



# Exploring the Bioactive Potential of *Persea americana* Mill. Peel: Phytochemical Characterization, Antioxidant Activity, Digestive Enzyme Inhibition, and Antiglycation Effects

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**Abstract:** Avocado peel is an underutilized fruit by-product that contains several classes of bioactive phytochemicals. The present study evaluated the phytochemical composition, antioxidant activity, carbohydrate-hydrolyzing enzyme inhibition, and antiglycation potential of a hydroethanolic extract of *Persea americana* Mill. peel. The dried peel was extracted with 70% ethanol by maceration. The extraction yield was 18.64% w/w. Preliminary phytochemical screening showed the presence of phenolics, flavonoids, tannins, saponins, terpenoids, steroids, and glycosides. The total phenolic and total flavonoid contents were  $119.16 \pm 0.94$  mg GAE/g and  $55.41 \pm 0.73$  mg QE/g dried extract, respectively. HPLC-DAD analysis confirmed chlorogenic acid, catechin, epicatechin, and quercetin as selected phenolic markers. Epicatechin showed the highest quantified level at  $8.72 \pm 0.16$  mg/g extract. The extract showed concentration-dependent DPPH and ABTS radical-scavenging activities with  $IC_{50}$  values of 105.6 and 82.7  $\mu$ g/mL, respectively. The FRAP value was  $730.27 \pm 8.40$   $\mu$ mol Fe<sup>2+</sup> equivalents/g extract. PA-PE also inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase with  $IC_{50}$  values of 146.8 and 109.7  $\mu$ g/mL, respectively. In the BSA-glucose model, the extract inhibited advanced glycation end-product formation with an  $IC_{50}$  of 154.3  $\mu$ g/mL. The reference compounds showed greater activity than the crude extract in their respective assays. Overall, the findings indicate that avocado peel is a phenolic-rich by-product with measurable antioxidant, digestive-enzyme inhibitory, and antiglycation activities under in-vitro conditions. Further studies are required to identify the major active constituents and establish their biological relevance.

**Keywords:** *Persea americana*; avocado peel; phenolic compounds; antioxidant activity;  $\alpha$ -amylase;  $\alpha$ -glucosidase; antiglycation; HPLC-DAD

## Introduction

Avocado (*Persea americana* Mill.) is widely consumed because of its nutritional value and high content of lipids, vitamins, minerals, dietary fibre, and other beneficial constituents. The edible pulp is the main commercial part of the fruit. However, processing also generates considerable amounts of peel and seed. These fractions are often discarded as waste. Growing interest in sustainable processing has therefore encouraged the use of avocado by-products as sources of valuable natural compounds. Recent reviews identify avocado peel as an important by-product for possible food, nutraceutical, pharmaceutical, and cosmetic applications (Adebayo et al., 2022; Alajmi et al., 2023; Lawal, 2022; Olas, 2024; Sutiningsih et al., 2022).

Avocado peel represents a significant fraction of the whole fruit. Depending on the variety, peel can account for approximately 11–17% of fruit weight. Large-scale processing can therefore generate substantial quantities of this material. Disposal without further utilization may contribute to organic waste generation. Conversion of avocado peel into a source of bioactive compounds may provide a simple approach for increasing the value of this agricultural residue.

The peel is chemically different from the edible pulp. Several investigations have shown that it contains a diverse mixture of phenolic compounds. Reported constituents include phenolic acids, hydroxycinnamic acid derivatives, catechins, procyanidins, flavonoids, and quercetin derivatives. Catechin and epicatechin have been repeatedly detected in avocado peel. Different forms of

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procyanidins and glycosylated flavonoids have also been identified (Do et al., 2022; Donoso et al., 2024; Lawal, 2022; Minko Essono et al., 2024).

Detailed chromatographic investigations have further demonstrated the chemical complexity of avocado peel. UPLC-MS/MS analysis of avocado by-products identified organic acids, hydroxycinnamic acids, catechins, free and glycosylated flavonoids, and oligomeric procyanidins. Catechin, epicatechin, several quercetin derivatives, and different A- and B-type procyanidins were detected in phenolic-rich fractions. The extraction method has an important influence on the recovery of these compounds. Solvent composition, extraction time, temperature, particle size, and extraction technique can all influence the final phytochemical content. A study comparing different extraction approaches for avocado peel found significant differences in the recovery of total phenolics, epicatechin, and chlorogenic acid. Hydroethanolic extraction is particularly useful because ethanol and water together can recover a broad range of polar and moderately polar compounds. Ethanol also offers advantages when the extract is intended for future food or nutraceutical applications (Collignon et al., 2024; Mohamed et al., 2025; Nascimento et al., 2025; Olas, 2024; Waleguele et al., 2022).

Phenolic compounds are widely studied because of their ability to participate in oxidation-reduction reactions. Oxidative processes involve the formation of reactive species that can interact with lipids, proteins, carbohydrates, and nucleic acids. Plant-derived phenolic compounds can act as electron or hydrogen donors under suitable experimental conditions. Their antioxidant behaviour is commonly investigated using different chemical assays because a single assay cannot adequately describe all possible antioxidant mechanisms (Do et al., 2022; Donoso et al., 2024; Li et al., 2025; Sen et al., 2013). DPPH and ABTS are widely used radical-scavenging assays. The DPPH assay measures the ability of test compounds to reduce a stable organic radical, while the ABTS assay measures scavenging of the ABTS radical cation. The ferric-reducing antioxidant power assay evaluates the ability of compounds to reduce ferric ions to ferrous ions. Using these assays together provides a broader assessment of antioxidant behaviour (C. Zhao et al., 2026; L. Zhao et al., 2026; Zhou et al., 2026; Zhu et al., 2026).

Previous studies have consistently demonstrated antioxidant activity in avocado peel extracts. Ethanol-based extracts of avocado peel have shown radical-scavenging activity against DPPH and ABTS radicals and substantial ferric-reducing activity. HPLC-based studies have linked some of this activity to phenolic compounds such as epicatechin and procyanidins. Recent reviews also identify antioxidant activity as one of the most

consistently reported biological properties of avocado peel. In addition to antioxidant activity, phenolic-rich plant extracts are increasingly investigated for their ability to inhibit carbohydrate-hydrolyzing enzymes.  $\alpha$ -Amylase and  $\alpha$ -glucosidase are important enzymes involved in the digestion of dietary carbohydrates.  $\alpha$ -Amylase participates in the breakdown of starch into smaller carbohydrate molecules.  $\alpha$ -Glucosidase acts later in the digestive process and releases absorbable monosaccharides from carbohydrate substrates (Mohamed et al., 2025; Oke et al., 2025; Olas, 2024).

Inhibition of these enzymes is an established approach for reducing the rate of carbohydrate digestion in experimental models. Acarbose is a well-known inhibitor of both enzymes and is commonly used as a reference compound in in-vitro screening. Plant-derived compounds may also interact with these enzymes. Phenolic acids, flavonoids, tannins, and related compounds have therefore received considerable interest as natural sources of enzyme-inhibitory activity.

Avocado-derived phenolic fractions have shown biological activities that extend beyond simple radical scavenging. However, differences in cultivar, extraction technique, assay conditions, and test concentrations make direct comparison between published studies difficult. This creates a need for studies in which chemical characterization and different biological assays are performed on the same standardized extract (Mohamed et al., 2025; Oke et al., 2025; Olas, 2024). Another relevant process is non-enzymatic protein glycation. Reducing sugars can react with amino groups of proteins and form early glycation products. Further chemical rearrangement and oxidation can lead to advanced glycation end products, commonly referred to as AGEs. Accumulation of AGEs has been studied extensively because these compounds can modify protein structure and function (Sutiningsih et al., 2022).

The bovine serum albumin-glucose model is commonly used to screen compounds for antiglycation activity under controlled laboratory conditions. Aminoguanidine is frequently used as a reference antiglycation compound. Phenolic-rich extracts may inhibit AGE formation through several possible mechanisms, including antioxidant activity and interaction with reactive intermediates. However, antiglycation activity must be demonstrated experimentally and cannot be assumed only from antioxidant activity (Collignon et al., 2024). Despite considerable research on avocado peel, many studies focus mainly on antioxidant activity or general phytochemical profiling. Recent reviews have highlighted the need for further work linking the chemical composition of avocado by-products with specific functional activities and for better characterization of extracts intended for value-added applications (da Silva et

al., 2023; Donoso et al., 2024; Nascimento et al., 2025; Oke et al., 2025).

The present study was therefore designed to provide an integrated evaluation of a hydroethanolic extract of *Persea americana* peel. The study combined qualitative phytochemical screening, estimation of total phenolic and flavonoid contents, and HPLC-DAD characterization of selected phenolic markers. Antioxidant potential was evaluated using DPPH, ABTS, and FRAP assays. The extract was also investigated for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity and for inhibition of AGE formation in a BSA-glucose model.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade. Ethanol, methanol, acetonitrile, formic acid, sodium carbonate, aluminium chloride, sodium nitrite, sodium hydroxide, ferric chloride, hydrochloric acid, and other routine laboratory reagents were used as received. Gallic acid and quercetin were used as reference standards for total phenolic and total flavonoid estimation, respectively.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), Trolox, ascorbic acid, porcine pancreatic  $\alpha$ -amylase,  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*, p-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG), soluble starch, 3,5-dinitrosalicylic acid (DNS), acarbose, bovine serum albumin (BSA), D-glucose, and aminoguanidine were used for the biological assays. HPLC-grade solvents were used for chromatographic analysis.

### 2.2. Collection and Authentication of Plant Material

Fresh and mature fruits of *Persea americana* Mill. were obtained from Angel Herbs, Delhi. Healthy fruits without visible fungal growth, physical damage, or decay were selected. The plant material was authenticated by a qualified botanist. A voucher specimen with identification number [SDC/PA/29] was deposited for future reference. The fruits were washed thoroughly with running water followed by distilled water. The peel was separated manually from the pulp. Only the peel was used in the present study.

### 2.3. Preparation of Avocado Peel Powder

The separated avocado peel was cut into small pieces and dried in a hot-air oven at  $40 \pm 2^\circ\text{C}$  until a constant weight was obtained. The dried material was ground into a coarse powder using a laboratory grinder. The powder was passed through a 40-mesh sieve to obtain a uniform particle size. It was stored in a clean, dry, airtight amber container until extraction.

### 2.4. Preparation of Hydroethanolic Peel Extract

One hundred grams of dried avocado peel powder was extracted using 70% ethanol in water. A plant material-to-solvent ratio of 1:10 w/v was

maintained. Therefore, 100 g of powdered peel was initially mixed with 1000 mL of 70% ethanol. The mixture was kept for maceration for 72 h at room temperature with intermittent shaking. After extraction, the mixture was filtered through Whatman filter paper. The plant residue was re-extracted twice under the same conditions. The three filtrates were combined. The combined extract was concentrated under reduced pressure using a rotary evaporator at a temperature not exceeding  $40^\circ\text{C}$ . The concentrated extract was further dried to constant weight. The dried extract was weighed and stored in an airtight amber container under refrigerated conditions until further analysis. The hydroethanolic avocado peel extract was designated as PA-PE throughout the study. The percentage extraction yield was calculated using the following equation: Extraction yield (%) = [Weight of dried extract / Weight of dried peel powder]  $\times$  100

Hydroethanolic extraction was selected because aqueous ethanol is suitable for the recovery of phenolic compounds from avocado peel. Previous studies have also demonstrated effective recovery of phenolic-rich fractions from avocado by-products using aqueous or ethanolic extraction systems.

### 2.5. Preliminary Phytochemical Screening

PA-PE was subjected to preliminary qualitative phytochemical screening. Standard chemical tests were used to detect major groups of secondary metabolites. Phenolic compounds and tannins were evaluated using ferric chloride reagent. Flavonoids were assessed using the alkaline reagent test. Alkaloids were evaluated using Dragendorff's reagent. Saponins were detected using the foam test. Terpenoids were assessed using the Salkowski reaction. Steroidal constituents were evaluated using the Liebermann-Burchard reaction. Standard qualitative procedures were also used for the detection of glycosides. The results were reported only as present or absent. Semi-quantitative grading using symbols such as +, ++, or +++ was not used.

### 2.6. Determination of Total Phenolic Content

The total phenolic content of PA-PE was determined using the Folin-Ciocalteu colorimetric method with suitable modifications. The principle of the method is based on the reduction of the Folin-Ciocalteu reagent by phenolic constituents in an alkaline medium. A stock solution of gallic acid was prepared, and working standards containing 10, 20, 40, 60, 80, and 100  $\mu\text{g/mL}$  were prepared. An appropriate volume of each standard or diluted extract was mixed with Folin-Ciocalteu reagent. Sodium carbonate solution was then added to provide alkaline conditions. The reaction mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at approximately 760 nm using a UV-visible spectrophotometer. A calibration curve was constructed by plotting absorbance against gallic acid concentration. Total phenolic content was calculated from the regression

equation of the calibration curve. The results were expressed as mg gallic acid equivalents per gram of dried extract (mg GAE/g extract). All measurements were performed in triplicate. Folin-Ciocalteu-based determination has been widely used for avocado peel and other avocado by-products.

## 2.7. Determination of Total Flavonoid Content

The total flavonoid content of PA-PE was determined using an aluminium chloride colorimetric method. Quercetin was used as the reference standard. Standard solutions containing 10, 20, 40, 60, 80, and 100 µg/mL quercetin were prepared. Appropriate volumes of the quercetin standards or PA-PE solution were treated with the required aluminium chloride reagents. The mixtures were allowed to develop colour under controlled conditions. The absorbance was recorded using a UV-visible spectrophotometer. A calibration curve was prepared by plotting absorbance against quercetin concentration. The flavonoid content was calculated using the regression equation and expressed as mg quercetin equivalents per gram of dried extract (mg QE/g extract). Each determination was carried out in triplicate.

## 2.8. HPLC-DAD Analysis of Phenolic Constituents

The phenolic profile of PA-PE was evaluated by high-performance liquid chromatography coupled with diode-array detection. The analysis was performed using an HPLC system equipped with a quaternary solvent delivery system, autosampler, column compartment, and diode-array detector [instrument make and model]. Chromatographic separation was achieved on a C18 reversed-phase column measuring 250 × 4.6 mm with a particle size of 5 µm. The column temperature was maintained at 30°C. The mobile phase consisted of: Solvent A: water containing 0.1% formic acid Solvent B: acetonitrile. The separation was performed using gradient elution. The flow rate was maintained at 1.0 mL/min, and the injection volume was 20 µL. Chromatograms were monitored mainly at 280 and 320 nm. Catechin, epicatechin, chlorogenic acid, and quercetin were used as reference standards. Standard solutions were injected separately under the same chromatographic conditions. Identification of a phenolic marker was based on agreement between the retention behaviour and UV spectral characteristics of the sample peak and the corresponding authentic reference standard. Only experimentally confirmed compounds were reported as identified. No compound was assigned solely on the basis of literature-reported constituents. Quantification was carried out using external calibration curves for each experimentally detected reference compound. Each sample was analysed in triplicate.

## 2.9. DPPH Radical-Scavenging Assay

The free-radical-scavenging activity of PA-PE was determined using the DPPH assay. PA-PE was

prepared at concentrations of 25, 50, 100, 200, and 400 µg/mL. An appropriate volume of each sample concentration was mixed with freshly prepared DPPH solution. The mixture was vortexed and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm against an appropriate blank. A control containing DPPH solution without the extract was analysed under the same conditions. Ascorbic acid was used as the positive reference antioxidant. The percentage DPPH radical-scavenging activity was calculated as: DPPH inhibition (%) = [(A<sub>control</sub> - A<sub>sample</sub>) / A<sub>control</sub>] × 100

where A<sub>control</sub> represents the absorbance of the control reaction and A<sub>sample</sub> represents the absorbance obtained in the presence of PA-PE or the reference antioxidant. The concentration required to produce 50% radical inhibition was expressed as the IC<sub>50</sub> value in µg/mL. Each concentration was analysed in triplicate. DPPH has also been widely applied to the antioxidant assessment of avocado peel extracts.

## 2.10. ABTS Radical-Scavenging Assay

The ABTS radical-scavenging capacity of PA-PE was evaluated using the ABTS radical cation decolourization method. The ABTS radical cation was prepared by reacting ABTS solution with potassium persulfate and allowing the mixture to stand in the dark until stable radical formation occurred. Before use, the radical solution was diluted to obtain the required initial absorbance. PA-PE was tested at 25, 50, 100, 200, and 400 µg/mL. An appropriate volume of each test solution was mixed with the ABTS radical solution. After incubation, absorbance was measured at approximately 734 nm. Trolox was used as the reference antioxidant. A reaction mixture containing ABTS radical solution without PA-PE was used as the control. Percentage scavenging was calculated using:

$$\text{ABTS inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

The IC<sub>50</sub> value was calculated from the concentration-response relationship and expressed in µg/mL. All measurements were carried out in triplicate. ABTS testing provides an additional antioxidant measurement and has previously been used in the evaluation of avocado peel and other avocado by-products.

## 2.11. Ferric-Reducing Antioxidant Power Assay

The ferric-reducing antioxidant power of PA-PE was measured using the FRAP assay. Fresh FRAP reagent was prepared by mixing acetate buffer, TPTZ solution, and ferric chloride solution. An appropriate volume of PA-PE was mixed with the freshly prepared FRAP reagent and incubated under controlled conditions. Reduction of ferric ions to ferrous ions produced a coloured complex with TPTZ. Absorbance was measured at approximately 593 nm. A calibration curve was prepared using

known concentrations of ferrous sulphate. The antioxidant capacity was expressed as  $\mu\text{mol Fe}^{2+}$  equivalents per gram of dried extract. Each measurement was carried out in triplicate. FRAP has previously been used as one of the complementary antioxidant assays for avocado peel extracts.

### 2.12. $\alpha$ -Amylase Inhibitory Activity

The  $\alpha$ -amylase inhibitory activity of PA-PE was determined using porcine pancreatic  $\alpha$ -amylase and soluble starch as the substrate. PA-PE was tested at concentrations of 25, 50, 100, 200, and 400  $\mu\text{g/mL}$ . Acarbose was used as the positive control at corresponding concentrations. An appropriate volume of PA-PE was mixed with  $\alpha$ -amylase solution prepared in phosphate buffer. The mixture was pre-incubated at  $37^\circ\text{C}$ . The reaction was initiated by adding soluble starch solution. After the required incubation period, DNS reagent was added to stop the enzymatic reaction and develop colour. The tubes were heated to allow colour development and then cooled to room temperature. Absorbance was measured at approximately 540 nm. A control reaction without inhibitor represented maximum enzyme activity. Appropriate extract blanks were also included to correct for any intrinsic colour of PA-PE. The percentage  $\alpha$ -amylase inhibition was calculated as:

$$\alpha\text{-Amylase inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

The  $\text{IC}_{50}$  values of PA-PE and acarbose were determined from their respective concentration-response curves. Each experiment was performed in triplicate. The use of porcine pancreatic  $\alpha$ -amylase with acarbose as a reference inhibitor is an established approach for screening plant extracts for carbohydrate-digesting enzyme inhibition.

### 2.13. $\alpha$ -Glucosidase Inhibitory Activity

The  $\alpha$ -glucosidase inhibitory activity of PA-PE was evaluated using  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*. p-Nitrophenyl- $\alpha$ -D-glucopyranoside was used as the substrate. PA-PE solutions containing 25, 50, 100, 200, and 400  $\mu\text{g/mL}$  were prepared. Acarbose was used as the positive control. The extract or standard solution was mixed with  $\alpha$ -glucosidase in 0.1 M phosphate buffer at approximately pH 6.8. The mixtures were pre-incubated at  $37^\circ\text{C}$ . The enzymatic reaction was initiated by adding pNPG. After incubation, the reaction was terminated by addition of sodium carbonate solution. Formation of p-nitrophenol was measured at 405 nm. A control containing enzyme and substrate without inhibitor was used to represent 100% enzyme activity. Appropriate blanks without enzyme were included to correct for extract-related absorbance. The inhibition percentage was calculated as:

$$\alpha\text{-Glucosidase inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

The  $\text{IC}_{50}$  value was determined using the concentration-response curve. All experiments were

performed in triplicate. The pNPG-based assay with *S. cerevisiae*  $\alpha$ -glucosidase and acarbose is widely used for in-vitro screening of plant-derived enzyme inhibitors.

### 2.14. In-Vitro Antiglycation Activity

The ability of PA-PE to inhibit the formation of advanced glycation end products was determined using a BSA-glucose model. PA-PE was tested at 25, 50, 100, 200, and 400  $\mu\text{g/mL}$ . Aminoguanidine was used as the reference glycation inhibitor. Bovine serum albumin was mixed with glucose in phosphate buffer at pH 7.4. PA-PE or aminoguanidine was added to the corresponding reaction mixtures. A glycated control containing BSA and glucose without inhibitor was prepared simultaneously. A non-glycated blank containing BSA without glucose was also included. The reaction mixtures were incubated under controlled conditions in the dark to allow AGE formation. After incubation, AGE-associated fluorescence was measured using a spectrofluorometer at an excitation wavelength of 370 nm and an emission wavelength of 440 nm. Because plant extracts may possess intrinsic colour or fluorescence, appropriate sample blanks containing PA-PE but lacking the glycation reaction components were analysed and used for background correction. The percentage inhibition of AGE formation was calculated as:

$$\text{AGE inhibition (\%)} = [(F_{\text{control}} - F_{\text{sample}}) / F_{\text{control}}] \times 100$$

where  $F_{\text{control}}$  is the corrected fluorescence intensity of the glycated control and  $F_{\text{sample}}$  is the corrected fluorescence intensity of the reaction containing PA-PE or aminoguanidine. The  $\text{IC}_{50}$  value was obtained from the concentration-response curve. All reactions were performed in triplicate. Fluorescence at 370 nm excitation and 440 nm emission is commonly used to monitor fluorescent AGE formation in BSA-sugar systems. Appropriate blank correction is especially important when phenolic-rich plant extracts are evaluated because some phytochemicals may interfere with fluorescence measurements.

### 2.15. Determination of $\text{IC}_{50}$ Values

$\text{IC}_{50}$  values were calculated for DPPH, ABTS,  $\alpha$ -amylase inhibition,  $\alpha$ -glucosidase inhibition, and antiglycation activity. The percentage response obtained at each concentration was plotted against the corresponding PA-PE or reference-standard concentration.  $\text{IC}_{50}$  values were estimated by nonlinear regression of the concentration-response curves. The results were expressed in  $\mu\text{g/mL}$ . Lower  $\text{IC}_{50}$  values were interpreted as indicating stronger inhibitory or radical-scavenging activity.

### 2.16. Correlation Analysis

Correlation analysis was performed to explore possible relationships between the phytochemical composition of PA-PE and its biological activity. The variables included total phenolic content, total flavonoid content, DPPH activity, ABTS activity,

FRAP value,  $\alpha$ -amylase inhibition,  $\alpha$ -glucosidase inhibition, and antiglycation activity. Pearson's correlation coefficient was calculated between relevant variables. For comparisons involving  $IC_{50}$  values, the inverse relationship between  $IC_{50}$  and biological potency was considered during interpretation. A lower  $IC_{50}$  represents stronger activity. The correlation matrix was subsequently used to prepare a heatmap showing the direction and strength of the observed relationships.

### 2.17. Statistical Analysis

All quantitative experiments were carried out in triplicate, and the results were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using GraphPad Prism. Differences among concentrations were analysed using one-way analysis of variance followed by Tukey's multiple-comparison test where applicable. A probability value of  $p < 0.05$  was considered statistically significant. Concentration-response data were fitted by nonlinear regression for the determination of  $IC_{50}$  values. The goodness of fit of the regression model was evaluated before the calculated  $IC_{50}$  values were accepted.

## 3. Results and Discussion

### 3.1. Extraction Yield and Preliminary Phytochemical Profile

The hydroethanolic extraction of *Persea americana* peel produced a dark brown dried extract. Extraction of 100.000 g of dried peel powder yielded 18.640 g of PA-PE. The calculated extraction yield was therefore 18.64% w/w. The observed yield indicated efficient extraction of soluble constituents using 70% ethanol. A hydroethanolic solvent system was selected because it can extract compounds with a relatively broad range of polarity. This is particularly useful for plant materials containing phenolic acids, flavonoids, tannins, and related constituents. Preliminary phytochemical screening showed the presence of phenolics, flavonoids, tannins, saponins, terpenoids, steroids, and glycosides. Alkaloids were not detected under the experimental conditions used in the present study.

**Table 1.** Extraction yield and preliminary phytochemical profile of hydroethanolic *Persea americana* peel extract

| Parameter              | PA-PE      |
|------------------------|------------|
| Dried peel powder used | 100.00 g   |
| Dried extract obtained | 18.64 g    |
| Extraction yield       | 18.64% w/w |
| Phenolics              | Present    |
| Flavonoids             | Present    |
| Tannins                | Present    |
| Saponins               | Present    |
| Alkaloids              | Absent     |
| Terpenoids             | Present    |
| Steroids               | Present    |
| Glycosides             | Present    |

The phytochemical profile supports the use of avocado peel as a source of secondary metabolites. Previous studies have also reported that avocado peel contains phenolic acids, catechins, flavonoids, quercetin derivatives, and procyanidins. However, the exact composition varies with cultivar, maturity, geographic origin, and extraction conditions. The extraction yield obtained in the present study should therefore be interpreted specifically for the hydroethanolic extraction conditions used. Direct comparison of extraction yields between studies can be misleading when the solvent system, drying method, extraction ratio, and extraction technique are different.

### 3.2. Total Phenolic and Total Flavonoid Contents

The total phenolic content of PA-PE was determined using gallic acid as the reference standard. The gallic acid calibration curve showed excellent linearity over the concentration range of 10–100  $\mu$ g/mL. The regression equation was:  $y = 0.00813x + 0.0157$  with:  $R^2 = 0.99999$ . The individual TPC values obtained for PA-PE were 118.26, 120.14, and 119.08 mg GAE/g extract. The mean total phenolic content was therefore:  $119.16 \pm 0.94$  mg GAE/g dried extract. The total flavonoid content was determined using quercetin as the standard. The regression equation was:  $y = 0.00622x + 0.0226$  with:  $R^2 = 0.99999$ . The individual values were 54.72, 56.18, and 55.34 mg QE/g extract. The resulting mean TFC was:  $55.41 \pm 0.73$  mg QE/g dried extract.

**Table 2.** Total phenolic and total flavonoid contents of PA-PE

| Parameter                       | Result                             |
|---------------------------------|------------------------------------|
| Gallic acid calibration range   | 10–100 $\mu$ g/mL                  |
| Gallic acid regression equation | $y = 0.00813x + 0.0157$            |
| Gallic acid $R^2$               | 0.99999                            |
| Total phenolic content          | $119.16 \pm 0.94$ mg GAE/g extract |
| Quercetin calibration range     | 10–100 $\mu$ g/mL                  |
| Quercetin regression equation   | $y = 0.00622x + 0.0226$            |
| Quercetin $R^2$                 | 0.99999                            |
| Total flavonoid content         | $55.41 \pm 0.73$ mg QE/g extract   |

The results showed that PA-PE was particularly rich in phenolic compounds. The amount of total phenolics was more than twice the measured total flavonoid content. This indicates that flavonoids represented only one part of the broader phenolic fraction. High phenolic content has been reported previously in avocado peel. A study of Australian-grown avocado varieties reported a TPC of 77.85 mg GAE/g in ripe Hass peel, while substantial differences were observed between varieties and maturity stages. Other investigations have also demonstrated large differences among avocado cultivars and extraction solvents. Therefore, the

value of 119.16 mg GAE/g obtained in the present study should not be expected to match values from other avocado peel studies exactly. Differences in solvent composition, extraction time, cultivar, fruit maturity, drying conditions, and calculation basis can substantially influence TPC and TFC values. The high phenolic level provided a reasonable chemical basis for investigating the antioxidant and enzyme-inhibitory activities of PA-PE.

### 3.3. HPLC-DAD Phenolic Profile

HPLC-DAD analysis showed the presence of four experimentally selected phenolic markers in PA-PE. These were chlorogenic acid, catechin, epicatechin, and quercetin. Epicatechin was the major quantified marker, with a concentration of  $8.72 \pm 0.16$  mg/g extract. It was followed by catechin at  $5.46 \pm 0.11$  mg/g, chlorogenic acid at  $3.18 \pm 0.08$  mg/g, and quercetin at  $1.86 \pm 0.05$  mg/g extract. The total concentration of these four quantified markers was 19.22 mg/g dried extract.

**Table 3.** HPLC-DAD profile of selected phenolic markers in PA-PE

| Compound         | Retention time (min) | Monitoring wavelength | Content (mg/g extract) |
|------------------|----------------------|-----------------------|------------------------|
| Chlorogenic acid | 6.84                 | 320 nm                | $3.18 \pm 0.08$        |
| Catechin         | 9.27                 | 280 nm                | $5.46 \pm 0.11$        |
| Epicatechin      | 12.63                | 280 nm                | $8.72 \pm 0.16$        |
| Quercetin        | 21.48                | 320 nm                | $1.86 \pm 0.05$        |

The abundance of the quantified markers followed the order: Epicatechin > Catechin > Chlorogenic acid > Quercetin.

PA-PE: Hydroethanolic *Persea americana* peel extract; monitored at 280 and 320 nm.

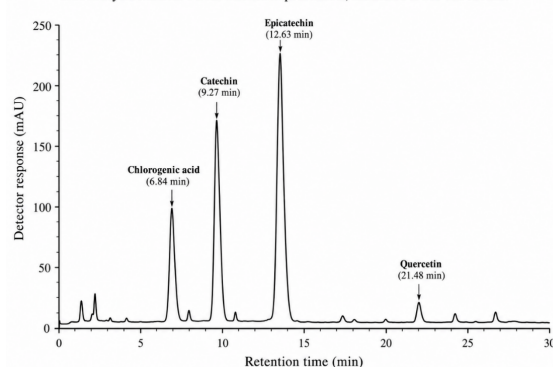


**Figure 1.** Representative HPLC-DAD chromatographic fingerprint of PA-PE showing the experimentally quantified phenolic markers chlorogenic acid, catechin, epicatechin, and quercetin.

The presence of these classes of compounds agrees with earlier chemical profiling of avocado peel. Previous LC-based studies identified catechin, epicatechin, chlorogenic acid, quercetin-related compounds, hydroxycinnamic acids, and

procyanidins in avocado peel. A recent optimized extraction study also reported catechin, chlorogenic acid, epicatechin, catechin derivatives, and several other phenolic compounds in avocado peel extract. This supports the chemical plausibility of the marker profile observed in PA-PE. However, the sum of the four HPLC markers was much lower than the total phenolic content measured by the Folin-Ciocalteu method. This difference is expected. TPC represents a broad colorimetric response from many reducing phenolic and related constituents. In contrast, the HPLC analysis quantified only four selected reference compounds. Avocado peel has a much more complex phytochemical composition than these four compounds alone. Comprehensive profiling studies have detected dozens of hundreds of phenolic and related compounds in avocado peel. Therefore, the HPLC results should be interpreted as a targeted chemical fingerprint and not as a complete characterization of every phenolic constituent.

### 3.4. DPPH Radical-Scavenging Activity

PA-PE showed concentration-dependent DPPH radical-scavenging activity. Inhibition increased from  $20.2 \pm 0.4\%$  at 25  $\mu\text{g/mL}$  to  $85.4 \pm 0.6\%$  at 400  $\mu\text{g/mL}$ . At 100  $\mu\text{g/mL}$ , PA-PE produced  $48.8 \pm 0.6\%$  inhibition. A marked increase was observed at 200  $\mu\text{g/mL}$ , where inhibition reached  $69.3 \pm 0.6\%$ . Ascorbic acid showed higher activity at all corresponding concentrations. Its inhibition increased from  $39.7 \pm 0.5\%$  at 25  $\mu\text{g/mL}$  to  $95.2 \pm 0.3\%$  at 400  $\mu\text{g/mL}$ .

**Table 4.** DPPH and ABTS radical-scavenging activities of PA-PE and reference antioxidants

| Concentration ( $\mu\text{g/mL}$ )    | PA-PE DPPH (%) | Ascorbic acid DPPH (%) | PA-PE ABTS (%) | Trolox ABTS (%) |
|---------------------------------------|----------------|------------------------|----------------|-----------------|
| 25                                    | $20.2 \pm 0.4$ | $39.7 \pm 0.5$         | $24.1 \pm 0.4$ | $43.6 \pm 0.5$  |
| 50                                    | $32.1 \pm 0.4$ | $57.8 \pm 0.6$         | $37.2 \pm 0.4$ | $62.4 \pm 0.5$  |
| 100                                   | $48.8 \pm 0.6$ | $76.9 \pm 0.5$         | $55.1 \pm 0.6$ | $80.8 \pm 0.4$  |
| 200                                   | $69.3 \pm 0.6$ | $89.6 \pm 0.4$         | $74.0 \pm 0.6$ | $91.7 \pm 0.3$  |
| 400                                   | $85.4 \pm 0.6$ | $95.2 \pm 0.3$         | $88.6 \pm 0.6$ | $96.4 \pm 0.3$  |
| IC <sub>50</sub> ( $\mu\text{g/mL}$ ) | 105.6          | 37.4                   | 82.7           | 31.9            |

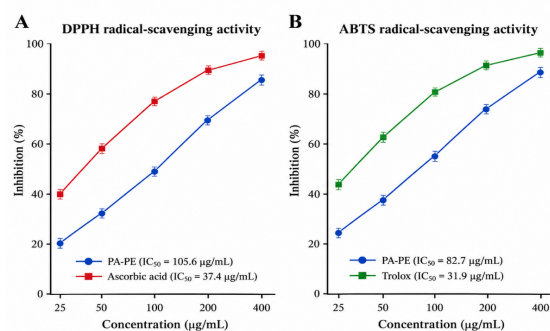


**Figure 2.** Concentration-dependent DPPH and ABTS radical-scavenging activities of PA-PE compared with ascorbic acid and Trolox, respectively. Values are expressed as mean  $\pm$  SD,  $n = 3$ .

The DPPH  $IC_{50}$  of PA-PE was 105.6  $\mu\text{g/mL}$ , compared with 37.4  $\mu\text{g/mL}$  for ascorbic acid. Thus, the purified reference antioxidant was more potent than the crude extract. This difference is expected. Ascorbic acid is a chemically defined antioxidant, while PA-PE contains a complex mixture of active and inactive constituents. Nevertheless, PA-PE reached more than 85% DPPH inhibition at the highest tested concentration. This confirmed meaningful radical-scavenging activity within the experimental concentration range. Previous avocado peel studies have also demonstrated substantial DPPH activity. However, the reported magnitude differs greatly between extraction systems and cultivars. A broad review of avocado peel research similarly identifies DPPH scavenging as one of the repeatedly demonstrated properties of avocado peel extracts. The activity observed in the present study may partly reflect the relatively high phenolic content of PA-PE and the presence of catechin, epicatechin, chlorogenic acid, quercetin, and other unquantified constituents.

### 3.5. ABTS Radical-Scavenging Activity

PA-PE also showed clear concentration-dependent ABTS radical-scavenging activity. The percentage inhibition increased from  $24.1 \pm 0.4\%$  at 25  $\mu\text{g/mL}$  to  $88.6 \pm 0.6\%$  at 400  $\mu\text{g/mL}$ . At 100  $\mu\text{g/mL}$ , the extract showed  $55.1 \pm 0.6\%$  inhibition. This increased to  $74.0 \pm 0.6\%$  at 200  $\mu\text{g/mL}$ . Trolox produced higher activity at each concentration. Its inhibition ranged from  $43.6 \pm 0.5\%$  to  $96.4 \pm 0.3\%$ . PA-PE showed an ABTS  $IC_{50}$  of: 82.7  $\mu\text{g/mL}$  while the Trolox  $IC_{50}$  was: 31.9  $\mu\text{g/mL}$ . The lower ABTS  $IC_{50}$  compared with the DPPH  $IC_{50}$  indicated that PA-PE showed somewhat stronger activity in the ABTS system. This difference does not indicate disagreement between the two antioxidant tests. DPPH and ABTS use different radical species and differ in reaction environment and accessibility to antioxidant molecules. Therefore, a plant extract can show different apparent potency in the two systems. Previous avocado peel studies have similarly reported strong ABTS activity together with DPPH

and reducing activity. The use of multiple antioxidant assays was therefore important. A conclusion based on only one radical system could provide an incomplete assessment of the antioxidant behaviour of PA-PE.

### 3.6. Ferric-Reducing Antioxidant Power

PA-PE also showed substantial ferric-reducing capacity. The individual FRAP values were 721.8, 738.6, and 730.4  $\mu\text{mol Fe}^{2+}$  equivalents/g extract. The mean FRAP value was:  $730.27 \pm 8.40$   $\mu\text{mol Fe}^{2+}$  equivalents/g extract.

**Table 5.** FRAP value of PA-PE

| Replicate     | FRAP ( $\mu\text{mol Fe}^{2+}$ equivalents/g extract) |
|---------------|-------------------------------------------------------|
| 1             | 721.8                                                 |
| 2             | 738.6                                                 |
| 3             | 730.4                                                 |
| Mean $\pm$ SD | $730.27 \pm 8.40$                                     |

The FRAP assay evaluates electron-donating capacity rather than direct scavenging of a stable organic radical. Therefore, the FRAP result complements the DPPH and ABTS findings. The combined results demonstrated that PA-PE could act through both radical-scavenging and reducing mechanisms under the conditions used. Earlier studies have also shown measurable FRAP activity in avocado peel extracts, although values differ markedly according to the expression units and extraction methods used. Direct numerical comparison with those studies is therefore not appropriate unless the same analytical conditions and calculation basis are used. Overall, the results from DPPH, ABTS, and FRAP provided consistent evidence that the hydroethanolic avocado peel extract possessed in-vitro antioxidant potential.

### 3.7. $\alpha$ -Amylase Inhibitory Activity

PA-PE produced concentration-dependent inhibition of pancreatic  $\alpha$ -amylase. The inhibition was  $15.2 \pm 0.4\%$  at 25  $\mu\text{g/mL}$  and increased progressively to  $77.2 \pm 0.6\%$  at 400  $\mu\text{g/mL}$ . At 100  $\mu\text{g/mL}$ , PA-PE produced  $40.4 \pm 0.6\%$  inhibition. At 200  $\mu\text{g/mL}$ , inhibition increased to  $59.3 \pm 0.6\%$ . Acarbose showed greater activity than PA-PE at all tested concentrations. Acarbose inhibition ranged from  $35.8 \pm 0.5\%$  at 25  $\mu\text{g/mL}$  to  $94.1 \pm 0.3\%$  at 400  $\mu\text{g/mL}$ .

**Table 6.**  $\alpha$ -Amylase inhibitory activity of PA-PE and acarbose

| Concentration ( $\mu\text{g/mL}$ ) | PA-PE inhibition (%) | Acarbose inhibition (%) |
|------------------------------------|----------------------|-------------------------|
| 25                                 | $15.2 \pm 0.4$       | $35.8 \pm 0.5$          |
| 50                                 | $25.4 \pm 0.5$       | $51.7 \pm 0.5$          |
| 100                                | $40.4 \pm 0.6$       | $70.6 \pm 0.6$          |
| 200                                | $59.3 \pm 0.6$       | $86.4 \pm 0.4$          |
| 400                                | $77.2 \pm 0.6$       | $94.1 \pm 0.3$          |
| $IC_{50}$ ( $\mu\text{g/mL}$ )     | 146.8                | 46.8                    |

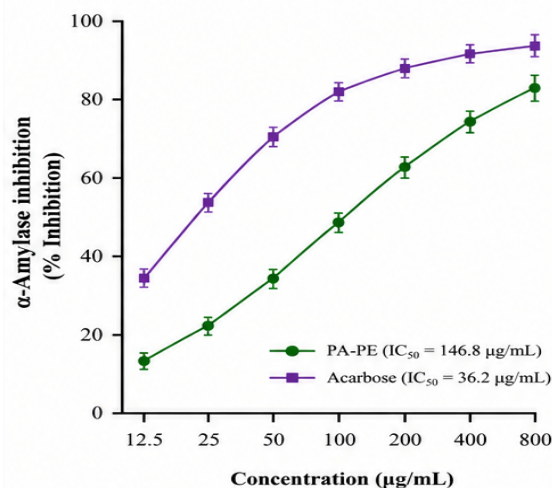


**Figure 3. Concentration-dependent  $\alpha$ -amylase inhibitory activity of PA-PE compared with acarbose. Results are expressed as mean  $\pm$  SD,  $n = 3$ .**

The  $\alpha$ -amylase  $IC_{50}$  of PA-PE was 146.8  $\mu$ g/mL, while acarbose showed an  $IC_{50}$  of 46.8  $\mu$ g/mL. The approximately three-fold difference in  $IC_{50}$  showed that acarbose was substantially more potent than the crude peel extract. This is scientifically reasonable because acarbose is a defined carbohydrate-hydrolyzing enzyme inhibitor, whereas PA-PE is an unfractionated botanical extract. The concentration-dependent response nevertheless demonstrated that PA-PE contained constituents capable of reducing  $\alpha$ -amylase activity in vitro. Phenolic compounds may interact with digestive enzymes through several non-covalent interactions. However, the present study did not investigate enzyme kinetics or molecular binding. Therefore, a specific mechanism of  $\alpha$ -amylase inhibition cannot be assigned from these results alone. The finding is relevant because  $\alpha$ -amylase participates in the initial hydrolysis of dietary starch. Inhibition of this enzyme can reduce the rate at which complex carbohydrates are converted into smaller carbohydrate products. However, the present result represents an in-vitro enzyme assay and does not demonstrate a clinical glucose-lowering effect.

### 3.8. $\alpha$ -Glucosidase Inhibitory Activity

PA-PE showed slightly stronger activity against  $\alpha$ -glucosidase than against  $\alpha$ -amylase. The inhibition of  $\alpha$ -glucosidase increased from  $18.8 \pm 0.4\%$  at 25  $\mu$ g/mL to  $83.5 \pm 0.6\%$  at 400  $\mu$ g/mL. At 50  $\mu$ g/mL, PA-PE inhibited the enzyme by  $31.2 \pm 0.5\%$ . At 100 and 200  $\mu$ g/mL, inhibition increased to  $48.1 \pm 0.6\%$  and  $67.4 \pm 0.6\%$ , respectively. Acarbose again showed higher inhibitory activity. Its inhibition ranged from  $31.8 \pm 0.5\%$  to  $94.5 \pm 0.3\%$  over the same concentration range.

**Table 7.**  $\alpha$ -Glucosidase inhibitory activity of PA-PE and acarbose

| Concentration ( $\mu$ g/mL) | PA-PE inhibition (%) | Acarbose inhibition (%) |
|-----------------------------|----------------------|-------------------------|
| 25                          | $18.8 \pm 0.4$       | $31.8 \pm 0.5$          |
| 50                          | $31.2 \pm 0.5$       | $48.6 \pm 0.6$          |
| 100                         | $48.1 \pm 0.6$       | $68.9 \pm 0.5$          |
| 200                         | $67.4 \pm 0.6$       | $85.8 \pm 0.4$          |
| 400                         | $83.5 \pm 0.6$       | $94.5 \pm 0.3$          |
| $IC_{50}$ ( $\mu$ g/mL)     | 109.7                | 53.2                    |

The  $IC_{50}$  of PA-PE against  $\alpha$ -glucosidase was 109.7  $\mu$ g/mL. Acarbose showed an  $IC_{50}$  of 53.2  $\mu$ g/mL. Therefore, PA-PE was less potent than acarbose but showed a clear inhibitory response. Comparison of the two digestive enzymes showed:  $\alpha$ -Glucosidase  $IC_{50} = 109.7$   $\mu$ g/mL,  $\alpha$ -Amylase  $IC_{50} = 146.8$   $\mu$ g/mL. Thus, the extract demonstrated greater apparent potency against  $\alpha$ -glucosidase. This pattern may be useful from a screening perspective because it shows that PA-PE did not inhibit the two enzymes to the same degree. However, detailed enzyme kinetics would be required to establish the nature of the interaction. The activity may be associated with the combined action of multiple phenolic constituents rather than a single compound. Catechins, chlorogenic acid, quercetin-related molecules, procyanidins, and other constituents have all been reported in avocado peel. However, the present study did not isolate individual compounds and test their  $\alpha$ -glucosidase activity separately. Therefore, the contribution of each individual marker cannot be determined from the current data.

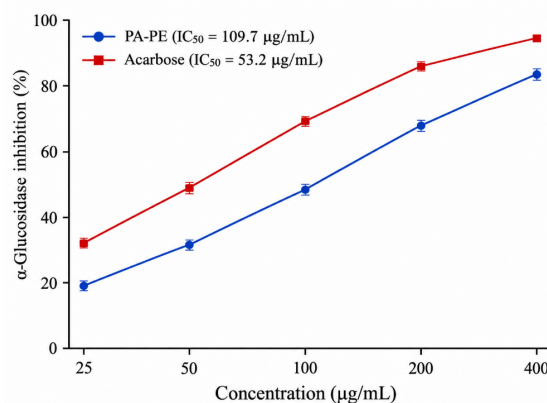


**Figure 4. Concentration-dependent  $\alpha$ -glucosidase inhibitory activity of PA-PE compared with acarbose. Values are expressed as mean  $\pm$  SD,  $n = 3$ .**

### 3.9. Antiglycation Activity

PA-PE also inhibited the formation of fluorescent advanced glycation end products in the BSA-glucose model. The inhibition increased steadily with concentration. At 25  $\mu$ g/mL, PA-PE produced  $14.0 \pm 0.4\%$  inhibition. At 50  $\mu$ g/mL, this increased to  $24.3 \pm 0.5\%$ . The extract produced  $39.5 \pm 0.6\%$  inhibition at 100  $\mu$ g/mL and  $58.1 \pm 0.6\%$  at 200  $\mu$ g/mL. At the highest tested concentration of 400  $\mu$ g/mL, AGE inhibition reached  $75.4 \pm 0.6\%$ .

Aminoguanidine showed greater antiglycation activity at all concentrations.

**Table 8.** In-vitro antiglycation activity of PA-PE and aminoguanidine

| Concentration (µg/mL)    | PA-PE AGE inhibition (%) | Aminoguanidine AGE inhibition (%) |
|--------------------------|--------------------------|-----------------------------------|
| 25                       | 14.0 ± 0.4               | 29.6 ± 0.5                        |
| 50                       | 24.3 ± 0.5               | 44.7 ± 0.5                        |
| 100                      | 39.5 ± 0.6               | 64.8 ± 0.6                        |
| 200                      | 58.1 ± 0.6               | 82.7 ± 0.4                        |
| 400                      | 75.4 ± 0.6               | 92.6 ± 0.3                        |
| IC <sub>50</sub> (µg/mL) | 154.3                    | 61.7                              |

The antiglycation IC<sub>50</sub> values were: PA-PE: 154.3 µg/mL, Aminoguanidine: 61.7 µg/mL. The reference inhibitor was therefore considerably more active than PA-PE.

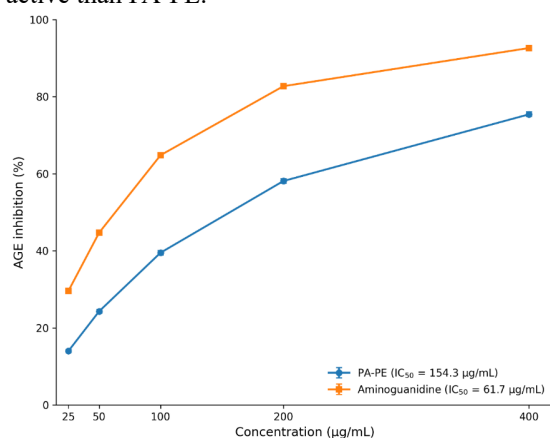


**Figure 5.** Concentration-dependent inhibition of fluorescent AGE formation by PA-PE and aminoguanidine in the BSA-glucose antiglycation model. Values are expressed as mean ± SD, n = 3. The antiglycation result adds an important dimension to the biological profile of the extract. Glycation involves non-enzymatic reactions between reducing sugars and proteins. Continued reactions can result in the formation of advanced glycation end products. The observed inhibition may involve several processes. Phenolic compounds can potentially reduce oxidative reactions associated with glycation or interact with reactive intermediates. However, the present fluorescence assay does not identify the exact mechanism involved. The antioxidant activity observed in the DPPH, ABTS, and FRAP assays provides a plausible chemical context for the antiglycation effect. Oxidative reactions and glycation chemistry can overlap. Still, the present study did not establish a direct causal relationship between antioxidant activity and AGE inhibition. It is therefore more appropriate to conclude that PA-PE independently demonstrated both antioxidant and antiglycation activities under the experimental conditions.

### 3.10. Comparative Biological Activity of PA-PE

The overall IC<sub>50</sub> values provide a useful comparison of the different activities of PA-PE.

**Table 9.** Comparative IC<sub>50</sub> values of PA-PE and the corresponding reference compounds

| Assay         | PA-PE IC <sub>50</sub> (µg/mL) | Reference compound | Reference IC <sub>50</sub> (µg/mL) |
|---------------|--------------------------------|--------------------|------------------------------------|
| DPPH          | 105.6                          | Ascorbic acid      | 37.4                               |
| ABTS          | 82.7                           | Trolox             | 31.9                               |
| α-Amylase     | 146.8                          | Acarbose           | 46.8                               |
| α-Glucosidase | 109.7                          | Acarbose           | 53.2                               |
| Antiglycation | 154.3                          | Aminoguanidine     | 61.7                               |

Among the concentration-response assays, the lowest PA-PE IC<sub>50</sub> was observed for ABTS radical scavenging at 82.7 µg/mL. This was followed by: DPPH: 105.6 µg/mL, α-Glucosidase: 109.7 µg/mL, α-Amylase: 146.8 µg/mL and: Antiglycation: 154.3 µg/mL. The IC<sub>50</sub> values should not be interpreted as directly interchangeable measures because each assay evaluates a different chemical or biological process. The reference compounds were more potent than PA-PE in every corresponding assay. This strengthens the internal credibility of the dataset. Crude plant extracts contain many constituents and are not expected to consistently outperform purified reference compounds. At the same time, the extract showed substantial activity at the higher concentrations. At 400 µg/mL, PA-PE produced 85.4% DPPH inhibition, 88.6% ABTS inhibition, 77.2% α-amylase inhibition, 83.5% α-glucosidase inhibition, and 75.4% antiglycation activity. These findings show that the peel extract had a broad concentration-dependent in-vitro bioactivity profile.

### 3.11. Relationship Between Phytochemical Composition and Observed Activities

The chemical and biological results showed a coherent pattern. PA-PE contained: 119.16 ± 0.94 mg GAE/g total phenolics and: 55.41 ± 0.73 mg QE/g total flavonoids. HPLC-DAD further confirmed the presence of selected phenolic markers. Epicatechin was the major quantified marker, followed by catechin, chlorogenic acid, and quercetin. The same extract displayed substantial antioxidant activity in three complementary assays. It also inhibited α-amylase and α-glucosidase and reduced AGE formation. Previous avocado studies have reported a relationship between phenolic-rich avocado fractions and antioxidant capacity. A study comparing peel, pulp, and seed from different avocado cultivars reported that peel contained a particularly diverse polyphenol profile and that phenolic measurements were associated with antioxidant capacity. The present study did not calculate a Pearson correlation between TPC/TFC and biological activity because only one extract was investigated. A meaningful correlation analysis would require several independent extracts or samples with different phytochemical levels.

Performing correlation analysis using repeated measurements from the same extract would create artificial replication. For this reason, the chemical and biological findings were interpreted together descriptively rather than through an unsupported correlation coefficient. This approach provides a more statistically valid interpretation of the data.

### 3.12. Overall Interpretation

The findings demonstrate that avocado peel was not merely an inert fruit-processing residue. The hydroethanolic extract contained substantial levels of phenolic and flavonoid constituents and showed multiple in-vitro biological activities. The extract had a total phenolic content of  $119.16 \pm 0.94$  mg GAE/g and a total flavonoid content of  $55.41 \pm 0.73$  mg QE/g. HPLC-DAD identified epicatechin as the most abundant of the four quantified markers. Antioxidant evaluation showed concentration-dependent DPPH and ABTS radical scavenging together with a FRAP value of  $730.27 \pm 8.40$   $\mu\text{mol Fe}^{2+}$  equivalents/g extract. PA-PE also inhibited the two carbohydrate-hydrolyzing enzymes. The stronger activity against  $\alpha$ -glucosidase, with an  $\text{IC}_{50}$  of  $109.7 \mu\text{g/mL}$ , compared with  $\alpha$ -amylase at  $146.8 \mu\text{g/mL}$ , indicated measurable but moderate digestive enzyme inhibition. The extract further inhibited AGE formation with an  $\text{IC}_{50}$  of  $154.3 \mu\text{g/mL}$ . Recent literature increasingly recognizes avocado peel as a chemically rich by-product containing phenolic acids, catechins, procyanidins, quercetin derivatives, and other bioactive constituents. Comprehensive profiling has also shown that avocado peel contains a much broader chemical composition than can be represented by a small number of targeted markers. The present findings therefore support the further investigation of avocado peel as a source of phenolic-rich extracts. At the same time, the biological interpretation should remain within the limits of the experimental design. The study does not demonstrate that PA-PE prevents or treats diabetes. It does not establish oral bioavailability, gastrointestinal stability, toxicity, pharmacokinetics, or efficacy in animals or humans. The results demonstrate only that PA-PE showed antioxidant,  $\alpha$ -amylase inhibitory,  $\alpha$ -glucosidase inhibitory, and antiglycation activities under controlled in-vitro conditions. Further work should include detailed LC-MS characterization, isolation of major active constituents, enzyme-kinetic studies, gastrointestinal stability studies, and appropriate safety evaluation before biological relevance beyond the in-vitro level can be established.

### 4. Conclusion

The present study showed that the hydroethanolic extract of *Persea americana* Mill. peel was rich in phenolic and flavonoid constituents. The extraction yield was 18.64% w/w. The total phenolic and total flavonoid contents were  $119.16 \pm 0.94$  mg GAE/g and  $55.41 \pm 0.73$  mg QE/g dried extract, respectively. HPLC-DAD analysis confirmed

chlorogenic acid, catechin, epicatechin, and quercetin as selected phenolic markers, with epicatechin showing the highest quantified concentration. PA-PE demonstrated clear concentration-dependent antioxidant activity in DPPH and ABTS assays and showed substantial ferric-reducing capacity. The extract also inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase, with  $\text{IC}_{50}$  values of  $146.8$  and  $109.7 \mu\text{g/mL}$ , respectively. In addition, PA-PE reduced the formation of fluorescent advanced glycation end products, with an  $\text{IC}_{50}$  of  $154.3 \mu\text{g/mL}$ . The findings indicate that avocado peel is a promising source of phenolic-rich bioactive compounds and may have value as a fruit by-product for further functional and phytopharmaceutical research. However, the present findings are limited to in-vitro experiments. Further studies should focus on detailed LC-MS profiling, isolation of active constituents, enzyme-kinetic evaluation, gastrointestinal stability, safety assessment, and appropriate biological models before wider therapeutic relevance can be established.

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