

Valorization of Buccaneer Avocado Agricultural Waste: Phytochemical Composition, Cosmeceutical Activities, and Cytotoxicity

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Abstract:

Objective: By-products from fruit processing, particularly residues of *Persea americana* Mill. (Buccaneer avocado), represent a valuable yet underutilized source of bioactive compounds for sustainable cosmeceutical development. This study aimed to characterize the phytochemical profiles and evaluate the cosmeceutical potential of Buccaneer avocado leaves, peels, and seeds.

Material and Methods: Buccaneer avocado by-products, including leaves, peels, and seeds, collected from Phetchabun Province, Thailand, were processed by washing, slicing, oven-drying, and grinding, and then extracted using ultrasonic-assisted extraction (UAE) with 60% ethanol. The extracts were characterized for extraction yield, total phenolic and flavonoid contents, and phytochemical composition using LC-MS/MS QTOF analysis. In vitro assays were conducted to evaluate antioxidant, anti-glycation, tyrosinase inhibitory, astringent, and anti-inflammatory activities. Cytotoxicity was assessed using L929 murine fibroblast cells, while anti-inflammatory activity was determined based on nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages.

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Results: The seed extract yielded the highest extraction efficiency (12.4%). The leaf extract exhibited the highest total phenolic (67.31 ± 6.64 mg GAE/g extract) and flavonoid contents (704.40 ± 8.76 mg QE/g extract). LC-MS/MS QTOF analysis indicated that the leaf extract contained the highest levels of key phytochemicals, including chlorogenic acid, p-coumaric acid, epigallocatechin, epicatechin, quercetin, rutin, luteolin, and proanthocyanidin derivatives. In vitro assays showed that the leaf extract exhibited the strongest antioxidant ($IC_{50} = 0.0131 \pm 0.0002$ mg/mL) and anti-glycation activity ($IC_{50} = 0.2044 \pm 0.0013$ mg/mL). The peel extract exhibited the highest tyrosinase inhibitory activity ($IC_{50} = 0.1708 \pm 0.0126$ mg/mL) and astringent activities ($54.63 \pm 0.32\%$). Both extracts were non-cytotoxic to L929 fibroblast cells at concentrations up to 0.50 mg/mL and significantly inhibited NO production by $74.8 \pm 3.8\%$ and $55.2 \pm 3.7\%$, respectively.

Conclusion: Buccaneer avocado residues from Phetchabun, Thailand, particularly the high-potential leaves and peels, are rich in bioactive compounds and exhibit significant cosmeceutical activities without cytotoxic effects, supporting their potential as sustainable cosmeceutical ingredients.

Keywords: anti-aging, anti-inflammatory activity, avocado by-products, cytotoxicity, non-conventional extraction techniques

Introduction

The growing demand for natural and sustainable ingredients has prompted the cosmetic and pharmaceutical industries to explore alternative sources of bioactive compounds¹. Agricultural by-products from food processing have recently attracted considerable attention as valuable sources for phytochemical recovery, offering opportunities for sustainable valorization and reduction of agro-industrial waste²⁻⁴. This strategy aligns with circular economy principles by converting waste into high-value functional ingredients for industrial applications¹.

Avocado (*P. americana* Mill.) is an economically important crop widely cultivated for its nutritional value and industrial applications⁵. In Thailand, the Buccaneer variety is increasingly grown in provinces such as Tak and Phetchabun. However, processing of avocado fruits generates considerable amounts of agricultural residues, including leaves, peels, and seeds. These by-products have been reported to contain a wide range of phytochemicals, particularly phenolics and flavonoids, which exhibit antioxidant and anti-aging activities⁶⁻⁹.

Phenolic compounds possess strong antioxidant properties, enabling them to neutralize reactive oxygen species and mitigate any oxidative damage linked to skin aging^{9,10}. Furthermore, avocado by-products^{1,3} have been reported to contain a variety of flavonoids. These compounds exhibit diverse biological activities, including anti-inflammatory¹, anti-glycation,¹¹ and tyrosinase inhibitory effects¹². Such activities are particularly valuable for cosmetic applications, especially in anti-aging and skin-whitening formulations¹², as well as in functional health products¹³.

Ultrasonic-assisted extraction (UAE) is recognized as an effective green extraction method that enhances phytochemical recovery by facilitating solvent penetration and cell-wall disruption²⁻³. This technique has been widely employed for the extraction of polyphenols from plant materials, including avocado by-products, due to its efficiency and reduced solvent usage^{1,3}.

Therefore, the present study examined the phytochemical composition, biological activities relevant to cosmeceutical applications, and cytotoxicity of extracts derived from Buccaneer avocado by-products using

UAE. The results should provide a scientific basis for the sustainable valorization of these materials as high-value ingredients in cosmeceutical formulations.

Material and Methods

Plant materials and sample preparation

Buccaneer avocado (*P. americana* Mill. cv. Buccaneer) by-products, including leaves, peels, and seeds, were collected from Phetchabun Province, Thailand, in June 2024. The samples were washed, sliced, and homogenized, followed by oven-drying at 40 °C to constant weight. The dried materials were then ground into a fine powder using a high-speed herbal grinder (1200 W; chamber dimensions: 11 cm diameter×9.5 cm height) and stored in zip-lock bags at 4 °C until further study.

Plant extraction

Ultrasonic-assisted extraction (UAE) was carried out with slight modifications to the established protocols of Tremocoldi et al. (2018)² and Petrantonaki et al. (2025)³. Powdered samples of Buccaneer avocado leaves, peels, and seeds were individually extracted in 60.0% (v/v) ethanol at a solid-to-solvent ratio of 1:10 (w/v). Sonication was performed in a 45 kHz ultrasonic bath for 30 min at 40–45°C. The extracts were subsequently filtered (Whatman No. 1), concentrated using a rotary evaporator, and the extraction yield was expressed as a percentage of dry weight.

Determination of phytochemical contents

Total phenolic content (TPC)

TPC was quantified using the Folin–Ciocalteu method¹⁴, employing gallic acid (GA) as the standard, and expressed as mg GA equivalents/ gram extract (mg GAE/g).

Total flavonoid content (TFC)

TFC was determined by the aluminum chloride colorimetric method¹⁵, using quercetin as the standard, and

results were expressed as mg quercetin equivalents per gram of extract (mg QE/g).

Total tannin content (TTC)

TTC was analyzed using a modified Folin–Ciocalteu method¹⁴ with tannic acid as the standard, and expressed as mg tannic acid equivalents per gram (mg TAE/g).

Chemical constituent analysis using LC-MS/MS QTOF

Phytochemical constituents, including phenolics, flavonoids, and tannins, were characterized using an Agilent 6545 LC/Q–TOF system (Agilent Technologies, USA). Chromatographic separation was achieved on a Zorbax Eclipse Plus C18 column (2.1×150 mm, 1.8 µm) with a 1 µl injection volume. The mobile phase comprised 0.1% formic acid in water (A) and acetonitrile (B), using a 33-min gradient elution program. Mass spectrometric analysis was performed in both positive and negative electrospray ionization modes, with a fragmentor voltage of 175 V and ramped collision energy. Compounds were tentatively identified by matching accurate m/z values and MS/MS fragmentation patterns with METLIN, HMDB, and PubChem databases using the molecular feature extraction (MFE) algorithm. Only features with a coelution score >70 and mass error within <±5 ppm were considered.

Determination of biological activities

Antioxidant activity (DPPH assay)

Radical scavenging activity was determined using the DPPH assay¹⁶, with trolox as the reference standard. Results were expressed as IC₅₀ (mg/mL).

Anti-glycation activity

Anti-glycation activity was evaluated using the Bovine Serum Albumin (BSA)–fructose model as described by Tantapakul et al. (2024)¹⁷ with quercetin as the standard. Results were expressed as IC₅₀ (mg/mL).

Anti-tyrosinase activity

Anti-tyrosinase activity was assessed using modified methods based on Chang (2009)¹² and Kanlayavattanakul et al. (2015)¹⁸ with kojic acid as the reference standard. Activity was expressed as IC₅₀ (mg/mL).

Astringent activity

Astringent activity was determined using a modified method of Kumar et al. (2017)¹⁹. Briefly, bovine hemoglobin solution (1 mg/mL) was mixed with each extract solution at a final concentration of 1 mg/mL in a 1:1 ratio, vortexed, and centrifuged at 1,000 × g at 25 °C for 10 min. The absorbance of the supernatant was measured at 407 nm using a UV-Visible spectrophotometer. Tannic acid (1 mg/mL) and deionized (DI) water were used as positive and negative controls, respectively. Astringent activity (%) was calculated as:

$$\text{Astringent activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} is the absorbance of the bovine hemoglobin solution alone and

A_{sample} is the absorbance of the mixture with the test sample.

Cellular study

Cytotoxicity

Cytotoxicity of Buccaneer avocado leaf and peel extracts on L929 fibroblast cells was assessed using the CCK-8 assay. Cells were seeded in 96-well plates at 5×10^4 cells/well and incubated for 24 h to allow attachment. The culture medium was replaced with fresh medium containing extract concentrations of 0.03–2.0 mg/mL. Cells treated with 10% DMSO served as a positive cytotoxic control, while 1.4% (v/v) ethanol was used as the vehicle control. After 24 h of exposure, morphological changes were observed under an inverted phase-contrast microscope. Subsequently, 10 µL of CCK-8 reagent was added to 100 µL of culture medium per well and incubated for 2 h

at 37 °C. Absorbance was measured at 450 nm using a microplate reader. Cell viability was calculated as a percentage relative to the control using the following equation:

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100$$

where A_{sample} is the absorbance of treated cells, A_{control} is the absorbance of untreated cells, and A_{blank} represents the absorbance of medium without cells.

Anti-inflammatory activity (nitric oxide assay)

Anti-inflammatory activity was assessed by quantifying nitric oxide (NO) production in LPS-stimulated RAW264.7 macrophages using the Griess reaction, as described by Wunoo et al. (2024)²⁰. Cells were seeded in 96-well plates at a density of 1×10^5 cells/well and incubated for 24 h. The cells were then stimulated with lipopolysaccharides (LPS, 1 µg/mL) in the presence or absence of extracts (0.03–0.50 mg/mL) for 24 h. Dexamethasone (Dex, 1 mM) was used as the positive control. NO levels in the culture supernatant were measured at 540 nm and quantified using a sodium nitrite standard curve, with results expressed as µM.

Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± S.D. (n=3). Statistical analysis was conducted using one-way analysis of variance (ANOVA) followed by the appropriate post hoc multiple comparison tests. Differences were considered statistically significant at p-value < 0.05.

Results

Extraction yield

Buccaneer avocado by-products were fractionated into leaves, peels, and seeds (Figure 1) and subjected to UAE. The seed fraction yielded the highest extract (12.4%), followed by leaves (11.6%) and peels (9.9%) (Figure 2).

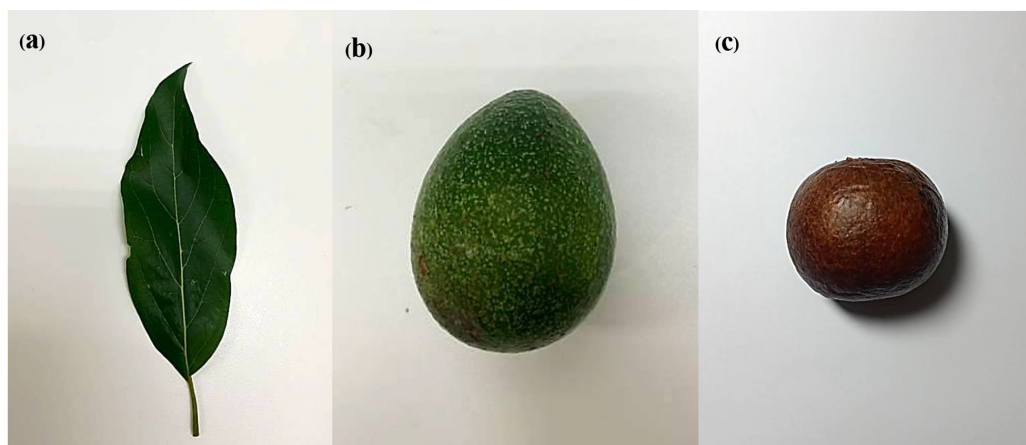


Figure 1 Buccaneer avocado plant materials used in this study: (a) leaf, (b) peel, and (c) seed.

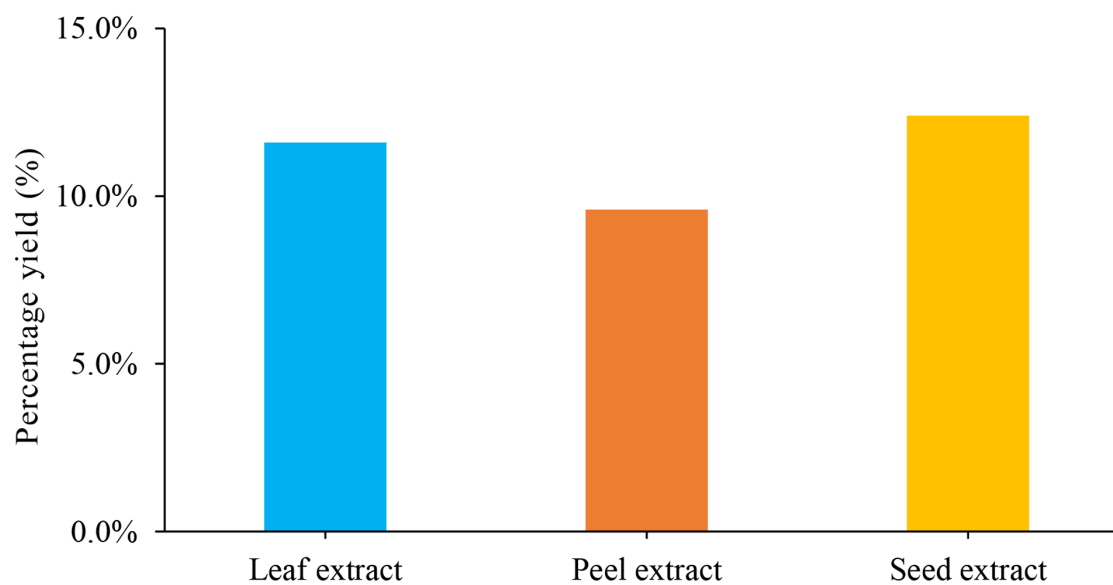


Figure 2 Percentage yield of extracts obtained from Buccaneer avocado leaf, peel, and seed. Data are presented as mean $\pm$ S.D. (n=3). No statistically significant differences were observed among extracts (p-value>0.05).

The extracts displayed distinct coloration, ranging from dark brown to greenish- and reddish-brown, reflecting differences in their phytochemical composition.

Phytochemical contents

The phytochemical contents of Buccaneer avocado extracts are summarized in Table 1. The leaf extract exhibited the highest TPC (67.31 ± 6.64 mg GAE/g extract), TFC (704.40 ± 8.76 mg QE/g extract), and TTC (81.11 ± 4.35 mg TAE/g), all significantly higher than those of the peel and seed extracts. The peel extract showed intermediate levels, while the seed extract contained the lowest phytochemical content.

Phytochemical constituents, including phenolics, flavonoids, and tannins, were characterized using LC-MS/MS QTOF analysis. The total ion chromatograms (TIC) of leaf, peel, and seed extracts revealed distinct chemical profiles. Tentative compound identification was performed based on retention time (RT), accurate mass, and MS/MS fragmentation patterns, and compared with spectral databases and published literature.

As presented in Table 2, a total of twenty-two compounds were identified, predominantly belonging to three major classes: phenolic compounds, flavonoids, and tannin-related constituents. The phenolic group included 3,4-dihydroxyphenylacetic acid, benzoic acid, 4-hydroxybenzoic acid, ferulic acid, chlorogenic acid, p-coumaric acid, 3-O-p-coumaroylquinic acid, scopoletin, and N-norarmepavine. Flavonoid comprised epigallocatechin, epicatechin, quercetin-3,4'-diglucoside, quercetin-3-O-arabinosyl-glucoside, rutin, quercetin-3-O-glucoside, quercetin-3-O-arabinoside, luteolin, quercetin, kaempferol 3-O- β -D-fucopyranoside, and vignaticol. Additionally, tannin-related compounds, namely proanthocyanidins A1 and B1, were detected.

Phytochemical profiling revealed distinct variations in compound distribution among plant parts. Compounds

such as 4-hydroxybenzoic acid and chlorogenic acid were detected across all extracts, whereas others exhibited part-specific occurrence. Epicatechin, quercetin-3,4'-diglucoside, quercetin-3-O-arabinosyl-glucoside, quercetin 3-O-glucoside, quercetin, kaempferol 3-O- β -D-fucopyranoside, and proanthocyanidins A1 and B1 were identified in both leaf and peel extracts, while p-coumaric acid and 3-O-p-coumaroylquinic acid were present in leaf and seed extracts. In contrast, benzoic acid, epigallocatechin, rutin, quercetin-3-O-arabinoside, luteolin, and vignaticol were exclusively detected in the leaf extract. These phytochemicals are widely recognized for their antioxidant and astringent properties^{8,19,24}.

Biological activities

The biological activities of the extracts are summarized in Table 3. The leaf extract exhibited the strongest antioxidant and anti-glycation activities, with IC_{50} values of 0.0131 ± 0.0002 mg/mL and 0.2044 ± 0.0013 mg/mL, respectively. In contrast, the peel extract demonstrated the highest tyrosinase inhibitory activity ($IC_{50} = 0.1708 \pm 0.0126$ mg/mL) and the greatest astringent activity ($54.63 \pm 0.32\%$) at 1.0 mg/mL, whereas the seed extract consistently showed the lowest activity across all assays.

Antioxidant activity (DPPH)

The leaf extract exhibited the highest antioxidant activity ($IC_{50} = 0.0131 \pm 0.0002$ mg/mL), indicating strong radical-scavenging capacity. In contrast, the peel and seed extracts showed lower activities, as reflected by higher IC_{50} values of 0.0429 ± 0.0003 mg/mL and 0.0853 ± 0.0015 mg/mL, respectively.

Anti-glycation activity

The leaf extract demonstrated the highest anti-glycation activity ($IC_{50} = 0.2044 \pm 0.0013$ mg/mL), indicating a strong capacity to inhibit protein glycation. The peel and

Table 1 Phytochemical contents of Buccaneer avocado by-products

Sample	Phytochemical Contents		
	TPC (mg GAE/g)	TFC (mg QE/g)	TTC (mg TAE/g)
Leaf extract	67.31±6.64 ^a	704.40±8.76 ^a	81.11±4.35 ^a
Peel extract	42.10±2.05 ^b	405.02±7.10 ^b	23.07±1.57 ^b
Seed extract	16.37±0.47 ^c	77.90±7.60 ^c	18.43±1.31 ^b

Note: data are presented as mean±S.D. (n=3). Different superscript letters (^{a,b,c}) within the same column indicate statistically significant differences (p-value<0.05). TPC=total phenolic content, TFC=total flavonoid content, TTC=total tannin content, GAE=gallic acid equivalents, QE=quercetin equivalents, TAE=tannic acid equivalents, S.D.=standard deviation

Table 2 Tentative identification of phenolic compounds, flavonoids, and tannin-related constituents in Buccaneer avocado extracts using LC-MS/MS QTOF analysis

No.	Compound	Formula	Score	Retention time (min)	Mode of Ionization	Theoretical mass	Observed m/z	Mass error (ppm)	Sample
Phenolics									
1	3,4-Dihydroxyphenylacetic acid	C ₈ H ₈ O ₄	83.56	5.120	(M-H) ⁻	168.0416	167.0342	-4.17	Seed
2	Benzoic acid	C ₇ H ₆ O ₂	77.53	7.966	(M+CH ₃ COO) ⁻	122.0370	181.0512	2.01	Leaf
3	4-Hydroxybenzoic acid	C ₇ H ₆ O ₃	83.13	8.083	(M-H) ⁻	138.0312	137.0241	-3.38	Leaf, Peel, and Seed
4	Ferulic acid	C ₁₀ H ₁₀ O ₄	80.95	8.085	(M-H) ⁻	194.0577	193.0507	-1.27	Seed
5	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	96.83	8.846	(M-H) ⁻	354.0940	353.0868	-2.94	Leaf, Peel, and Seed
6	p-Coumaric acid	C ₉ H ₈ O ₃	92.14	9.140	(M-H) ⁻	164.0466	163.0394	-4.27	Leaf, Seed
7	3-O-p-Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	97.50	11.019	(M-H) ⁻	338.0994	337.0922	-2.28	Leaf, Seed
8	Scopoletin	C ₁₀ H ₁₀ O ₄	89.50	17.712	(M-H) ⁻	202.1208	201.1137	1.24	Peel
9	N-norarmepavine	C ₁₆ H ₂₁ NO ₃	89.42	10.660	(M+H) ⁺	299.1535	300.1610	4.48	Seed
Flavonoids									
10	Epigallocatechin	C ₁₅ H ₁₄ O ₇	84.05	1.744	(M-H) ⁻	306.0753	305.0666	4.25	Leaf
11	Epicatechin	C ₁₅ H ₁₄ O ₆	96.48	11.019	(M-H) ⁻	290.0779	289.0708	-4.04	Leaf, Peel
12	Quercetin-3,4'-diglucoside	C ₂₇ H ₃₀ O ₁₇	95.69	12.310	(M-H) ⁻	626.1467	625.1395	-2.61	Leaf, Peel
13	Quercetin-3-O-arabinosyl-glucoside	C ₂₆ H ₂₈ O ₁₆	96.45	13.250	(M-H) ⁻	596.1363	595.1291	-2.34	Leaf, Peel
14	Rutin	C ₂₇ H ₃₀ O ₁₆	93.97	14.982	(M-H) ⁻	610.1515	609.1442	-3.14	Leaf
15	Quercetin-3-O-glucoside	C ₂₁ H ₂₀ O ₁₂	97.03	15.599	(M-H) ⁻	464.0943	463.0871	-2.44	Leaf, Peel
16	Quercetin-3-O-arabinoside	C ₂₀ H ₁₈ O ₁₁	95.64	16.421	(M-H) ⁻	434.0836	433.0763	-3.01	Leaf
17	Luteolin	C ₁₅ H ₁₀ O ₆	94.00	16.421	(M-H) ⁻	286.0469	285.0396	-3.03	Leaf
18	Quercetin	C ₂₁ H ₂₀ O ₁₁	96.81	16.451	(M-H) ⁻	448.0994	447.0922	-2.54	Leaf, Peel
19	Kaempferol 3-O-β-D-fucopyranoside	C ₂₁ H ₂₀ O ₁₀	95.07	17.068	(M-H) ⁻	432.1042	431.0970	-3.33	Leaf, Peel
20	Vignaticol	C ₂₇ H ₄₄ O	70.56	21.941	(M-H) ⁻	384.3406	383.3328	3.57	Leaf
Tannins									
21	Proanthocyanidins B1	C ₃₀ H ₂₆ O ₁₂	96.57	7.731	(M-H) ⁻	578.1411	577.1337	-2.36	Leaf, Peel
22	Proanthocyanidin A1	C ₃₀ H ₂₄ O ₁₂	75.98	13.367	(M-H) ⁻	576.1260	575.1176	-1.40	Leaf, Peel

seed extracts showed lower activities, with IC_{50} values of 0.2401 ± 0.0329 mg/mL and 0.5906 ± 0.0356 mg/mL, respectively.

Anti-tyrosinase activity

The peel extract exhibited the strongest tyrosinase-inhibitory activity ($IC_{50} = 0.1708 \pm 0.0126$ mg/mL), whereas the leaf extract showed moderate inhibition ($IC_{50} = 0.5450 \pm 0.0138$ mg/mL).

Astringent activity

The peel extract exhibited high astringent activity ($54.63 \pm 0.32\%$) at a test concentration of 1 mg/mL, which was superior to that of the leaf and seed extracts.

Cellular study

Cytotoxic evaluation of avocado leaf and peel extracts

Cytotoxicity on L929 fibroblast cells was evaluated using microscopic observation and the CCK-8 assay (Figure 3). Untreated cells displayed normal morphology (Figure 3a), whereas treatment with 10% DMSO (positive control) induced severe morphological alterations, including cell shrinkage and detachment, accompanied by a significant reduction

in viability ($27.87 \pm 2.59\%$, p -value < 0.001). In contrast, the vehicle control (1.4% ethanol) showed no cytotoxic effects, with viability comparable to untreated cells ($98.2 \pm 2.4\%$, p -value > 0.05). Both Buccaneer avocado leaf and peel extracts (0.03 – 0.50 mg/mL) maintained cell viability close to 100% (Figure 3b and 3c). These findings demonstrate that the extracts are non-cytotoxic to L929 fibroblast at concentrations up to 0.50 mg/mL, supporting their suitability for further biological evaluation.

Anti-inflammatory activity

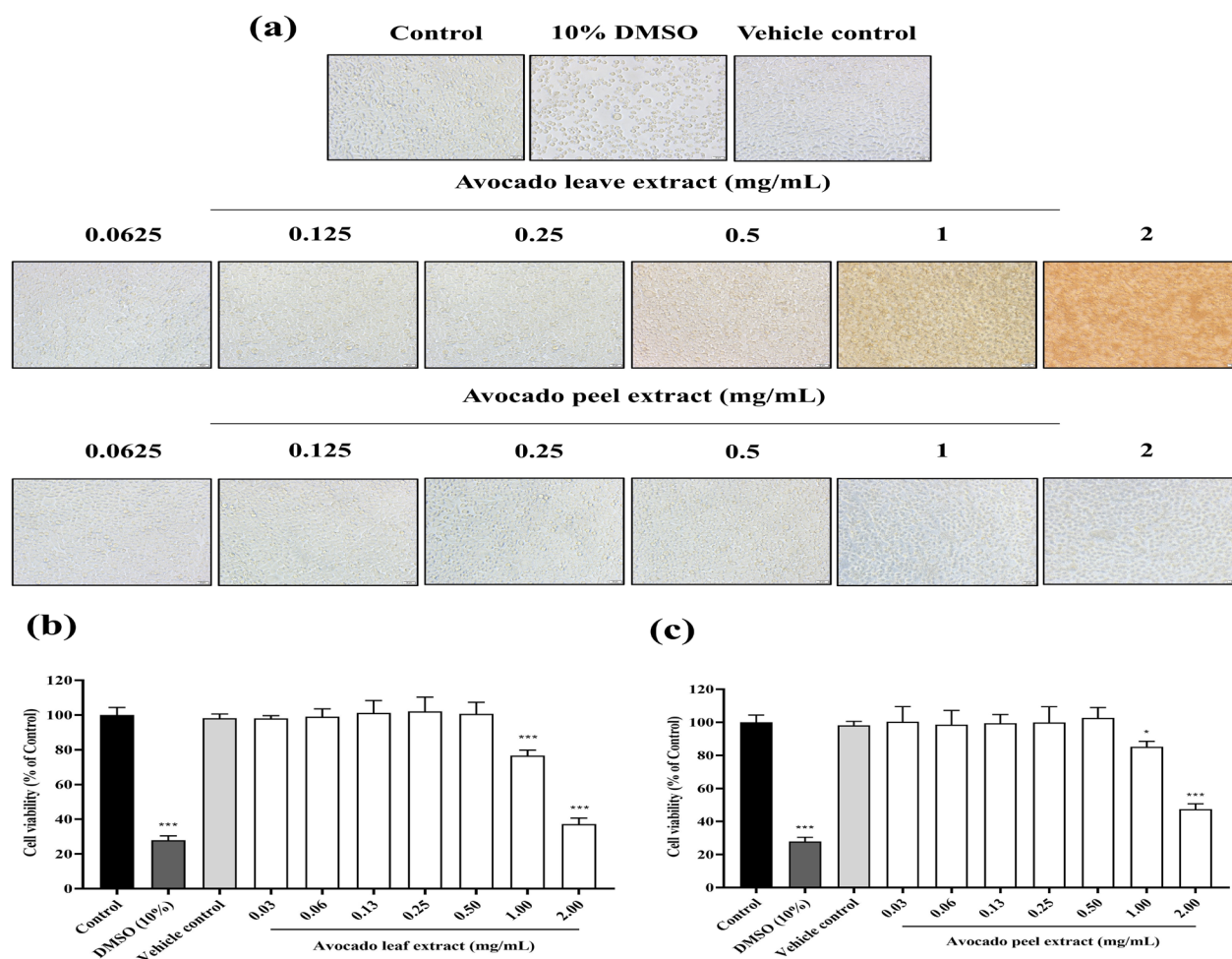
The effects of Buccaneer avocado leaf and peel extracts on RAW 264.7 macrophage viability and NO production are presented in Figure 4. Treatment with 10% DMSO significantly reduced cell viability ($11.2 \pm 6.4\%$, p -value < 0.001) (Figure 4a and 4b), whereas the vehicle control showed no cytotoxicity ($100.0 \pm 4.8\%$, p -value > 0.05). Both extracts (0.03 – 0.50 mg/mL) maintained high cell viability, indicating non-cytotoxic effects. LPS stimulation (1 μ g/mL) significantly increased NO production compared with the untreated control (62.18 ± 1.45 μ M vs 2.71 ± 1.19 μ M, $p < 0.001$), confirming successful induction of inflammation. Both extracts significantly inhibited NO production in a concentration-dependent manner, with pronounced effects at

Table 3 Biological activities of Buccaneer avocado by-products

Sample	Biological Activities			
	Anti-oxidant (IC_{50} , mg/mL)	Anti-glycation (IC_{50} , mg/mL)	Anti-tyrosinase (IC_{50} , mg/mL)	Astringent (%)
Leaf extract	0.0131 ± 0.0002^b	0.2044 ± 0.0013^b	0.5450 ± 0.0138^b	12.91 ± 0.50^c
Peel extract	0.0429 ± 0.0003^c	0.2401 ± 0.0329^b	0.1708 ± 0.0126^a	54.63 ± 0.32^b
Seed extract	0.0853 ± 0.0015^d	0.5906 ± 0.0356^c	1.8142 ± 0.1579^c	8.01 ± 0.22^d
Trolox	0.0053 ± 0.0003^a	Not tested	Not tested	Not tested
Quercetin	Not tested	0.0018 ± 0.0001^a	Not tested	Not tested
Kojic acid	Not tested	Not tested	0.0152 ± 0.0001^a	Not tested
Tannic acid	Not tested	Not tested	Not tested	97.58 ± 0.11^a

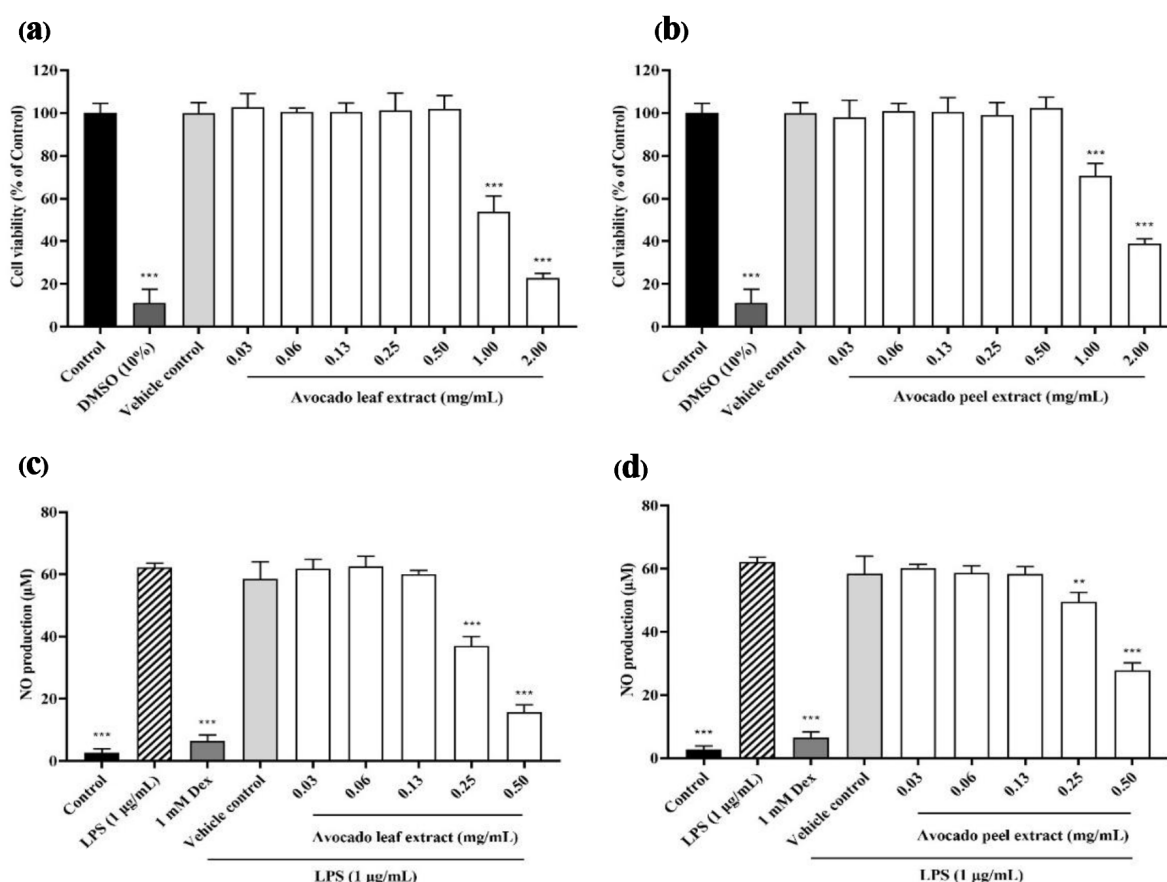
Note: values are expressed as mean $\pm$ S.D. ($n=3$). Different superscript letters (^a-d) indicate significant differences among extracts (p -value < 0.05).

IC_{50} =half-maximal inhibitory concentration, S.D.=standard deviation



DMSO=dimethyl sulfoxide, S.D.=standard deviation

Figure 3 Cytotoxic evaluation of Buccaneer avocado leaf and peel extracts on L929 fibroblast cells. (a) Representative microscopic images showing L929 cell morphology after treatment with untreated control, 10% DMSO (positive control), vehicle control (1.4% v/v ethanol, final concentration), and Buccaneer avocado extract. (b) Cell viability of L929 cells treated with leaf extract. (c) Cell viability of L929 cells treated with peel extract. Cell viability was expressed as a percentage relative to untreated control cells. Data are presented as mean $\pm$ S.D. (n=3) *p-value <0.05 and ***p-value <0.001 versus control group.



NO=nitric oxide, LPS=lipopolysaccharide, S.D.=standard deviation

Figure 4 Effects of Buccaneer avocado leaf and peel extracts on cell viability and nitric oxide (NO) production in RAW 264.7 murine macrophage cells. Cell viability following treatment with different concentrations (0.03–2.0 mg/mL) of Buccaneer avocado leaf extract (a) and peel extract (b). Inhibitory effects of Buccaneer avocado leaf extract (c) and peel extract (d) on nitric oxide (NO) production in lipopolysaccharide (LPS)–stimulated RAW 264.7 cells. NO levels were measured and expressed as μM . Data are presented as mean $\pm$ S.D. (n=3) **p-value<0.01 and ***p-value<0.001 versus the control group.

0.25 and 0.50 mg/mL ($p < 0.01$ – 0.001). At 0.50 mg/mL, NO inhibition reached $74.8 \pm 3.8\%$ (leaf) and $55.2 \pm 3.7\%$ (peel), with IC_{50} values of 0.32 ± 0.03 mg/mL and 0.46 ± 0.02 mg/mL, respectively.

Discussion

Extraction yield

The seed fraction exhibited the highest extraction yield (12.4%), which may be attributed to its role as a storage tissue rich in starch and lipids. UAE was conducted using 60% ethanol, consistent with previous reports by Petrantonaki et al. (2025)³, Tremocoldi et al. (2018)², and Rodríguez-Martínez et al. (2021)¹³, demonstrating that ethanol–water mixtures provide optimal polarity for the simultaneous extraction of polar and non-polar phytoconstituents and enhance the recovery of polyphenolic compounds. This intermediate solvent polarity facilitates the concurrent extraction of hydrophilic and lipophilic constituents and is appropriate for the phytochemical profile of Buccaneer avocado. Variations in yield compared with earlier studies are likely due to genetic differences and environmental factors influencing plant metabolic composition and tissue structure.

Phytochemical contents

The TPC values obtained in this study are consistent with those reported for avocado peels and seeds. Tremocoldi et al. (2018)² reported 63.5–120.3 and 57.3–59.2 mg GAE/g, respectively, using UAE with 80% ethanol, while Petrantonaki et al. (2025)³ reported ranges of 3.72–103.14 and 17.18–53.84 mg GAE/g using UAE with 50–100% ethanol. Similarly, Segovia et al. (2014)⁷ reported 35–47 mg GAE/g DW in seeds, using RSM with 26–94% ethanol. In comparison, the TFC of the leaf extract (704.40 mg QE/g) demonstrates efficient flavonoid recovery and is comparable to values reported for peel extracts (119–986.26 mg QE/g) by Dibacto et al. (2021)²¹ using maceration with aqueous 50–100% ethanol.

These phytochemicals are widely recognized for their antioxidant and astringent properties^{8,19,24}.

Antioxidant activity (DPPH)

Similar IC_{50} ranges have been reported for avocado seed extracts by Ong et al. (2022)²² (0.011–0.023 mg/mL; PHWE) and Peccin et al. (2022)²³ (0.013–0.077 mg/mL; ethanolic maceration). These results suggest that the antioxidant activity of the present extract is comparable to, or slightly higher than, previously reported values.

DPPH radical scavenging is predominantly governed by a Hydrogen Atom Transfer (HAT) mechanism²⁴, in which antioxidants convert the violet DPPH radical into a stable pale–yellow form. The superior activity of the leaf extract is attributed to its enrichment in flavonoids such as quercetin, rutin, and luteolin (Table 2), whose ortho–dihydroxy (catechol) B–ring structures facilitate the formation of resonance–stabilized phenoxyl radicals²⁴. In contrast, the lower TPC and TFC values of the peel and seed extracts (Table 1) are consistent with their reduced activities, as reflected by higher IC_{50} values (0.0429 ± 0.0003 mg/mL and 0.0853 ± 0.0015 mg/mL), respectively. Moreover, glycosylation of flavonoids in the peel, such as quercetin–3,4′–diglucoside, may hinder hydrogen–donating capacity compared to the aglycone–rich extract¹⁴. Collectively, these findings highlight the potential of the extracts as functional ingredients for scavenging reactive oxygen species (ROS) and mitigating oxidative stress, in agreement with previous reports on avocado by-products^{22,23}.

Anti-glycation activity

This effect is attributed to the suppression of advanced glycation end–products (AGEs) formation via two primary mechanisms: (1) scavenging reactive dicarbonyl intermediates, such as methylglyoxal, which are key precursors in glycation pathways, consistent with previous reports on rutin metabolites as non–oxidative glycation

inhibitors²⁵, and (2) protecting protein amino groups from reactions with reducing sugars.

The anti-glycation activity of the leaf extract is attributed, in part, to the presence of quercetin and chlorogenic acid, which inhibit glycation-induced protein cross-linking through the trapping of reactive carbonyl species^{26,27}. In contrast, the lower activities observed in the peel ($IC_{50}=0.2401\pm0.0329$ mg/mL) and seed ($IC_{50}=0.5906\pm0.0356$ mg/mL) extracts are consistent with their reduced polyphenolic density. Collectively, these findings highlight the potential of the extract as an anti-aging agent by attenuating oxidative damage and preserving skin structural integrity and elasticity¹¹.

Anti-tyrosinase activity

Compared with previously reported values, Laksmiani et al. (2020)²⁸ reported an IC_{50} of 0.093 ± 0.002 mg/mL for avocado seed extract, indicating that the peel extract obtained in this study demonstrates substantially higher inhibitory activity. The potent effect is attributed to the presence of flavonoids, including quercetin, epicatechin, and quercetin glycosides (quercetin-3-O-glucoside and quercetin-3-O-arabinosyl-glucoside), as identified by LC-MS/MS profile (Table 2).

The tyrosinase inhibitory activity of these compounds is mediated through a dual-action mechanism. First, flavan-3-ol compounds, such as catechin, act as structural analogues of L-DOPA and competitively inhibit dopachrome formation by occupying the enzyme's active site, thereby preventing substrate oxidation^{11,28}. Second, the polyhydroxylated structure of flavan-3-ol facilitates the chelation of binuclear copper ions at the catalytic center of tyrosinase¹². As these metal ions are essential cofactors for enzymatic activity, their sequestration results in effective enzyme inactivation.

In contrast, although the leaf extract exhibited higher TFC (Table 1), its predominant flavonoids, including quercetin, quercitrin, and quercetin glycosides (e.g., quercetin

3-O- α -D-arabinopyranoside), contain bulky sugar moieties at the C-3 position, which may induce steric hindrance and limit its effectiveness to the tyrosinase active site. Conversely, the peel extract, enriched with epicatechin and quercetin glycosides (quercetin-3-O-glucoside and quercetin-3-O-arabinosyl-glucoside) (Table 2), may exhibit more favorable spatial orientation and stronger interactions with the enzyme. This structural advantage likely contributes to its superior tyrosinase inhibitory activity and highlights its potential application as a high-performance skin-lightening agent^{12,28}.

Astringent activity

This effect is strongly associated with its polyphenolic profile identified via LC-MS/MS analysis (Table 2). Astringency is primarily governed by the formation of insoluble polyphenol-protein complexes¹⁹, initiated through interactions between bioactive metabolites and hemoglobin, leading to protein aggregation and subsequent precipitation during centrifugation²⁹.

The efficacy observed in the peel extract is attributed to epicatechin and proanthocyanidins A1 and B1. These compounds possess multiple hydroxyl groups and aromatic rings that facilitate strong multi-point interactions with the peptide backbone of hemoglobin through hydrogen bonding and hydrophobic interactions. Notably, proanthocyanidins exhibit high protein-binding affinity and act as molecular bridges, promoting the formation of large, insoluble aggregates²⁹.

In contrast, although the leaf extract exhibited a higher TFC (Table 1), its phytochemical profile is dominated by glycosylated flavonoids, including kaempferol 3-O- β -D-fucopyranoside, quercetin-3-O-arabinosyl-glucoside, and quercetin-3-O-glucoside. The presence of bulky sugar moieties, particularly the arabinosyl-glucoside group, may introduce steric hindrance that limits accessibility of the flavonoid core to protein-binding sites. This structural constraint likely reduces binding affinity toward bovine hemoglobin compared with the peel extract, which contains

more structurally compatible glycosides. Collectively, these findings highlight the superior astringent potential of the peel extract and support its application in cosmetic formulations for pore-minimizing and skin-toning purposes.

Cytotoxicity

These findings demonstrate that the extracts are non-cytotoxic to L929 fibroblast at concentrations up to 0.50 mg/mL, supporting their suitability for further biological evaluation.

The evaluation of cytotoxicity is essential for determining the safety and biocompatibility of plant-derived extracts for biomedical and cosmetic applications. In this study, Buccaneer avocado leaf and peel extracts exhibited no cytotoxic effects on L929 fibroblast cells at concentrations up to 0.50 mg/mL, as indicated by preserved cell morphology and high viability ($100.7 \pm 6.7\%$ and $102.7 \pm 6.2\%$, respectively; Figure 3). L929 fibroblasts are widely used for cytocompatibility assessment due to their critical role in extracellular matrix synthesis and skin wound repair^{20,30}. The absence of cytotoxicity suggests favorable compatibility with skin-related cells. These findings are consistent with previous studies reporting the dermatological benefits of Buccaneer avocado-derived products, including enhanced skin hydration, collagen content, and elasticity for peel extracts, as well as improved fibroblast proliferation and wound healing for leaf extracts³¹.

Anti-inflammatory activity

These findings demonstrate that both extracts exhibit significant anti-inflammatory activity, supporting their potential application as bioactive ingredients in cosmetic formulations.

Chronic inflammation is a major contributor to skin aging, as excessive production of inflammatory mediators and reactive oxygen species can induce matrix metalloproteinases (MMPs) expression, leading to accelerated extracellular matrix degradation. Therefore, natural compounds that modulate or suppress inflammatory

responses may help prevent skin damage and delay skin aging processes³². In this study, both Buccaneer avocado leaf and peel extracts exhibited significant anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages without cytotoxicity. Notably, the leaf extract showed stronger anti-inflammatory activity, reducing nitric oxide (NO) production by $74.7 \pm 3.83\%$ compared with $55.2 \pm 3.7\%$ for the peel extract at 0.5 mg/mL (Figure 4c and 4d). This enhanced effect is likely attributed to its higher phenolic content, including total phenolics, flavonoids, and tannins (Table 1). LC-MS/MS analysis further identified chlorogenic acid, quercetin, luteolin, and rutin (Table 2), which are known to inhibit NO production via the suppression of iNOS expression and the modulation of NF- κ B and MAPK signaling pathways in activated macrophages^{32,33}. Overall, the anti-inflammatory activity is likely driven by synergistic interactions among these polyphenols, supporting their potential application as natural anti-inflammatory and anti-aging cosmetic ingredients.

Conclusion

This study demonstrates that Buccaneer avocado by-products, particularly leaves and peels, are valuable sources of bioactive compounds. UAE-derived extracts revealed that the leaf extract was rich in phenolics and flavonoids and exhibited strong antioxidant and anti-glycation activities. In contrast, the peel extract displayed superior tyrosinase-inhibitory and astringent properties. LC-MS/MS QTOF analysis confirmed the presence of phenolic acids, flavonoids, and tannins, which are likely responsible for the observed biological activities. Both extracts were non-cytotoxic to L929 fibroblast cells at 0.50 mg/mL and effectively inhibited nitric oxide production in RAW 264.7 macrophages, indicating anti-inflammatory potential.

The present findings provide scientific evidence supporting the potential of Buccaneer avocado by-products as functional ingredients in cosmetic formulations, particularly for skin-lightening, anti-aging, and anti-

inflammatory applications. Future research should focus on in vivo efficacy studies, formulation development, and stability assessment of the bioactive compounds to support their practical application in the cosmetic industry.

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