.0 pa.s with a flow rate of 40 mlmin^{-1}. The oven operated from a temperature of 200 °C until it heated to 330 °C at a rate of 3.0°Cmin^{-1}.

This temperature was maintained for 5 minutes and the detector operated at a temperature of 320°C [4]. Phytocompounds were determined by the ratio between the area and mass of internal standard and the area of the identified phytocompounds and the concentration was expressed in µg/g.

2.3 Non-enzymatic Antioxidant Content

2.3.1 Determination of Vitamin A

Vitamin A was determined by the calorimetric methods of Kirk and Sawyer [5]. A measured weight (1.0g) of the sample and standard was mixed with 3.0 ml of absolute alcohol and 3.0 ml of 50 KOH solutions was added to it and boiled gently for 30 minutes under efflux. After washing with distilled water, vitamin-A was extracted with 3 x 50 ml of diethyl ether. The extract was evaporated to dryness at low temperature and then dissolved in 10 ml of isopryl alcohol. 1.0 ml of standard vitamin A solution prepared and that of the dissolved extract were transferred to separate curettes and their respective absorbance were read in a spectrophotometer at 325 nm with reagent blank at zero.

Calculation:

$$\text{Concentration of Vitamin A in Sample} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

2.3.2 Determination of Vitamin C (Ascorbic Acid)

This was determined by the titrimetric method reported by Kirk and Sawyer [5]. A weighted sample was homogenized in 6 % EDTA/CA solution. The homogenate was filtered and used for analysis. 20 ml of 30 % KI was added to the homogenate followed by 100 ml of distilled water. 1.0 ml of 1.0 % starch solution was added to it and it was titrated against 0.1 M $CuSO_4$ solution. The end point was marked by a black coloration. A reagent blank was also titrated. Vitamin content was calculated based on the relationship below.

1.0 ml of 0.1 mole $CuSO_4$ = 0.88mg vitamin c.

$$\text{Vitamin C mg/100} = \frac{100 \times 0.888 \times \text{titre} - \text{blank}}{\text{Weight of Sample}}$$

2.3.3 Determination of Vitamin E (Tocopherol)

Vitamin E was determined by the Futter-Mayer Colometric method with association of other vitamin chemists in Kirk and Sawyer method [5]. 1.0 g of the sample was mixed with 10 ml of ethanoic sulphuric acid and boiled gently under reflux for 30 minutes. It was transferred to a separating funnel and treated with 3 x 30 ml diethyl ether and recovering ether layer each time, the ether extract was transferred to a desiccator and dried for 30 minutes and later evaporated to dryness at room temperature. The dried extract was dissolved in 10 ml of pure ethanol. 1.0 ml of the dissolved extract and equal volume of standard vitamin E were transferred to separate tubes. After continuous addition of 5.0 ml of absolute alcohol and 1.0 ml of concentrated nitric acid solution, the mixtures were allowed to stand for 5 minutes and the respective absorbance was measured in spectrophotometer meter at 410 nm with blank reagent at zero.

2.4 Antioxidant Enzyme Assay

2.4.1 Determination of catalase activity

Catalase activity was determined by measuring the decrease in absorbance at 240 nm in UV spectrophotometer (Ultrospec 3100 UV/Visible spectrophotometer, Amershan Bioscience) by monitoring the decomposition of H_2O_2 [6]. The reaction mixture contained 0.1 ml of suitably diluted serum in phosphate buffer (50.0 Mm, ph 7.0) and 2.9 ml of 30 Mm H_2O_2 in phosphate buffer. The reference reagent contained 0.1 ml of buffer and 2.9 ml of 30.0 mM H_2O_2 in buffer. An extinction coefficient for H_2O_2 at 240 nm of 40 M was used for the calculation. The specific activity of catalase was expressed as moles of H_2O_2 reduced/min/mg. protein.

2.4.2 Determination of Superoxide Dismutase (SOD)

SOD was assayed according to the standard method described by Kakkar and other chemists [7]. The assay of SOD was based on the inhibition of the formation of NADH-phenaine methosulphate-nitroblue tetralium frmazon. The colour formed at the end of the reaction was extracted into butanol and measured at 560 nm.

2.4.3 Determination of Glutathione Peroxidase (GPx)

Glutathione peroxidase was assayed by the standard procedure adopted by Reddy and other researchers [8]. In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product

called purpurogalli was followed spectro-photometrically at 430 nm.

2.4.4 Total antioxidant capacity (TAC) assay

The Total Antioxidant Capacity (TAC) of the extract (*Persea americana*) in different extracting solvents (absolute ethanol, 70% and 50% ethanol) was determined by the phosphomolybdate method [9].

2.4.5 Heavy metal analysis

Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of American Public Health Association [10].

3. RESULTS AND DISCUSSION

The waste peel of *Persea americana* was investigated to obtain phytochemical contents as well as the presence of some important minerals, vitamins and antioxidant enzymes activities.

3.1 Phytocompounds

The quantitative phytochemical analysis of *Persea americana* fruit peel is graphically represented in Fig. 1. The peel of *Persea americana* possess mainly flavonoids followed by alkaloids (phenolic compounds), among the studied plant phytochemical compounds.

The high presence of flavonoids in the peels of *Persea americana* is likely to be responsible for the free radical scavenging effect. Flavonoids and polyphenols are major group of compounds that may act as primary free radical scavengers [11]. This also suggests that *Persea americana* fruit peels have the potential to inhibit activities of α-amylase and α-glucosidase enzyme. This could be exploited in pharmacological production of anti-diabetic drugs. Flavonoids have diverse favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc [12]. They are associated with a broad spectrum of health-promoting effects and an indispensable component in a variety of pharmaceutical, medicinal and cosmetic applications. This is because of their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme functions [12]. Natural and synthetic antioxidants have been used successfully as prophylactic or therapeutic agents for these human diseases caused by oxidative stress [2].

The presence of alkaloids and phenols (Fig. 1) in the peel also contributed to the antioxidant activity of *Persea americana* fruit peel, since plants extract containing alkaloids and phenols are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements [11]. They are also known for their biological activities which include anti-oxidant activity, muscle relaxant property, anti-microbial, amoebicidal, anti-cancer and anti-diabetic activities [13].

The tannin content of *Persea americana* although in small concentration, contributed to the antioxidant activity of the peel. Polyphenols (such as tannins) are a major group of compounds that act as primary antioxidants or free radial scavengers, hence block the action of free radicals, which have been implicated in the pathogenesis of many diseases and in the ageing process [11]. Tannins could be an effective ameliorative agent of the kidney; it has also shown to be potential anti-viral, anti-bacterial and anti-parasitic agents [14].

The presence of saponins also contributed to the antioxidant activity in *Persea americana* fruit peel and it's in agreement with other reported work about the Health benefits of saponins such as anti-carcinogenic, antitumor, antivirus, anti-hepatic and anti-hepatoprotective properties [15]. They possess hypocholesterolemic, immunostimulatory, and anticarcinogenic properties. In addition, they reduce the risk of heart diseases in humans consuming a diet rich in food legumes containing saponins. Saponin-rich foods are important in human diets to control plasma cholesterol, preventing peptic ulcer and osteoporosis and to reduce the risk of heart disease [16].

The presence of oxalate and phytates from the result were found to be relatively low in concentration. However, the presence of these anti-nutrients also contributed to the antioxidant activity of *Persea americana* fruit peel. Phytic acid has been recognized as a potent antioxidant because of its inhibitory effect on iron catalyzed hydroxyl radical via the Fenton-type reaction. Iron-mediated oxidative damage involved in the progression of Parkinson's disease are reported to be inhibited by phytates [11]. These discovered phytochemical compounds in *Persea americana* fruit peel are known to possess

biological properties such as cardiovascular protection and improvement of endothelial functions, as well as inhibition of angiogenesis and cell proliferation activities as also stated in a previous work [17].

3.2 Minerals

Copper, zinc, iron and manganese which are essential in antioxidant enzyme metabolism were the minerals found in the *Persea americana* fruit peel (Table 1). The minerals concentrations are within the standard for human mineral requirements (ppm) range according to FAO/WHO [17]. No selenium was detected in *Persea americana* peel extract. In living organisms, Umerie and Ekuna reported that, these minerals serve as enzyme cofactor for antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase [17].

3.3 Vitamins

The result for vitamin assay in Table 2 showed lipid soluble vitamins (A and E), to be present in very small quantities when compared to the values of FAO/WHO standard [18]. These vitamins have been shown to elicit good radical scavenging properties [19]. Vitamin A is an important antioxidant, a property shared with vitamins E and C, respectively. It is also important for growth and development, the maintenance of immune function and maintenance of epithelial cell integrity, good vision, reproduction as well as lipid metabolism [20].

Vitamin E is the major lipid-soluble component in the cell antioxidant defense system and has numerous important roles within the body because of its antioxidant activity [21]. Vitamin E main function is as an antioxidant, in which it helps protect the cells of the body against damage that can lead to health problems such as cancer. Vitamin E helps prevent the oxidation of LDL or bad cholesterol which contributes to plaque buildup in the arteries. It boosts the immune system and reduces the risk of cataracts [21].

The result from Table 2 showed that ascorbic acid or vitamin C was present in high concentration which contributed majorly to the antioxidant activity of the fruit peel of *Persea americana*. The high level of vitamin C seen in the result shows that *Persea americana* peel is a good source of vitamin C which is a cofactor for enzymes involved in regulating other antioxidants. It is one of the nutrient non-enzymatic anti-oxidants [22]. As a general rule, it was detected that vitamin C acts as a pro-oxidant at low doses and acts as an antioxidant in high doses [23]. It also has an important role in the maintenance of a healthy immune system and its deficiency causes immune insufficiency and multiple infections. The ascorbic acid level is lowered in various body fluids during bacterial infections [23].

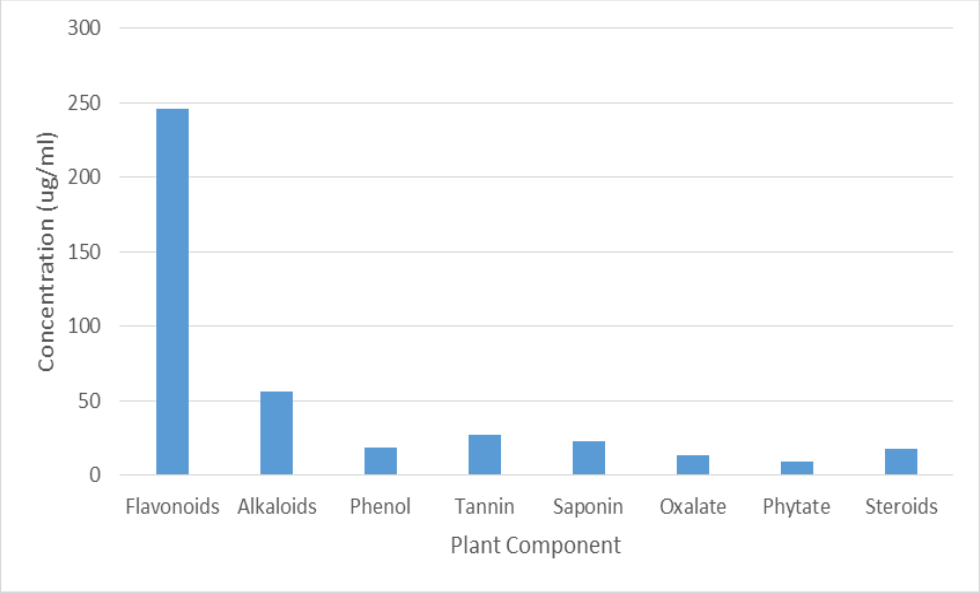

Fig. 1. Graphical representation of results of the quantitative phytochemical analysis of *Persea americana* peel

Table 1. Result for metal analysis of Zinc, Copper, Iron and Manganese

Parameters	Concentration (ppm)	FOA/WHO standard for human mineral requirements (ppm)
Zinc ppm	0.564	3.0
Copper ppm	0.083	2.0
Iron ppm	0.144	3.27
Manganese ppm	0.139	0.5
Selenium ppm	0 ppm	4.2

Table 2. Result of Non-enzymatic assay of *Persea americana* fruit peel

Parameters	*Persea americana* (mg/100g)	FAO/WHO standard for human vitamin requirements
Vitamin A	0.06806	0.18 - 0.45
Vitamin E	0.0022935	0.2 - 2.7
Vitamin C (Ascorbic acid)	470.8	5.0 -70

Values are mean of duplicate determination

3.4 Anti-oxidant Enzymes

Total SOD activity standard can vary from 20-90 units/mg protein, catalase activity can vary from 5 to 30 ml/mg protein and GPx activity is in the range of 14-30 units/mg protein. The concentration of anti-oxidant enzymes SOD was high when compared to the standard, while that of CAT, and GPx were relatively low compared to their standards as can be seen in Table 3. The increased activity of SOD enzyme in the *Persea americana* peels suggest that it is a good source of antioxidant. SODs form the frontline of defense against reactive oxygen species (ROS)-mediated injury [24]. These proteins catalyze the dismutation of superoxide anion free radical (O^{-2}) into molecular oxygen and hydrogen peroxide and decrease O^{2-} level which mages the cells at excessive concentration [25]. CAT is one of the most important antioxidant enzyme scavenging the active oxygen species in plant cells SOD, CAT, Glutathione peroxidase are known as the first line of defense antioxidants. [26].

Table 3. Result of Antioxidant enzyme activity of *Persea americana* fruit peel

Parameters	*Persea Americana*
Catalase (CAT)	0.0273 umol/ml
Superoxide Dismutase (SOD)	768.9 units /mg
Glutathione peroxidase (GPx)	267.132 umol/ml

Values are mean of duplicate determination

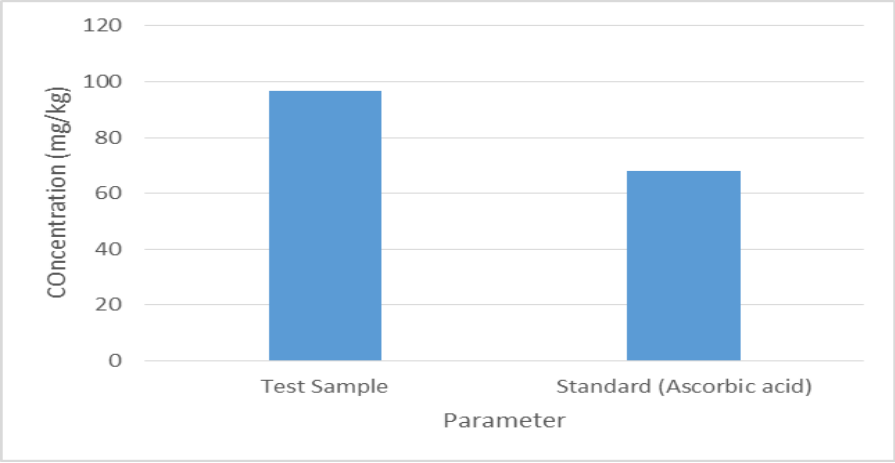

Fig. 2. Graphical representation of Total Antioxidant Capacity (TAC) of *Persea americana* compared with ascorbic acid as a standard

3.5 Total Antioxidant Capacity (TAC)

The results for the Total Antioxidant Capacity (Fig. 2**)** of the fruit peel of *Persea americana* was high when compared to the ascorbic acid. The observed high total antioxidant capacity of the fruit peels of *Persea americana* could be due to the presence of the different phenolic compounds.

4. CONCLUSION

Plants produce large amount of antioxidants to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity. Increasing knowledge of antioxidant phyto-constituents and their inclusions can give sufficient support to human body to fight against those diseases.

The result obtained in the present investigation revealed the presence of important phytochemicals, vitamins, minerals and antioxidant enzymes in *Persea americana* fruit peels. The concentration of phytochemicals, vitamins, minerals and the activity of antioxidant enzymes suggests that the fruit peels could serve as a viable source of natural antioxidant that could be of great importance as therapeutic agents in preventing or slowing the progress of ageing and age associated oxidative stress related degenerative diseases. Thus, they might have potentials as nutraceticals for preparation of functional foods.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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