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Phytochemical profile, antioxidant properties, and anticancer activity of *Mauritia flexuosa* L. from the Ecuadorian Amazon.

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Abstract:

A comprehensive study was performed to elucidate the metabolite composition, antioxidant activity, and anti-breast cancer potential of peel, pulp, and seed extracts from *Mauritia flexuosa* L., a palm native to the Ecuadorian Amazon. Antioxidant capacity was determined using DPPH, ABTS, and FRAP assays, supported by electrochemical analyses. Metabolite profiling by UPLC-ESI-QTOF-MS revealed diverse phenolic constituents. The antiproliferative effects of the extracts against the T-47D breast cancer cell line were evaluated by MTT assay, with apoptosis induction confirmed by western blotting. Among the tested extracts, the peel demonstrated the strongest cytotoxic activity. Molecular docking and molecular dynamics simulations indicated that the predominant phenolic compounds may inhibit CYP1A1 and CYP1B1 enzymes, providing mechanistic insight into the observed antiproliferative effects. Collectively, these findings highlight *M. flexuosa* as a promising natural source of bioactive compounds with potential applications in the development of novel agents against breast cancer.

Keywords: *Mauritia flexuosa* L., Phytochemical profile, antioxidant activity, T-47D, western blot analysis, CYP1A1, CYP1B1.

1. Introduction

Breast cancer remains one of the most prevalent and lethal malignancies in women worldwide, accounting for approximately 30% of newly diagnosed cancer cases[1], [2]. Epidemiological studies have shown that cytochrome P450 (CYP) enzymes are expressed in breast cancer tissues. This ubiquitous enzyme family catalyzes oxidation–reduction reactions and plays a pivotal role in xenobiotic metabolism[3]. Among them, CYP1A1 and CYP1B1 possess a high capacity for metabolizing xenobiotics, making them promising targets for anticancer drug development[4]. However, cancer cells often reprogram their metabolic pathways to produce specialized metabolites and signaling molecules that sustain tumor growth and promote immune evasion[5]. Resistance to conventional therapies remains a critical clinical challenge, as tumor cells develop adaptive mechanisms that lead to treatment failure.

This resistance is further compounded by adverse effects such as endocrine dysfunction and cardiotoxicity, which limit therapeutic options and underscore the need for more selective and effective treatment strategies[6], [7].

Natural compounds have gained increasing attention for their therapeutic potential in cancer and neurodegenerative diseases[8], [9], [10], [11]. Phenolic compounds, in particular, are a diverse group of specialized metabolites characterized by an aromatic ring with one or more hydroxyl groups. Approximately 8,000 phenolic structures have been identified in plants[12], encompassing major classes such as flavonoids, phenolic acids, lignins and lignans, tannins, xanthones, stilbenes, and coumarins[13]. Recent pharmacological studies have demonstrated the chemomodulatory effects of dietary phenolics, highlighting their potential role in cancer prevention and therapy[14], [15], [16].

The Amazon rainforest, the most biodiverse wilderness on Earth, harbors an estimated 16,000 tree species[17], [18]. Naturally occurring swamp forests dominated by palm species cover approximately 150,000 km² of the basin[19]. Among these, *Mauritia flexuosa* L.—commonly known as morete, muriti, buriti, or aguaje—is one of the most versatile medicinal palms of the Arecaceae family, thriving in river floodplains and upland depressions. This species is native to several Amazonian countries, including Trinidad and Tobago, Venezuela, Brazil, Ecuador, Peru, Colombia, and Bolivia[20], [21]. In the Ecuadorian Amazon, *M. flexuosa* L. is particularly abundant in the western region. The palm can grow up to 30–40 meters in height, with a stem diameter ranging from 13 to 55 cm, and bears reddish-brown fruits approximately 5 cm in size. At maturity, the fruit pulp is orange, aromatic, and characterized by a sweet flavor and oily, pasty texture[20], [22], [23].

M. flexuosa L. is considered a keystone species of notable economic and ecological importance, widely utilized for nutritional, cosmetic, medicinal, and cultural purposes[24]. Pharmacologically, it represents a valuable source of bioactive compounds. The fruit pulp contains tetraterpenoids, xanthophylls, tocopherols, carotenoids, and vitamins A, B, and C, along with a range of potent flavonoids such as rutin, lutein, luteolin, kaempferol, catechin, and quercetin[25]. Traditionally, oil extracted from the fruit has been used in indigenous medicine, and recent studies have demonstrated its photoprotective[26], antidiabetic[27], and anticancer[28] properties.

The present study aimed to evaluate the antioxidant and antiproliferative activities of *Mauritia flexuosa* L. extracts against the T-47D breast cancer cell line, alongside a detailed analysis of their phytochemical composition. Targeted phytochemicals in the peel, pulp, and seed extracts were quantified using ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS). Our findings revealed that differences in the concentration of identified phytochemicals across the fruit parts were closely associated with their antioxidant capacity and antiproliferative effects. Furthermore, in an effort to explore the untapped anticancer potential of *M. flexuosa* L., we conducted *in silico* analyses to preliminarily assess the interactions between selected metabolites and the CYP1A1 and CYP1B1 enzymes, which are implicated in breast cancer pathophysiology.

2. Materials and methods

2.1 Reagents

The following compounds were obtained from Sigma-Aldrich (St. Darmstadt, Germany): DPPH radical, ferric chloride, ethanol, methanol, aluminum chloride, sodium nitrite, gallic acid, dimethyl sulfoxide (DMSO), and tetrabutylammonium hexafluorophosphate (Bu₄NPF₆), all with a purity greater than 99%. Authentic standards of phenolic compounds, including (-) epicatechin, (+) catechin (Fig. 13), rutin, and quercetin, as well as ascorbic acid (> 99%), trans-resveratrol (> 99%), and neochlorogenic acid (> 99%) were also obtained from Sigma-Aldrich. Chromatography solvents were of reversed-phase high-performance liquid chromatography grade, meeting the requirements of the analyses performed.

Ultrapure water was obtained by purifying deionized water using a Thermo Scientific™ Barnstead™ GenPure™ water purification system (conductivity: 18 MΩ cm⁻¹).

2.2 Collection and extraction of fruit materials

Mauritia flexuosa L. fruits (Fig. 1) were purchased in 2022 from a local supplier in Napo, Ecuador (1°02'19.2" S, 77°45'41.8" W). The fruits were placed in sterile plastic bags and transported to the laboratory in an icebox for further analysis. The peel, pulp, and seed were separated and analyzed individually. Samples were lyophilized using an SP Scientific-Genevac freeze dryer (BTP-9E LOVE) for 72 h until a constant weight was achieved. The lyophilized material was then stored in an ultra-low freezer at -20 °C, ground into a fine powder using a grinder, and kept protected from light until analysis. For extraction, the powdered samples were weighed and mixed with a hydroethanolic solution (ethanol:water, 30:70, v/v) at room temperature, using a solid-to-solvent ratio of 1:10 (w/v). The mixtures were intermittently shaken for 120 min in the dark, after which the supernatants were collected into 5 mL amber vials and stored at -4 °C until analysis [29].



Fig. 1. Fresh *Mauritia flexuosa* L. fruits from the Ecuadorian Amazon.

2.3 Determination of TPC

TPC was evaluated using the adapted Folin–Ciocalteu reagent method[29]. Briefly, 0.1 mL of the hydroethanolic extract was added to a volumetric flask containing 0.5 mL of the Folin–Ciocalteu reagent and 7 mL of water. The mixture was thoroughly shaken to ensure proper mixing. Next, 1.5 mL of a 10% (w/v) sodium carbonate solution was introduced, and the solution was diluted to a final volume of 10 mL, followed by vortex mixing. The reaction mixture was left in the dark for 30 min at room temperature. The absorbance of the resulting solution was measured using a UV–Vis spectrophotometer (UV-DR6000, Hach, Loveland, CO) at a wavelength of 765 nm. The TPC results were expressed as milligrams of gallic acid equivalent per 100 g⁻¹ of fruit (d.w.) and compared to gallic acid standards.

2.4 Determination of TFC

TFC was quantified using the aluminum chloride colorimetric assay, as described by Chang et al.[30]. A calibration curve was prepared by diluting quercetin in methanol. In brief, 0.1 mL of the hydroethanolic extract or quercetin solution was mixed with 0.1 mL of 10% (w/v) aluminum chloride solution and 0.1 mL of 0.1 mM potassium acetate solution. The mixture was incubated for 30 min at room temperature. The absorbance was measured at 415 nm using a UV–Vis spectrophotometer (UV-DR6000, Hach, Loveland, CO). Results were expressed in mg of quercetin equivalent per gram of sample (d.w.).

2.5 Metabolomic profile using UPLC-MS

Metabolomics data acquisition of the 70% hydroethanolic extract of *Mauritia flexuosa* L. (peel, pulp, and seed) was carried out using reverse-phase chromatography on a Waters Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 µm particle size; Waters, Milford, MA, USA). Freeze-dried extracts (5 mg) were reconstituted in 1 mL of a water:ethanol mixture (1:1, v/v) and centrifuged at 10,000 rpm for 3 minutes; the resulting supernatants were collected for MS analysis. A pooled QC sample, generated by mixing equal aliquots of each extract, was periodically injected throughout the sequence to monitor analytical reproducibility. Solvent and process blanks were included to identify and exclude background signals. Each sample was injected at a volume of 3 µL. Chromatographic separation was achieved using a mobile phase composed of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile, following a gradient elution program: 0–1 min, 1% B; 1–10 min, 1–20% B; 10–20 min, 20–25% B; 20–25 min, 25–35% B; 25–30 min, 35–50% B; and 30–34 min, 1% B. The flow rate was maintained at 0.2 mL min⁻¹. Full-scan mass spectra were acquired in negative electrospray ionization (ESI) mode over an m/z range of 100–1200 Da.

The electrospray ionization (ESI) parameters for both full-scan and data-dependent acquisition (DDA) modes were as follows: capillary voltage, 0.5 kV; sample cone voltage, 40 V; source offset, 80 V; source temperature, 120 °C; desolvation temperature, 450 °C; nitrogen desolvation gas flow, 900 L h⁻¹; cone gas flow, 0 L h⁻¹; and argon as the collision gas. Identification of (-)-epicatechin, (+)-catechin, rutin, quercetin, trans-resveratrol, ascorbic acid, and neochlorogenic acid was achieved by matching retention times and ESI–MS spectra with authentic standards, corresponding to Level 1 identification according to the Metabolomics Standards Initiative (MSI). Both MS and DDA data were acquired in resolution mode using collision energies of 10, 20, and 40 eV. Mass calibration was performed using an external reference (LockSpray™) with continuous infusion of leucine-enkephalin (1 ng µL⁻¹) at 10 µL min⁻¹. The MS¹ survey scan time was set to 0.2 s and the MS² scan time to 0.1 s. The five most intense precursor ions detected during MS¹ acquisition were selected for collision-induced dissociation (CID). Data acquisition and processing were performed using MassLynx v4.1 software (Waters, Milford, MA, USA). In DDA mode, the mass ranges were set to m/z 100–1200 for MS¹ and m/z 50–1200 for MS².

2.6 Data processing and metabolomic identification

Data preprocessing was conducted using MZmine version 4.3.0 (MZmine 4 Documentation) for non-targeted metabolomics analysis of data-dependent acquisition (DDA) datasets. The processing parameters included a minimum MS¹ peak height of 200 (amplitude) and an MS/MS abundance threshold of 20 (amplitude). Peaks detected in blank samples were removed to reduce background noise. For putative metabolite annotation without reference standards, spectral data were processed through the Global Natural Products Social Molecular Networking (GNPS2) platform (GNPS2 Documentation, ccms-ucsd.github.io). Raw Waters data files were first converted to .mgf or .mzML formats following recommended protocols (Mass Spectrometry File Conversion – GNPS Documentation). Mass tolerances for precursor and fragment ions were set at 0.5 Da. A molecular network was constructed by retaining edges supported by at least three matched fragment ions and a cosine similarity score >0.70. The resulting feature list was then filtered to retain only metabolites with a mass error ≤10 ppm and a cosine score ≥0.70. These features were classified as Level 2 identifications according to the Metabolomics Standards Initiative (MSI), corresponding to putatively annotated compounds based on spectral similarity to reference MS/MS libraries. Features not meeting these criteria were classified as Level 3 identifications, reflecting putatively characterized compound classes based on similarity to known chemical classes in public MS/MS databases. The final feature list generated in MZmine (.txt format) was imported into the notame R package for further processing, including normalization and batch drift correction. The processed dataset was subsequently exported to MetaboAnalyst 6.0 for downstream statistical analysis.

2.7 Antioxidant capacity determination

The antioxidant capacity of *M. flexuosa* L. extracts were assessed using the DPPH radical scavenging

assay, FRAP assay, and electrochemical methods, including CV and DPV.

2.7.1 DPPH scavenging assay

The DPPH assay was performed following the method described by Stafussa et al.[29]. Briefly, 0.5 mL of hydroethanolic extract was diluted with 2.75 mL of ethanol and 1.75 mL of DPPH solution (0.06 mM in 80% ethanol). The mixture was maintained under dark conditions for 30 min at room temperature[31]. The absorbance was measured at 517 nm using a UV–Vis spectrophotometer (UV-DR6000, Hach, Loveland, CO). A Trolox standard solution was used to construct calibration curves, and the results were expressed as mg of TEAC per 100 g⁻¹ of d.w. of fruit. The percentage of radical scavenging activity of the extracts was calculated using the following equation (1):

$$DPPH(\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \quad (1)$$

where Abs control is the absorbance of the DPPH solution with ethanol and Abs sample is the absorbance of the DPPH solution with the sample extract or standard. The IC₅₀ values were calculated using GraphPad Prism® software using the following equation (2):

$$y = \frac{100}{(1 + 10^{((\log IC_{50} - x) \cdot HillSlope)})} \quad (2)$$

The TEAC was calculated as follows (3):

$$TEAC = \frac{IC_{50} Trolox}{IC_{50} sample} \quad (3)$$

2.7.2 ABTS assay

The ability of *M. flexuosa* L. extracts to inhibit ABTS radicals was estimated following the method described by Re et al.[32]. Trolox (10–320 µM) was used as the control. A free radical solution was prepared by mixing 7.07 mM ABTS and 2.45 mM potassium persulfate and allowing it to stand in the dark for 16 h at room temperature until the absorbance reached 0.7 at 734 nm. Extract samples or standard solutions (150 µL at different concentrations) were added to the free radical solution (2850 µL) and mixed thoroughly. The absorbance was measured at 734 nm, and the TEAC value was calculated using the same equation as that for the DPPH free radical scavenging activity.

2.7.3 FRAP assay

The FRAP assay of the hydroethanolic extract was performed following the established protocol[33]. The reaction mixture consisted of 300 µL of FRAP reagent, 30 µL of distilled water, and 10 µL of the sample. The mixture was incubated at 37 °C for 4 min, and the absorbance was measured at 600 nm. The results were expressed as mmol Fe²⁺ g⁻¹ d.w.

2.7.4 Antioxidant capacity as measured by CV

The antioxidant capacity of *M. flexuosa* L. extracts against electrochemically generated superoxide anion radicals (O₂^{•-}) were assessed by monitoring changes in the O₂/O₂^{•-} CV response with increasing extract concentrations. The degree of superoxide radical scavenging was represented by plotting the dimensionless parameter $((Ip_a^0 - Ip_a^s)/Ip_a^0)$ vs. extract concentration, where Ip_a^0 is the peak current in the absence of the extract, and Ip_a^s is the anodic peak current in the presence of the extract. The concentration of the extract needed to decrease the anodic peak current of the O₂^{•-} by 50% is referred to as the AI₅₀, which is inversely proportional to radical scavenging activity[34].

CV was performed following the method of Barrietos et al[35] using a three-electrode system comprising a glassy carbon working electrode (3 mm in diameter), a saturated calomel reference electrode, and a graphite rod counter electrode connected to a potentiostat (CHInstruments). The working solution contained 0.05 M tetrabutylammonium hexafluorophosphate in dimethylformamide. Prior to each measurement, the working electrode was polished with alumina of decreasing particle size (0.3, 0.1, and 0.05 μm), rinsed with deionized water at 18 m Ω , sonicated for 1 min, rinsed with acetone, and dried with dry air compressed. The electrochemical cell was saturated with O₂ by bubbling the gas for 1 min. Voltammetric signals were recorded at room temperature using a scan rate of 0.1 V s⁻¹ and a potential range between -0.2 and -1 V.

2.7.5 Antioxidant capacity as measured by DPV

The antioxidant activity of *M. flexuosa* L. extracts was further evaluated using an EI determined by DPV. The EI provides an effective measure of the antioxidant capacity of a chemical species and is calculated using the following equation[36](4):

$$EI = \frac{I_{pa,1}}{E_{pa,1}} + \frac{I_{pa,2}}{E_{pa,2}} + \frac{I_{pa,3}}{E_{pa,3}} + \dots + \frac{I_{pa,n}}{E_{pa,n}} \quad (4)$$

where I_{pa} and E_{pa} correspond to current and potential values, respectively, for each major anodic peak observed in the voltammograms. DPV was conducted using the same electrochemical setup as described previously. A 0.1 M acetate buffer (pH = 5) was used as the electrolytic solution. Prior to each measurement, the solution was purged with nitrogen for 30 s. Electrochemical measurements were recorded within a potential range of -1.2 to 1.5 V (vs. Ag/AgCl), with a scan rate of 17 mV s⁻¹.

2.8 Cell line and culture conditions

The T-47D ductal carcinoma cell line (HTB-133) was used to represent a luminal A subtype of breast cancer (ER+, PR+/-, and HER2-)[37]. The cell line was purchased from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI medium supplemented with penicillin G (100 units/mL), streptomycin (100 $\mu\text{g mL}^{-1}$), amphotericin B (0.25 $\mu\text{g mL}^{-1}$), 2 mM L-glutamine, and 10% fetal bovine serum (v/v, Invitrogen) in a humidified incubator (37 °C, 5% CO₂).

2.8.1 Cell proliferation assay

Cell viability was evaluated using the MTS assay (Gibco), which quantifies the inhibition of cancer cell growth based on mitochondrial respiratory activity. T-47D cells (3 × 10³ cells per well) were seeded into 96-well plates and allowed to adhere for 24 h. Subsequently, cells were treated with *M. flexuosa* L. extracts (peel, pulp, and seed). Each concentration and condition was tested in triplicate and independently replicated three times. DMSO (0.1% v/v) was used as a vehicle control, while 1 μM doxorubicin served as a positive control. Following 24 h of treatment, MTS reagent (5 mg mL⁻¹) was added after 20 h of incubation, and cells were further incubated for 4 h at 37 °C. Absorbance was measured at 570 nm with a reference wavelength of 650 nm. Cell viability was calculated using the following equation (Eq. 5):

$$viability(\%) = \frac{Abs_{oftreatedcells}}{Abs_{ofcontrolcells}} \times 100\% \quad (5)$$

The IC₅₀ was calculated using GraphPad Prism version 10.

2.8.2 Observation of cell morphological changes

T-47D cells were seeded at a density of 2×10^4 cells mL^{-1} in chamber slides with cover glasses (Lab-Tek). After 24 h of attachment, the cells were treated for 24 h with the control, 200 $\mu\text{g/mL}$ of *M. flexuosa* L. peel extract, or 1 μM doxorubicin. Subsequently, the cells were fixed with methanol and stained with crystal violet. Morphological changes induced by the treatments were observed under a Zeiss Axioscope 7 microscope, and images were captured.

2.8.2 Western blotting

Total protein extraction, quantification, and immunoblotting were performed according to the method described by Bailon et al.[38], with slight modifications. Briefly, 30 μg of total protein was separated using 15% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was blocked and incubated overnight at 4 °C with primary antibodies from Santa Cruz Biotechnology (H2AX: sc-517336; p21: sc-817; Bcl-2: sc-783; Actin: sc-58673) and Cell Signaling Technology (cleaved caspase-3 (Asp175): #9661S; Bax: #2774S) at the manufacturer-recommended dilutions. Afterward, the blots were incubated with secondary antibodies from Santa Cruz Biotechnology (m-IgG 1 BP-HRP: sc-525408; mouse anti-rabbit IgG-HRP: sc-2357) at a 2:10,000 dilution for 2 h. The blots were developed using the SuperSignal® West Pico chemiluminescent substrate (Millipore/Sigma-Aldrich) and quantified using LI-COR software. The blots were developed using Immobilon® Crescendo Western HRP Substrate (Millipore, Cat. No. WBLUR0500). Chemiluminescent signals were captured using a C-DiGit® Blot Scanner (LI-COR), with a fixed acquisition area of 10.0×8.5 cm and a resolution of 196 μm . Data quantification and image export were performed using Image Studio™ Software v4.0.21 (LI-COR). To ensure accurate molecular weight determination, membranes were photographed under white light to visualize the ladder positions prior to the chemiluminescent substrate incubation.

2.9 Computational study

To study the possible pharmacological activity of the *Mauritia flexuosa* L. metabolites, a computational study using molecular docking and molecular dynamics simulation techniques was done. The target metabolites, identified and quantified using UPLC-QTOF-MS, were selected and docked in the active site of Cytochrome P450 (CYP) 1A1[39] and Human CYP 1B1[40], [41] as they have been reported to be target for anticancer drugs. To model these enzymes, the Homo sapiens Cytochrome P450 (CYP) 1A1[42] and CYP 1B1[43] X-ray structures were selected. Furthermore, the co-crystallized ligands for both enzymes (2-phenyl-4h-benzo[h]chromen-4-one) were redocked as control. Docking studies were performed, reducing the area of calculation to the active site, and setting conditions as follows: exhaustiveness = 32, spacing = 1 Å, ligand flexibility = full. All files for the receptor and the ligands were prepared using AutodockTools[44] and molecular docking run using Autodock VINA[45].

For molecular dynamics simulations, the pose with the lowest docking score was chosen as initial structure. The topology of the receptors was built using AMBER99SB-ILDN forcefield[46] while the ligands' topology calculated from the ACPYPE server[47] using the Generalised Amber Force Field (GAFF)[48]. The receptor-ligand complex (system) was solvated using a TIP3 water model and neutralized using the required Cl^- or Na^+ atoms. The system was energy minimized and equilibrated for 100 ps at 300K using previously described NVT and NPT ensembles[48], [49]. The simulation was run for 200 ns at 1 Barr and 300K using Gromacs 2019[50] software. Finally, the free energy of binding was estimated from the produced trajectory using the g_mmpbsa tool which applies the Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) method[51].

2.10 Statistical analysis

All experiments were performed in triplicate and the data presented as mean $\pm$ standard deviation. Statistical analyses were conducted using R software. One-way analysis of variance was used for assessing the significance between different extracts, and pairwise difference was tested using Tukey's

multiple consistency tests. The correlations between variables were calculated using Pearson's correlation coefficient. Statistical significance was set at $p \leq 0.05$.

3. Results and discussion

3.1 Total flavonoid and phenolic content

Phenolic compounds are well known for their potent antioxidant activity and involvement in diverse subcellular processes. Among these, phenolic acids and flavonoids are particularly notable for their antioxidant potential. Accordingly, fruits rich in phenolic compounds are regarded as promising sources of natural antioxidants[52], [53]. In the present study, statistically significant differences in total phenolic content (TPC) and total flavonoid content (TFC) were observed among different parts of *M. flexuosa* L. fruit (Table 1). Notably, the peel exhibited the highest TPC, with a value of 18.30 ± 0.19 mg GAE g⁻¹ dry weight (d.w.). Similarly, TFC was highest in the peel (1.2 ± 0.03 mg QE g⁻¹ d.w.), followed by the seed and pulp. TPC was lowest in the seed, intermediate in the pulp, and highest in the peel. In contrast, TFC was lowest in the pulp, slightly higher in the seed, and peaked in the peel. The TPC value in the *M. flexuosa* L. pulp extract was slightly lower than that reported by Naranjo et al.[21] for the same species in the Ecuadorian Amazon, which may be attributed to factors such as environmental conditions and fruit ripeness.

Table 1. Total flavonoid and phenolic content

	Total phenolic content (GAE mg g ⁻¹ d.w.)	Total flavonoid content (QE mg g ⁻¹ d.w.)
Peel	18.30 ± 0.19^a	1.20 ± 0.03^a
Pulp	3.60 ± 0.11^b	0.80 ± 0.03^b
Seed	2.20 ± 0.05^c	1.00 ± 0.05^c

Note: Values are expressed as mean $\pm$ SD (n = 3). Superscript lowercase letters ("a", "b", "c") within the same column indicate statistically significant differences among the different parts of *Mauritia flexuosa* L. (peel, pulp, and seed), as determined by Tukey's multiple comparisons test ($p < 0.05$). GAE = gallic acid equivalent; QE = quercetin equivalent.

3.2 Metabolomic profile

Table 2 presents the components identified by LC-MS/MS, categorized by different energy levels: 10 eV (GNPS - 10 eV; ID = efb3cccc99eb4b93ba29d84ceba1ce6d), 20 eV (GNPS - 20 eV; ID = 5cbd50000bc24767bef9967f1192e4ac) and 40 eV (GNPS - 40 eV; ID = 6b9b95d606204708805df2de0794a3ed). Among the identified compounds, 7 were classified at level 1 with authentic standards, 20 were identified at level 2 using public libraries, and 09 were assigned to level 3 based on putative chemical classes, where the chemical class could be assigned through spectral matches with public MS/MS libraries.

Table 2. Compounds identified in hydroethanolic extracts of *Mauritia flexuosa* L. using UPLC-MS/MS

IL	Rt (min)	[M-H] ⁻	Molecular Formula	Error ppm	MS2 Score	Identification	Metabolite classification
2	1.075	111.0078	C ₅ H ₄ O ₃	-6.70	0.87	3-Furoic acid	Organoheterocyclic compounds
2	2.762	113.0246	C ₄ H ₄ O ₄	-	0.91	Fumarate	Organic acids and derivatives
2	0.538	115.0032	C ₄ H ₄ O ₄	5.73	0.86	Fumaric acid	Organic acids and derivatives
3	1.240	128.0340	C ₆ H ₁₁ NO ₂	-	0.88	Pipecolate	Organic acids and derivatives
1	2.472	175.0244	C ₆ H ₈ O ₆	0.57	-	Ascorbic acid	Organic acids and derivatives

1	3.347	227.0686	C ₁₄ H ₁₂ O ₃	12.47	-	Trans-resveratrol	Phenylpropanoids and polyketides
2	4.568	135.044	C ₈ H ₈ O ₂	-8.36	0.73	4'-Hydroxyacetophenone	Organic oxygen compounds
3	0.448	146.96	C ₉ H ₈ O ₂	-	0.84	Cinnamic acid	Phenylpropanoids and polyketides
3	3.685	151.003	C ₈ H ₈ O ₃	-	0.73	Vanillin	Benzenoids
3	4.147	163.061	C ₉ H ₈ O ₃	-	0.88	2-hydroxycinnamic acid	Phenylpropanoids and polyketides
2	3.787	164.071	C ₉ H ₁₁ NO ₂	10.23	0.71	Phenylalanine	Lipids and lipid-like molecules
3	2.744	163.061	C ₉ H ₈ O ₃	-	0.88	4-Methoxysalicylic acid	Phenylpropanoids and polyketides
3	2.923	173.008	C ₇ H ₁₄ N ₂ O ₃	-	0.74	N-Acetylmethionine	Organic acids and derivatives
2	1.360	173.008	C ₆ H ₆ O ₆	-6.49	0.80	Aconitic acid	Organic acids and derivatives
2	0.849	173.044	C ₇ H ₁₀ O ₅	-8.78	0.84	(-)-Shikimic acid	Organic oxygen compounds
2	5.402	175.039	C ₁₀ H ₈ O ₃	-5.96	0.85	6-Hydroxy-4-methylcoumarin	Phenylpropanoids and polyketides
2	2.989	179.034	C ₉ H ₈ O ₄	-5.32	0.82	Cis-Caffeic acid	Phenylpropanoids and polyketides
3	2.134	191.309	C ₇ H ₁₂ O ₆	-	0.77	Quinate	Organic oxygen compounds
2	1.069	191.019	C ₆ H ₈ O ₇	-3.64	0.94	Citric acid	Organic acids and derivatives
2	4.335	193.049	C ₁₀ H ₁₀ O ₄	-8.28	0.77	Trans-Ferulic acid	Phenylpropanoids and polyketides
2	4.112	203.081	C ₁₁ H ₁₂ N ₂ O ₂	-7.73	0.89	Tryptophan	Organoheterocyclic compounds
2	4.105	206.08	C ₁₁ H ₁₃ NO ₃	-10.81	0.91	N-Acetyl-L-phenylalanine	Organic acids and derivatives
2	2.708	218.102	C ₉ H ₁₇ NO ₅	-5.93	0.77	D-(+)-Pantothenic acid	Organic acids and derivatives
2	4.577	243.066	C ₁₄ H ₁₂ O ₄	-1.06	0.75	E-Piceatannol	Phenylpropanoids and polyketides
2	7.488	257.081	C ₁₅ H ₁₄ O ₄	-3.53	0.81	2',4',6'-Trihydroxydihydrochalcone	Phenylpropanoids and polyketides
2	3.493	287.055	C ₁₅ H ₁₂ O ₆	-3.77	0.95	Dihydrokaempferol	Phenylpropanoids and polyketides
1	3.670	289.071	C ₁₅ H ₁₄ O ₆	-2.54	-	Catechin	Phenylpropanoids and polyketides
1	3.482	289.071	C ₁₅ H ₁₄ O ₆	-2.54	-	(+)-Epicatechin	Phenylpropanoids and polyketides
3	3.332	293.123	C ₁₄ H ₁₈ N ₂ O ₅	-	0.73	Glutamylphenylalanine	Organic acids and derivatives
1	5.557	301.0333	C ₁₅ H ₁₀ O ₇	-6.83	-	Quercetin	Phenylpropanoids and polyketides
2	2.743	329.087	C ₁₄ H ₁₈ O ₉	-8.45	0.89	Vanillic acid 4-beta-D-glucoside	Phenylpropanoids and polyketides
1	2.997	353.087	C ₁₆ H ₁₈ O ₉	-2.20	-	Chlorogenic Acid	Phenylpropanoids and polyketides
2	3.693	577.135	C ₃₀ H ₂₆ O ₁₂	-0.21	0.72	Procyanidin B2	Phenylpropanoids and polyketides
2	3.825	577.135	C ₃₀ H ₂₆ O ₁₂	-0.21	0.83	Procyanidin B1	Phenylpropanoids and polyketides
1	3.728	609.1460	C ₂₇ H ₃₀ O ₁₆	0.14	-	Rutin	Phenylpropanoids and polyketides
2	3.695	865.198	C ₄₅ H ₃₈ O ₁₈	-0.59	0.70	Procyanidin C1	Phenylpropanoids and polyketides

IL, identification level according to Summer et al. (2007); RT, retention time in minutes; MS2 score for level 2 identification refers to GNPS modified cosine score

We used the principal component analysis (PCA) and a Biplot plot to explore the metabolomic relationships among the different parts of *Mauritia flexuosa* L (Fig. 2).

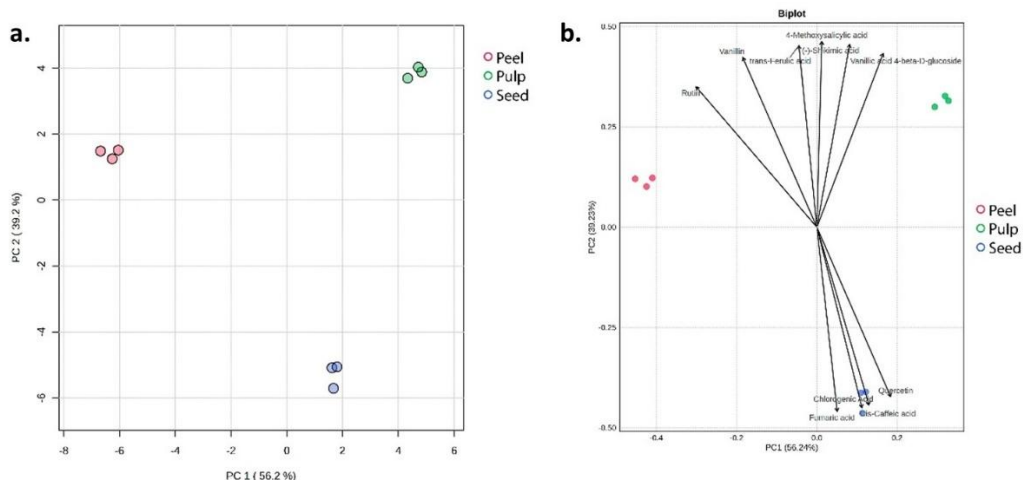


Fig. 2 | Principal component analysis. a. Principal component analysis (PCA) score plot; b. corresponding biplot illustrating the relationships between samples and variables.

The clear overlap of the pooled quality control samples (Supplementary Fig. 1) demonstrates both the high performance of the mass spectrometer and the consistency of the data preprocessing. This analytical robustness is further supported by the PCA score plot, in which the first principal component (PC1) accounts for 56.2% of the total variance and the second principal component (PC2) explains an additional 39.2%. The plot shows a well-defined separation among the three sample types. Peel samples cluster on the negative side of PC1, Seed samples group near the origin on the positive side of PC1, and Pulp samples are distributed along the positive axis of PC2. This distinct clustering pattern reflects clear metabolic differences among the three sample types. Similarly, the biplot analysis identified metabolites such as *cis*-caffeic acid, chlorogenic acid, quercetin, and fumaric acid as strongly associated with the differentiation between Seed and the other fruit parts (peel and pulp), with these compounds being predominantly accumulated in the Seed samples.

The variability in the abundance of identified metabolites across the different fruit tissues was explored using a heatmap (Fig. 3) combined with hierarchical cluster analysis (HCA). Unlike the group separation suggested by the PCA, the HCA based on annotated metabolites revealed a different clustering pattern, in which Seed samples appear metabolically more like pulp than to peel.

The horizontal dendrogram organizes the metabolites into two major clusters, reflecting distinct abundance of profiles among the sample types. The upper cluster comprises metabolites that show moderate to high abundance in pulp, lower levels in seed, and are largely absent in peel. This cluster includes several compounds such as 3-furoic acid, citric acid, quinate, and fumarate, encompassing chemical classes like organic acids, organic oxygen compounds, and phenylpropanoids. These metabolites are often associated with fruit acidity, flavor, and antioxidant properties, which aligns with their accumulation in the pulp a tissue specialized in attracting frugivores for seed dispersal[54].

In contrast, the lower cluster includes metabolites that are highly enriched in peel samples, but present at much lower levels in pulp and seed. This cluster is largely composed of phenylpropanoids and polyketides, particularly flavonoids such as catechin, epicatechin, quercetin, and rutin, along with procyanidins (B1, B2, and C1). These compounds are well recognized for their antioxidant, anti-inflammatory, and antimicrobial roles in plant defense [55]. Their high abundance in peel aligns with their function in protecting reproductive tissues from oxidative damage and microbial attack [56]. This distinct clustering of metabolites highlights the tissue-specific metabolic specialization within the fruit and underscores the importance of chemical classification in interpreting metabolomic data. These

insights contribute to a deeper understanding of the ecological roles of secondary metabolites in plant biology and offer a valuable framework for identifying candidates with nutritional or pharmacological potential[57].

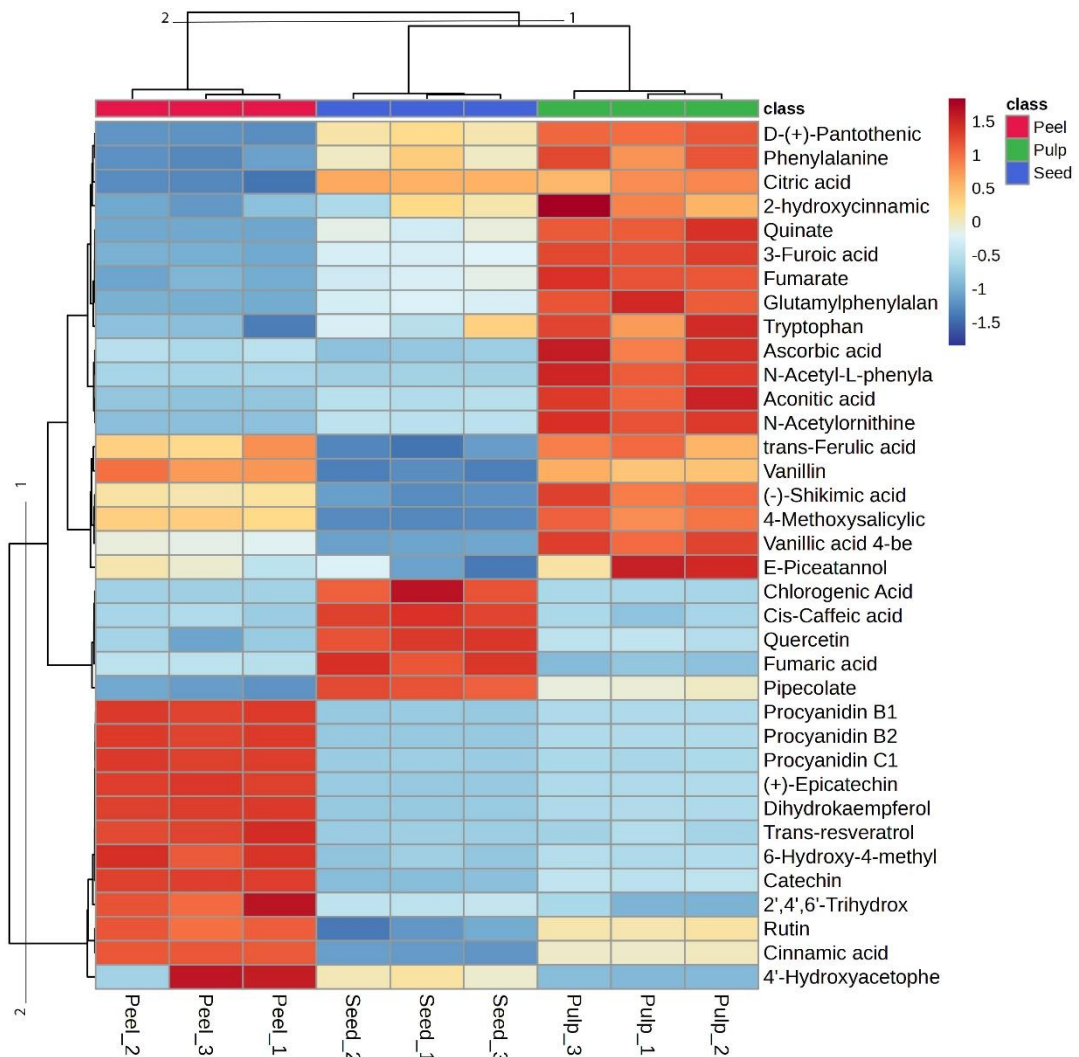


Fig. 3. Heatmap and HCA of LC-MS analysis of *Mauritia flexuosa* L.

3.3 Antioxidant capacity

Reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), are continuously produced during normal cellular metabolism. If not neutralized, these ROS can cause oxidative damage to essential cellular components, including lipids, proteins, carbohydrates, and DNA molecules[58], [59]. Numerous plant extracts have demonstrated the ability to reduce ROS-associated diseases due to the presence of various bioactive compounds[21]. In this study, the antioxidant properties of the hydroethanolic extracts of *M. flexuosa* L. were evaluated using a combination of assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and electrochemical methods such as the antioxidant index 50 (AI₅₀, defined as the extract concentration required to reduce the anodic peak current by 50%) and electrochemical index (EI). Table 3 shows the antioxidant capacity of the hydroethanolic extracts. The

test results were compared to the standard Trolox, with results expressed in terms of Trolox equivalent antioxidant capacity (TEAC) for the DPPH and ABTS assays.

The highest antioxidant potential was observed in the peel extract, with a DPPH half-maximal inhibitory concentration (IC_{50}) of $31.20 \pm 0.70 \mu\text{g mL}^{-1}$ and an ABTS IC_{50} of $35.23 \pm 0.50 \mu\text{g mL}^{-1}$. In contrast, the seed extract exhibited the lowest potential (DPPH $IC_{50} = 626.90 \pm 4.40 \mu\text{g mL}^{-1}$ and ABTS $IC_{50} = 975.53 \pm 5.34 \mu\text{g mL}^{-1}$). For comparison, the positive control Trolox had a DPPH IC_{50} of $14.90 \pm 0.4 \mu\text{g mL}^{-1}$ and an ABTS IC_{50} of $137.40 \pm 1.90 \mu\text{g mL}^{-1}$. The FRAP assay to examine radical scavenging activity further confirmed these trends, with the peel extract showing the highest radical scavenging activity ($0.57 \pm 0.02 \text{ mmol Fe}^{2+}\text{g}^{-1} \text{ d.w.}$), followed by the pulp ($0.04 \pm 0.00 \text{ mmol Fe}^{2+}\text{g}^{-1} \text{ d.w.}$) and seed extracts ($0.02 \pm 0.00 \text{ mmol Fe}^{2+}\text{g}^{-1} \text{ d.w.}$)(Table 2) These findings are consistent with those of previous studies that have also demonstrated the strong antioxidant capacity of *M. flexuosa* L.[20], [21], [60].

Table 3. Antioxidant properties of hydroethanolic extracts of *Mauritia flexuosa* L. (peel, pulp, and seed)

	DPPH (TEAC)	ABTS (TEAC)	FRAP (mmol Fe^{2+} $\text{g}^{-1} \text{ d.w.}$)	IC_{50} DPPH ($\mu\text{g mL}^{-1}$)	IC_{50} ABTS ($\mu\text{g mL}^{-1}$)	AI_{50} (mg L^{-1})	EI ($\mu\text{A V}^{-1}$)
Peel	248.28 ± 5.30	507.80 ± 7.10	0.57 ± 0.02	31.20 ± 0.70	35.23 ± 0.50	1.45 ± 0.02	32.13 ± 0.27
Pulp	11.10 ± 0.20	22.20 ± 0.30	0.04 ± 0.00	329.70 ± 4.90	380.10 ± 4.83	1.46 ± 0.08	5.18 ± 0.00
Seed	3.96 ± 0.03	5.60 ± 0.03	0.02 ± 0.00	626.90 ± 4.40	975.53 ± 5.34	1.55 ± 0.17	3.39 ± 0.02

*Note: DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), FRAP: ferric-reducing antioxidant power, AI_{50} (antioxidant index 50), EI (Electrochemical Index).

Natural antioxidants exhibit redox properties, making electrochemical analysis a valuable tool for their evaluation[61]. In this study, the antioxidant capacity of the hydroethanolic extracts was evaluated using cyclic voltammetry (CV) via a superoxide anion scavenging assay. As shown in Fig. 4, the anodic peak of the superoxide anion radical decreases with the dose-dependent addition of the extracts to the electrolytic medium, leading to a reduction in the intensity of the Ip_a^0 . The dimensionless parameter $((Ip_a^0 - Ip_a^s)/Ip_a^0)$ was plotted against extract concentration (Fig. 5), revealing a linear relationship indicative of the consumption of $\text{O}_2^{\cdot-}$. From the plots, the AI_{50} was calculated, with lower AI_{50} values corresponding to higher antioxidant capacity. The AI_{50} values are presented in Table 4. The antioxidant capacity of the peel extract was the highest, with an AI_{50} of $1.45 \pm 0.02 \text{ mg L}^{-1}$, compared to the seed ($1.46 \pm 0.08 \text{ mg L}^{-1}$) and pulp extracts ($1.55 \pm 0.17 \text{ mg L}^{-1}$).

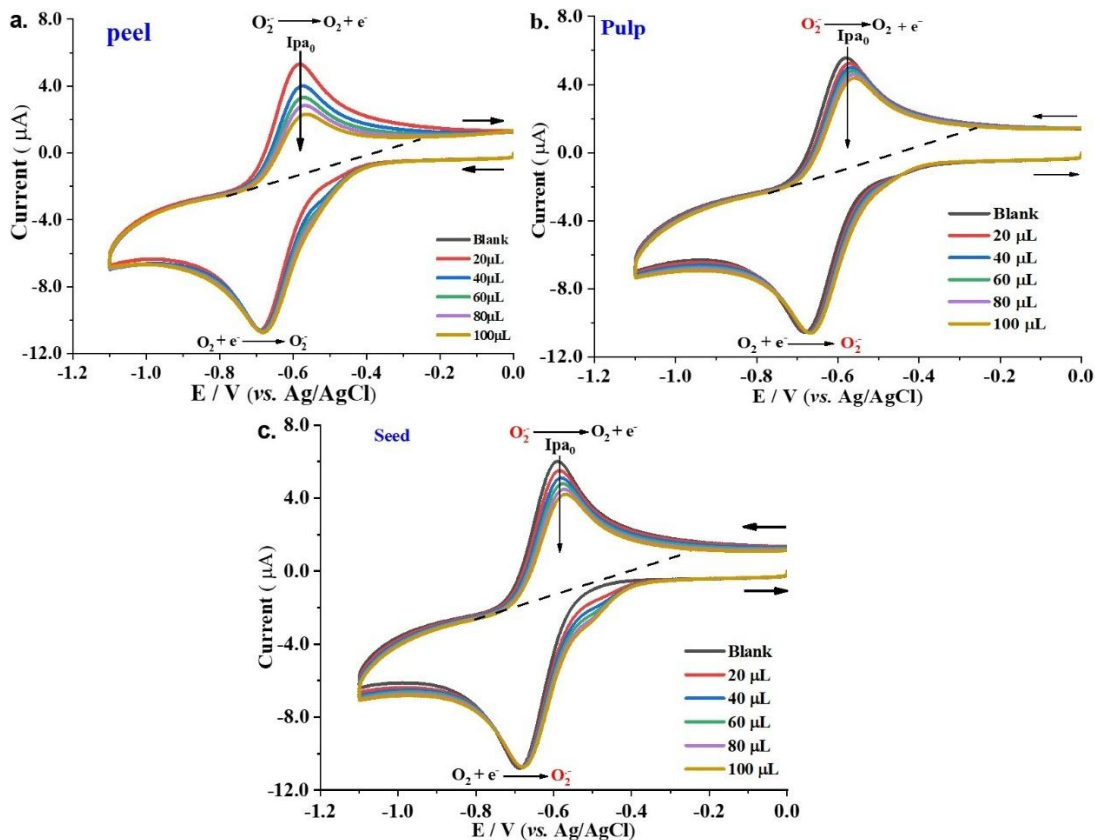


Fig. 4 | Cyclic voltammograms showing superoxide anion ($\text{O}_2^{\bullet-}$) formation on a glassy carbon electrode in the presence of *Mauritia flexuosa* L. hydroethanolic extracts.

Cyclic voltammograms recorded upon successive additions of extracts from: **a**, peel; **b**, pulp; and **c**, seed. Measurements were performed in DMS containing 0.05 M Bu_4NPF_6 at a scan rate of 0.1 V s^{-1} .

450

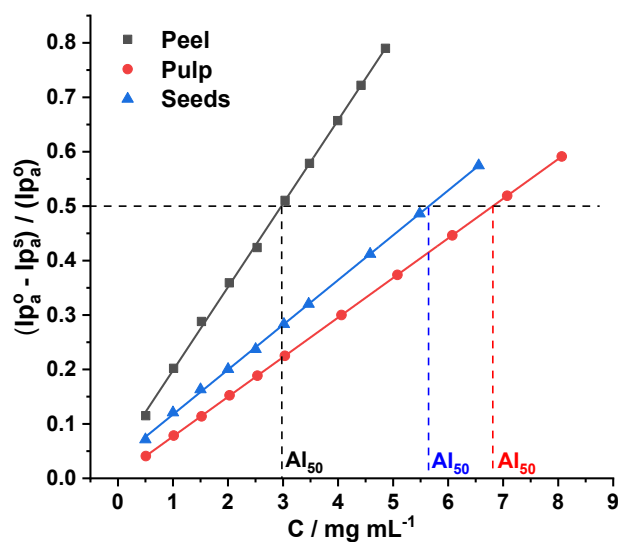


Fig. 5 | Dimensionless parameter $((I_{p_a}^0 - I_{p_a}^s) / I_{p_a}^0)$ as a function of *Mauritia flexuosa* L. extract concentration for peel, pulp, and seed.

To further investigate the antioxidant properties of the hydroethanolic extracts of *M. flexuosa* L., the EI was evaluated using differential pulse voltammetry (DPV). Substrates with lower oxidation potentials ($E_{pa} < 0.45$ V) demonstrated significantly higher electron-donating ability, reflected by higher peak currents, indicating a greater presence of electroactive species. Conversely, substrates with higher oxidation potentials (E_{pa} values > 0.45 V), acted as prooxidants. Thus, EI is directly proportional to the antioxidant capacity of the extracts[62], [63], [64]. Fig. 6 shows the DPV behavior of *M. flexuosa* L. (peel, pulp, and seed).

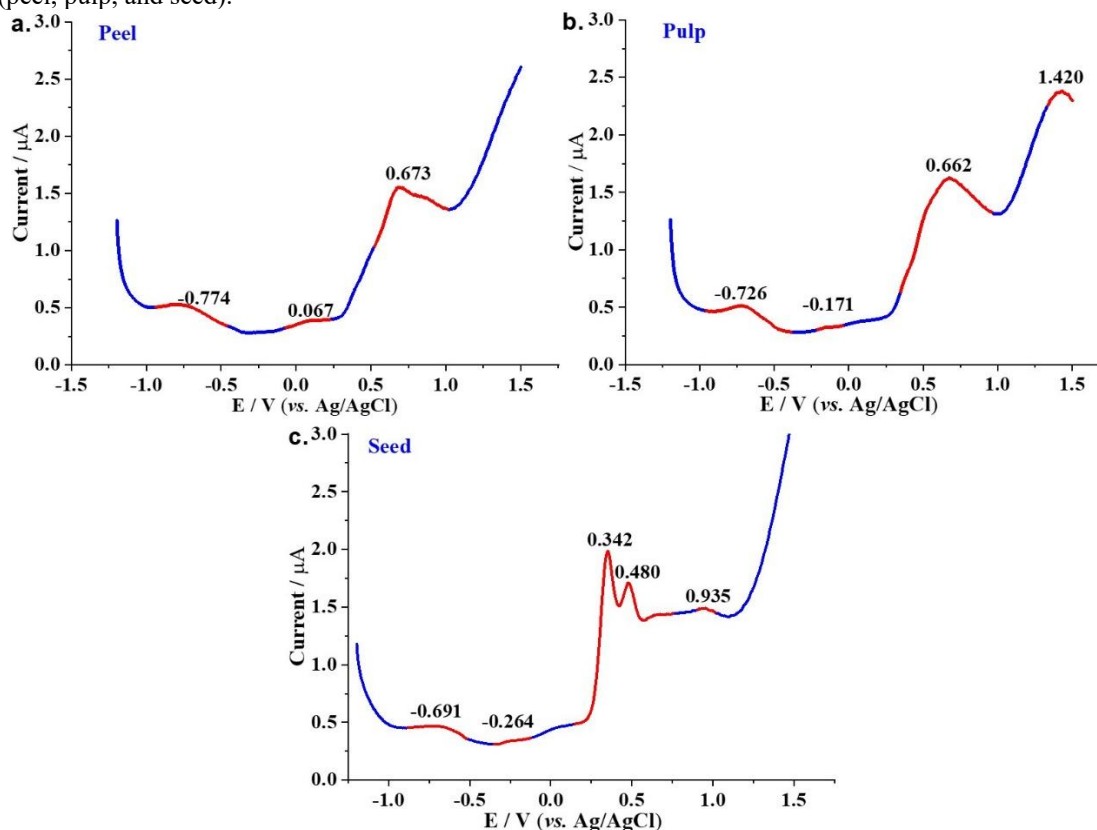


Fig. 6 | Differential pulse voltammograms of hydroethanolic extracts of *Mauritia flexuosa* L.: a. peel, b. pulp, and c. seed in a buffer acetate solution (pH 5.0) characterized using a glassy carbon electrode.

The DPV results for the peel extract (Fig. 6a) showed an anodic peak at approximately 0.342 V (vs. Ag/AgCl), with a shoulder at 0.480 V (vs. Ag/AgCl). The appearance of shoulder peaks is likely related to matrix effects and the presence of non-phenolic compounds with inherent electroactivity[62]. The pulp extract (Fig. 6b) exhibited two distinct anodic peaks at -0.726 and 0.662 V (vs. Ag/AgCl), while the seed extract (Fig. 6c) presented one peak at 0.673 V (vs. Ag/AgCl). The EI values of the pulp, peel, and seed extracts, shown in Table 4, ranged from 3.39 ± 0.02 to $32.13 \pm 0.27 \mu\text{A V}^{-1}$. The peel extract had the highest EI value ($32.13 \pm 0.27 \mu\text{A V}^{-1}$), followed by the pulp and seed extracts. These results are consistent with the antioxidant capacity determined using DPPH, ABTS, and FRAP assays.

3.4 Correlation coefficient analysis

Pearson's correlation coefficients for TFC, TPC, DPPH, ABTS, FRAP, DPPH IC_{50} , ABTS IC_{50} , AI_{50} , and EI are provided in Table 4. The analysis revealed a positive linear correlation between TPC and the antioxidant assays, which is consistent with the literature[65], [66], [67], [68]. Specifically, Pearson's

correlation analysis indicated a strong positive linear correlation between TPC and the antioxidants assays of *M. flexuosa* L. (TPC–DPPH: $R^2 = 0.998$, TPC–ABTS: $R^2 = 0.999$, TPC–FRAP: $R^2 = 0.999$, TPC–EI: $R^2 = 1.000$). In contrast, the AI_{50} method, where lower values indicate higher antioxidant capacity, showed a strong negative linear correlation with the TPC, TFC, DPPH, ABTS, FRAP, and EI values. These findings align with previous studies that have shown a significant correlation between TPC, spectrophotometric assays, and electrochemical measurements for evaluating antioxidant activity[62], [69].

Table 4. Pearson’s correlation coefficients between antioxidant capacity assays and phenolic content and flavonoid content.

	TFC	TPC	DPPH	ABTS	FRAP	DPPH IC ₅₀	ABTS IC ₅₀	AI ₅₀	EI
TFC	1.000								
TPC	0.824	1.000							
DPPH	0.856	0.998	1.000						
ABTS	0.851	0.999	1.000	1.000					
FRAP	0.850	0.999	1.000	1.000	1.000				
DPPH IC ₅₀	-0.501	-0.903	-0.876	-0.881	-0.882	1.000			
ABTS IC ₅₀	-0.363	-0.827	-0.792	-0.798	-0.800	0.988	1.000		
AI ₅₀	-0.091	-0.639	-0.602	-0.600	-0.602	0.907	0.961	1.000	
EI	0.837	1.000	0.999	1.000	1.000	-0.893	-0.621	-0.621	1.000

3.5 Cell viability and apoptosis induction

We investigated the efficacy of the peel extract of *Mauritia flexuosa* L. on the proliferation of Ductal breast cancer T-47D, owing to their antioxidant potential, and bioactive compounds. The cell proliferation assay determined an IC₅₀ of 161 $\mu\text{g mL}^{-1}$ (Fig. 7a). The morphological changes at the highest concentration of the analyzed extract, as well as the controls, are shown in Fig. 7a-d.

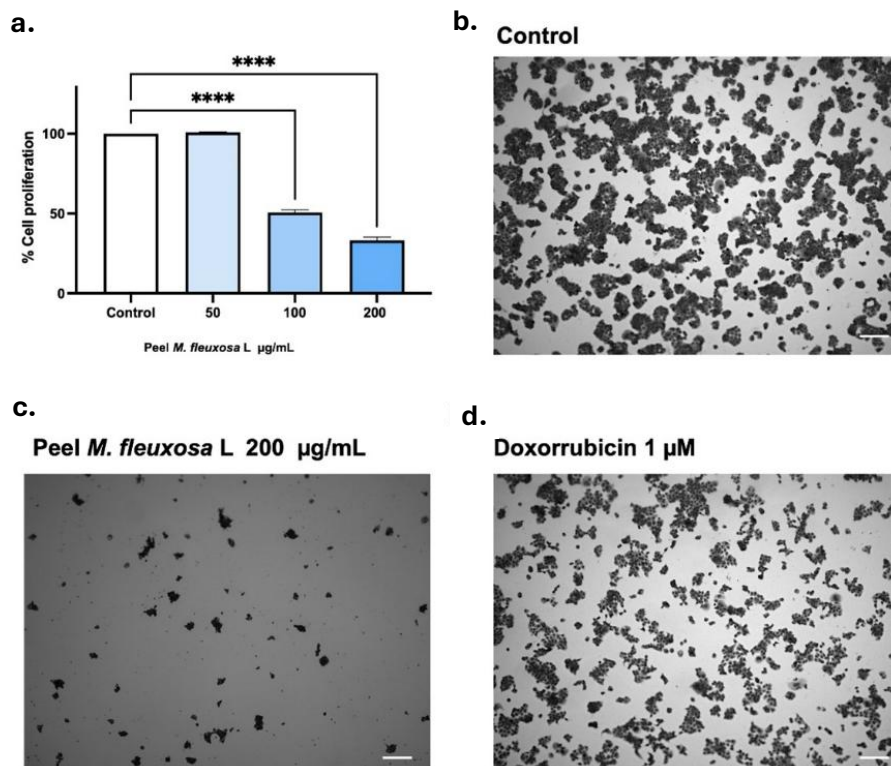


Fig. 7 | Inhibitory effect of *M. flexuosa* L. peel extract on T-47D cell proliferation.

a, Cell proliferation assay. Statistical significance was assessed using Dunnett's multiple comparisons test ($p < 0.0001$). **b–d**, Microscopic images of T-47D cells after 24 h of treatment: **b**, control; **c**, 200 $\mu\text{g mL}^{-1}$ *M. flexuosa* peel extract; and **d**, doxorubicin 1 μM . Magnification 40 $\times$.

The type of cell death induced in T-47D breast cancer cells was evaluated using three different doses of the peel extract. Protein modulation related to cell death, DNA damage, and repair is shown in Fig. 8a and 8b. Increasing the concentration of *M. flexuosa* L. peel extract led to an increase in γH2AX expression, a protein associated with double-strand DNA breaks and repair. At the lowest dose, there was a higher induction of p21—a protein involved in cell cycle arrest—compared to the control, but its expression decreased at higher doses, which coincides with the induction of the pro-apoptotic protein Bax and a decrease in Bcl-2, an anti-apoptotic protein, highlighting a significant difference in the Bax/Bcl-2 ratio. Furthermore, the extract induced a decrease in procaspase-3 levels, along with the induction of cleaved caspase-3 at the highest dose, indicating apoptosis induction in T-47D cells. These anticancer effect may be attributed to the secondary metabolites in the extract, including (+) catechin[70], rutin[71], and trans-resveratrol [72], [73], [74], which have been reported to exert chemopreventive and chemotherapeutic effects on cancer cell lines. In the specific case of trans-resveratrol, it has been found to induce apoptosis in similar cells in the estrogen receptor and human epidermal growth factor receptor type 2-positive breast cancer cells[75].

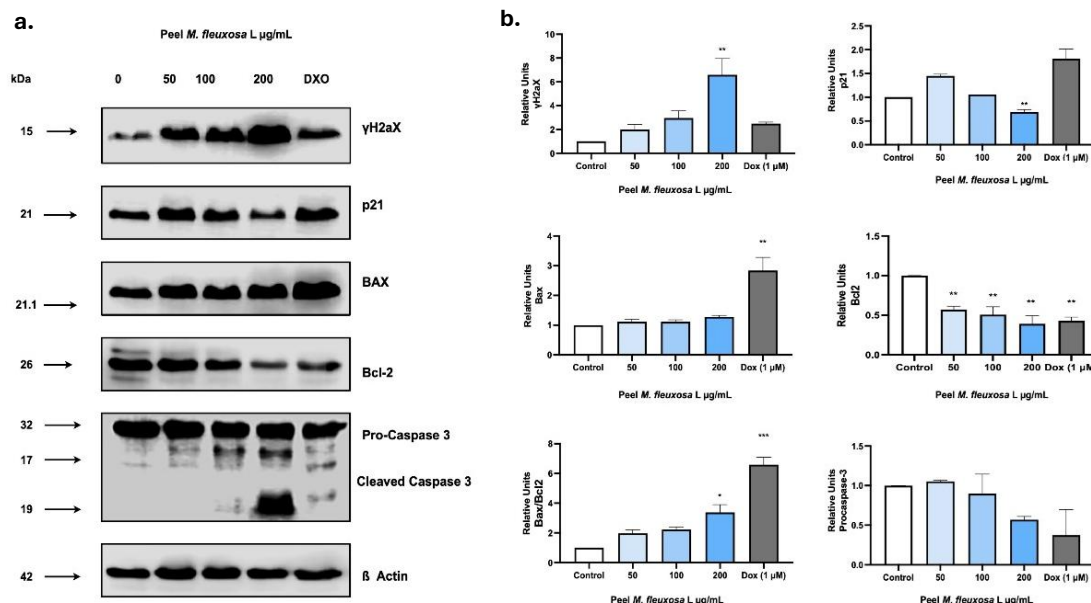


Fig. 8 | Western blot analysis of DNA damage and apoptosis-related proteins in T-47D cells treated with *M. flexuosa* L. peel extract for 24 h. a, Representative immunoblots for γH2AX, p21, Bcl-2, Bax, procaspase-3, and cleaved caspase-3. β-Actin served as the loading control (Full, uncropped gel and blot images are provided in **Supplementary Fig. 2 – Supplementary Fig.6**). **b,** Quantification of protein expression levels. Data is presented as mean ± SEM of relative band intensities. Statistical significance was evaluated using Dunnett's multiple comparisons test (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3.6 Computational study

For molecular docking studies, target metabolites from *Mauritia flexuosa* L. (Peel in Fig. 9 and Neochlorogenic acid) and the co-crystallized ligand (CL) from the two CYPs were chosen (Table 5). Docking calculations showed that the three Procyanidins (B1, B2, and C1) produced the highest score, being positive for both CYPs. Furthermore, rutin also produced a positive score against CYP1B1 and the highest negative score for CYP1A1. Looking at the docking conformations produced, it is observed that these molecules are too big to fit into the active site of the two studied CYP, making them unlikely to be able to bind. All the other molecules present negative docking scores and manage to fit in the active site of both CYPs. For CL, the docking score is the lowest, which was expected as the enzyme plasticity is shaped to accept this ligand. Further pose analysis showed the docking result superimposed the ligand's electron density obtained from the X-ray experiment. Looking at the docking score between different CYPs, it seems all the molecules may bind with a similar affinity to both enzymes as differences in their docking score are between 0.1 and 0.6 kcal mol⁻¹. To study a possible binding between the metabolites and the selected CYPs, molecular dynamics studies were performed in molecules with docking scores lower than -8.5 kcal mol⁻¹. Epicatechin, 6-hydroxy-4-methylcoumarin, and trihydroxydihydrochalcone were not considered as they have been already studied in previous research[76], [77], [78], while rutin and the three procyanidin were not considered because it is unlikely to bind those CYPs. CL was also simulated as a control.

Table 5. Docking results for the interaction between metabolites in *Mauritia flexuosa* L. and CYPs

Compound	CYP1A1 score (kcal mol ⁻¹)	CYP1B1 score (kcal mol ⁻¹)
(-) Epicatechin	-10.0	-10.3

(+) Catechin	-10.7	-10.8
Rutin	-3.3	0.2
4-Hydroxyacetophenone	-7.4	-7.4
Cinnamic Acid	-8.2	-7.9
Procyanidin B1	7.7	12.5
Procyanidin B2	7.9	10.8
Procyanidin C1	31.3	40.5
Resveratrol	-10.3	-10.3
Trihydroxydihydrochalcone	-10.1	-10.2
Dihydrokaempferol	-11	-11.6
Neochlorogenic acid	-9.3	-8.9
CL	-14.1	-14.3

Results of the simulation show that the RMSD for CYP1A1 complexes with (+) catechin, neochlorogenic acid, 6-hydroxy-4-methylcoumarin, trihydroxydihydrochalcone, and CL (Fig. 9a) are stable in the 200 ns of simulation. In CYP1B1, for all five systems, it is mostly stable with some fluctuation in the first 30 ns. RMSD values fluctuate around 0.25 nm for all the systems in the two targets. This shows the proteins do not present notable conformational changes when bound to different ligands compared to their co-crystallized compound (CL).

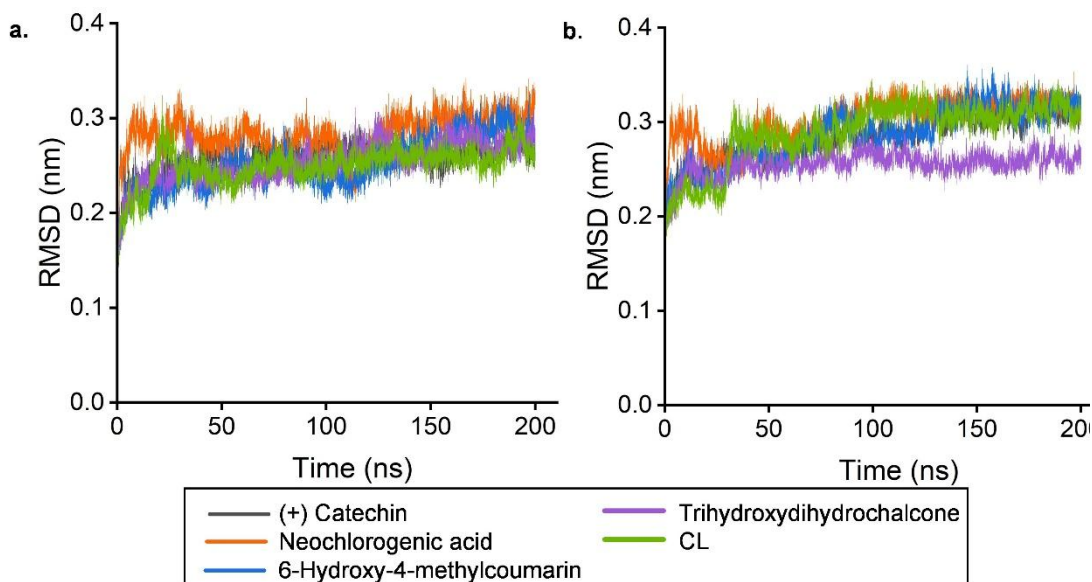


Fig. 9 | Molecular dynamics analysis. Root-mean-square deviation (RMSD) profiles of **a.** CYP1A1 and **b.** CYP1B1 in complex with the target metabolites from *Mauritia flexuosa* L.—(+)—catechin, neochlorogenic acid, 6-hydroxy-4-methylcoumarin, trihydroxydihydrochalcone, and CL—monitored over a 200 ns molecular dynamics simulation.

Upon analyzing the RMSD values of the different ligands across the systems, the complex formed between (+)-catechin and neochlorogenic acid with CYP1A1 exhibited an RMSD of approximately 0.3 nm (Fig. 10a). As expected, CL displayed a lower RMSD (~0.15 nm) with CYP1A1, consistent with its role as a known substrate. In comparison, trihydroxydihydrochalcone showed a similar RMSD to CL,

whereas 6-hydroxy-4-methylcoumarin exhibited the highest deviations, with RMSD values peaking at 0.76 nm and stabilizing around 0.58 nm. For CYP1B1 (Fig. 10b), (+)-catechin showed the lowest RMSD, suggesting a strong and stable interaction with the enzyme. Both 6-hydroxy-4-methylcoumarin and trihydroxydihydrochalcone presented RMSD values approximately 0.1 nm higher than that of (+)-catechin, remaining stable during the final 100 ns of the simulation. Neochlorogenic acid underwent more pronounced conformational changes, reaching an RMSD of 0.5 nm, though it remained stable in that conformation thereafter. CL, while showing lower RMSD values than (+)-catechin, exhibited greater fluctuations, indicative of a less stable interaction.

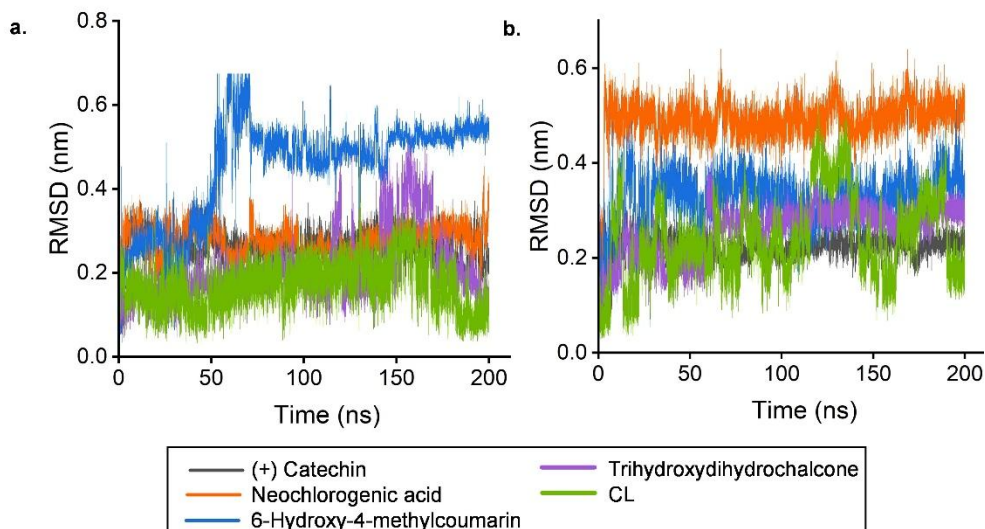


Fig. 10 | Molecular dynamics analysis. RMSD profiles of (+)-catechin, neochlorogenic acid, 6-hydroxy-4-methylcoumarin, trihydroxydihydrochalcone, and CL in complex with **a.** CYP1A1 and **b.** CYP1B1 over a 200 ns molecular dynamics simulation.

A hydrogen bond analysis shows that in general, neochlorogenic acid, 6-hydroxy-4-methylcoumarin, trihydroxydihydrochalcone, and CL present the same number of HB when interacting with both CYP (Fig. 11). For (+) catechin, around two extra HB are formed when interacting with CYP1A1 in comparison with CYP1B1.

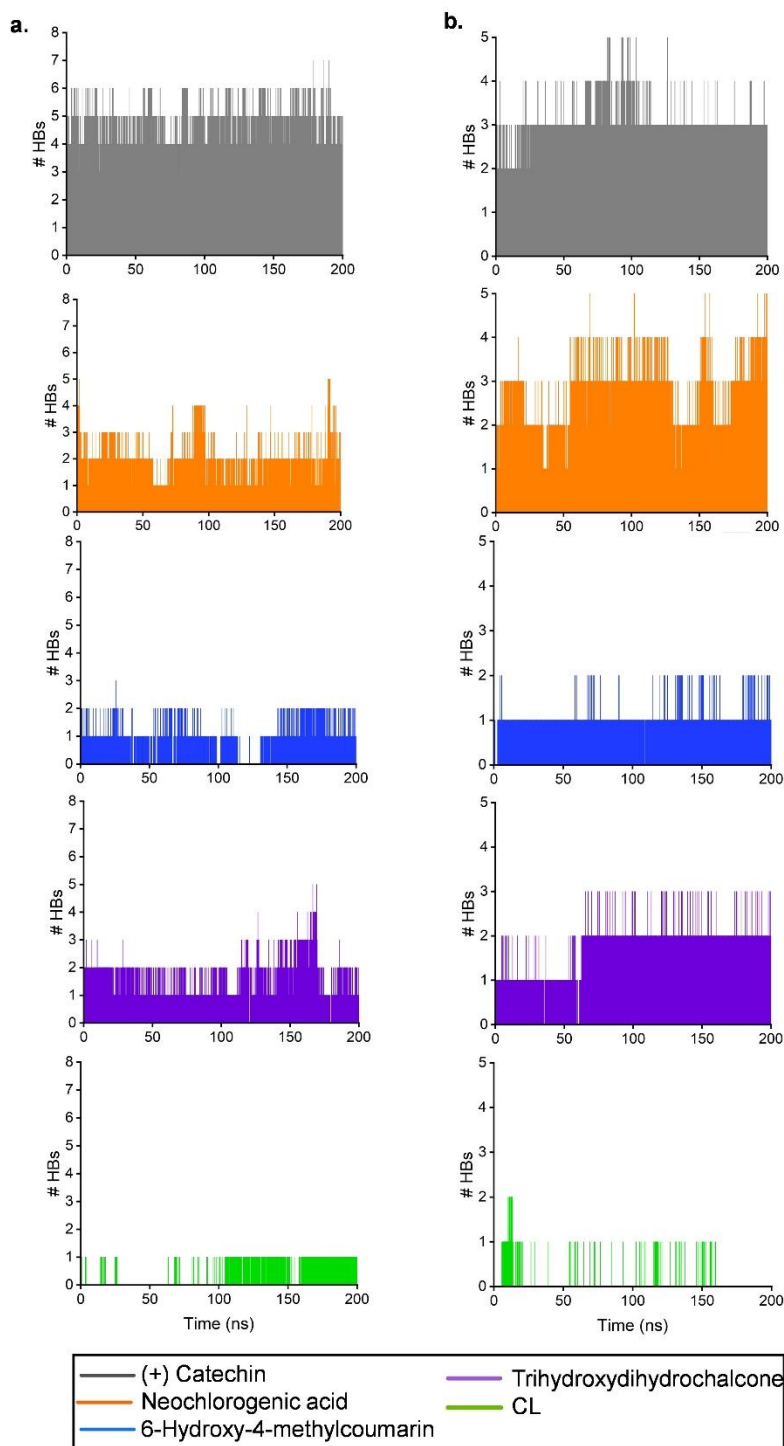


Fig. 11 | HB analysis: a. CYP 1A1 and b. CYP 1B1 in complex with (+) catechin, neochlorogenic acid, 6-hydroxy-4-methylcoumarin, trihydroxydihydrochalcone and CL during 200 ns simulation.

Analysing interactions with CYP1A1, (+) catechin forms in average 4 HB with a maximum of 7. Analysing the structure at the end of the simulations, catechin forms HB with S116, D313, and T321.

Furthermore, two HBs with the main chain of L312 and A317 were observed. In neochlorogenic acid, an average of 2 HBs are formed throughout the simulation. Structural analysis shows these HBs are with S122 and N255. For 6-hydroxy-4-methylcoumarin, 1 HB is formed in average which according to the structural analysis is with S116. Although trihydroxydihydrochalcone also forms one HB on average, at the end of the simulation, 3 HB with N222, G316, and D320 are observed. Finally, CL forms one HB on average with N222. Interestingly, the conformation of CL at the end of the simulation is in a similar pose as the electron density in the crystal structure showing the reliability of this simulation method and that even though this ligand did not make a lot of HBs, it managed to stay stable in the pocket maybe due to hydrophobic interaction and pi staking (Fig. 12).

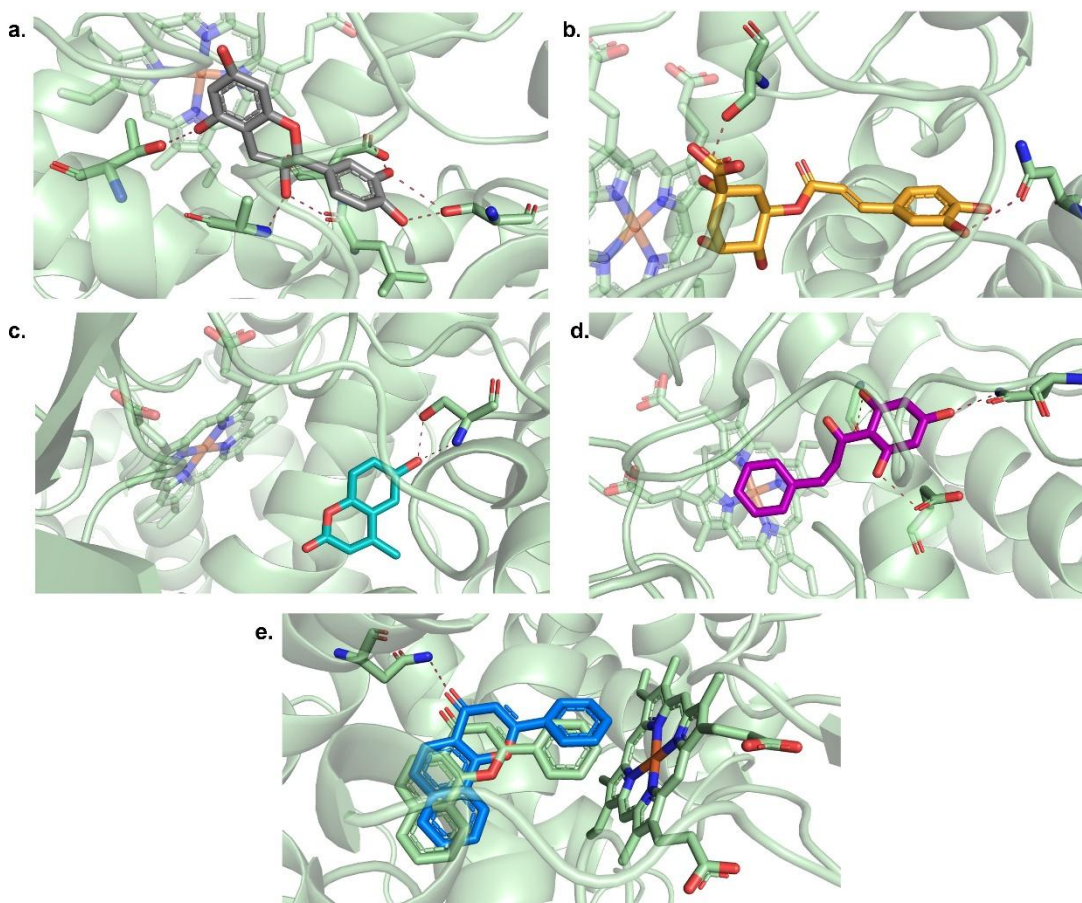


Fig. 12 | Structural analysis: a. (+) catechin, b. neochlorogenic acid, c. 6-hydroxy-4-methylcoumarin, d. trihydroxydihydrochalcone, and e. CL with CYP 1A1 at the end of the simulation time.

In CYP1B1, catechin presents on average 3HBs. Structural analysis shows those three HBs with N228, N265, and D326. Analysing the final structure in neochlorogenic acid, it present HBs with N265, and Q332 which are likely to be the 2HBs formed on average in the HB graph. Furthermore, a couple of HBs with the main chain of L224, S226, and H227 were also observed although they are in flexible loops they will tend to appear and disappear during the trajectory. In 6-hydroxy-4-methylcoumarin, same as CYP1A1, one HB is formed on average which according to structural analysis at the end of the simulation is with D333. 6-hydroxy-4-methylcoumarin also forms a HB with D333, and with N228, which agrees with its 2 HB formed on average during the simulation. For CL, HBs are almost nonexistence during the simulation where some sporadic ones can be observed from time to time. This correlates with the structural analysis where no HBs are observed. Still, same as with CYP1A1, close alignment with the

experimental crystal structure was observed (Fig. 13).

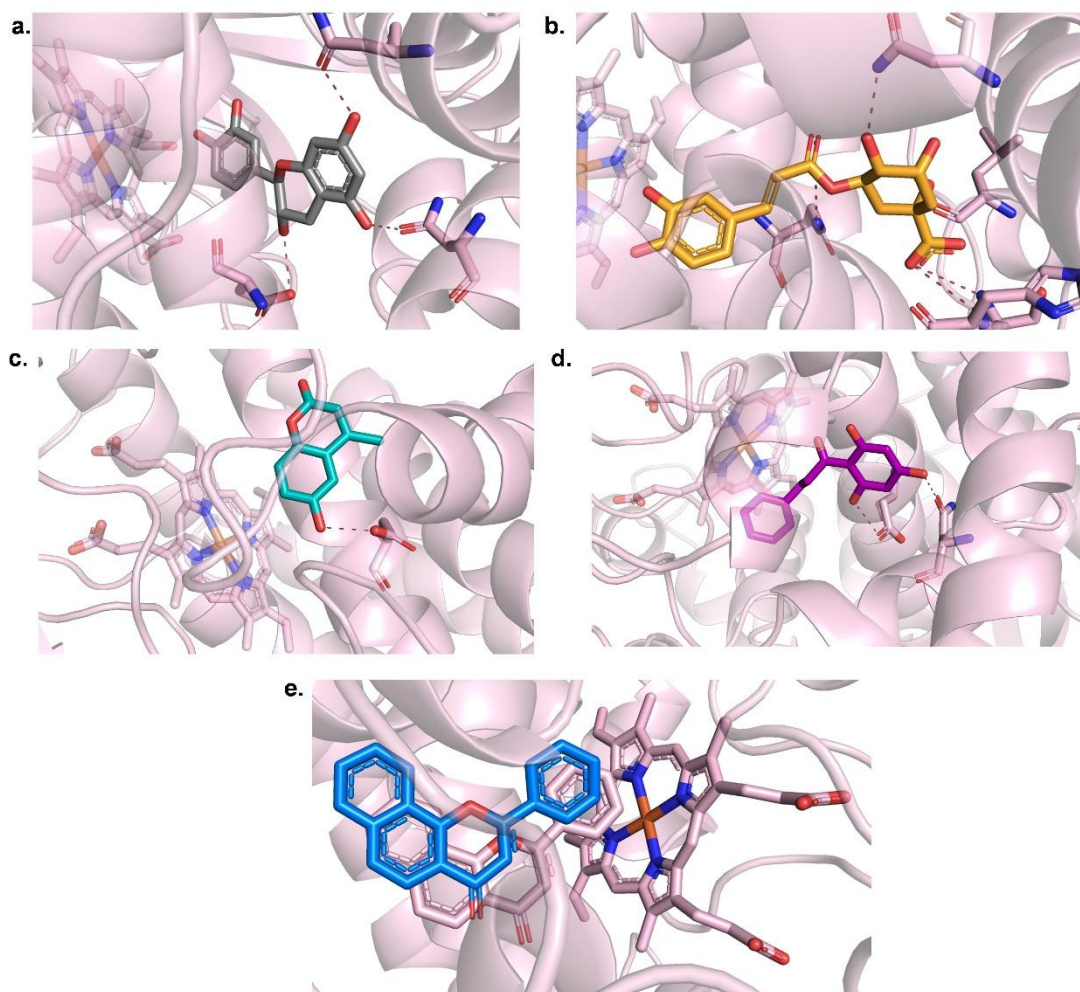


Fig. 13 | Structural analysis: a. (+) catechin, b. neochlorogenic acid, c. 6-hydroxy-4-methylcoumarin, d. Trihydroxychalcone, and e. CL with CYP 1B1 at the end of the simulation time.

Finally, the free binding energy was estimated (Table 6). The results show that all the energies are negative which may suggest a positive interaction between all the ligands and the enzymes. Comparing the four ligands with CL, it is observed that (+) catechin presents a similar binding energy while the others have binding energies higher than $-20 \text{ kcal mol}^{-1}$ except for neochlorogenic acid in CYP1B1. Interestingly, for CYP1A1, the difference in binding energy between catechin and CL is less than 1 kcal mol^{-1} , which may suggest the capacity of this ligand to inhibit the enzyme. For CYP1B1, the same molecule has a binding energy around 4 kcal mol^{-1} lower meaning a possible tighter interaction with this other enzyme. Epicatechin, also present in the metabolites and already studied in previous research, shows negative binding energies ($-16.21 \text{ kcal mol}^{-1}$ for CYP1A1, and $-20.25 \text{ kcal mol}^{-1}$ for CYP1B1), although both are higher than CL. Previous reports also revealed significant interaction of catechin[79] and neochlorogenic acid[80] against cancer target proteins. In summary, these results suggest that even though all studied ligands may interact with the CYPs, catechin is more likely to be the one responsible for the pharmacological activity that *Mauritia flexuosa L.* may have.

Table 6. van de Walls, electrostatic, SASA, and binding energy (BE) (in kcal mol^{-1}) of the studied compounds.

Enzyme	Compound	van der Waals energy	Electrostatic energy	SASA energy	Binding energy
CYP1A1	(+) catechin	-36.54	-13.09	-3.58	-24.59
	neochlorogenic acid	-45.79	-10.28	-4.56	-19.51
	6-hydroxy-4-methylcoumarin	-24.11	-5.95	-2.74	-12.92
	trihydroxydihydrochalcone	-32.43	-5.77	-3.82	-17.03
	CL	-41.65	-0.30	-3.91	-24.02
CYP1B1	(+) catechin	-41.79	-15.09	-3.65	-27.70
	neochlorogenic acid	-51.04	-12.60	-4.66	-21.86
	6-hydroxy-4-methylcoumarin	-25.65	-9.91	-2.80	-15.20
	trihydroxydihydrochalcone	-32.71	-13.42	-3.80	-17.80
	CL	-42.40	-2.79	-3.85	-23.86

Finally, the structure of the studied molecules was analyzed. Interestingly, the structure of catechin and epicatechin, and 6-hydroxy-4-methylcoumarin (Fig. 14) possess similar features with CL. The presence of two aromatic rings and a pyranose may be key features for binding. This suggests that indeed these molecules may serve as CYP1A1 and 1B1 inhibitors.

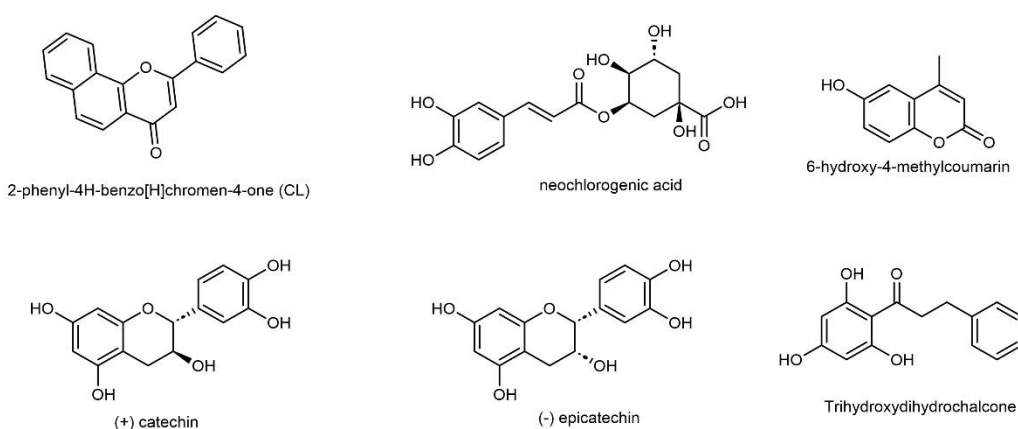


Fig. 14 | Structure of the ligands in this study.

4. Conclusions

This study aimed to evaluate the antioxidant and anti-breast cancer potential of *M. flexuosa* L. extracts. The peel exhibited the highest concentrations of total phenolic compounds, flavonoids, and anthocyanins. Its antioxidant capacity was consistently demonstrated across multiple assays, including TPC, DPPH, ABTS, erythrocyte inhibition (EI), and AI_{50} . Notably, *M. flexuosa* L. peel extracts were identified as a promising natural source of trans-resveratrol, catechin, and rutin. These findings suggest that *M. flexuosa* L. may serve as a valuable reservoir of anticancer agents, particularly against breast adenocarcinoma. Overall, the bioactive compounds present in *M. flexuosa* L. showed significant antioxidant and antiproliferative properties, supporting their potential application in functional foods or nutraceuticals with therapeutic relevance. *In silico* molecular docking and molecular dynamics analyses further revealed that catechin, neochlorogenic acid, 6-hydroxy-4-methylcoumarin, and trihydroxydihydrochalcone fit within the active sites of CYP1A1 and CYP1B1, suggesting a possible mechanism of action. Future *in vivo* studies are warranted to confirm the antiproliferative effects of these bioactive compounds and to explore their potential in alternative medicine.

Abbreviations

UPLC-ESI-QTOF-MS	Ultra-performance liquid chromatography coupled to electrospray ionization quadrupole time-of-flight mass spectrometry
DPPH	2,2-diphenyl-1-picrylhydrazyl radical
ABTS	2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	ferric reducing antioxidant power
TEAC	Trolox equivalent antioxidant capacity
AI ₅₀	antioxidant index 50
EI	electrochemical index
CV	cyclic voltammetry
DPV	differential pulse voltammetry
IC ₅₀	half-maximal inhibitory concentration
TPC	total phenolic content
MPA	metaphosphoric acid
TFC	total flavonoid content
LOD	limit of detection
LOQ	limit of quantification
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt
DMSO	dimethyl sulfoxide
ROS	reactive oxygen species

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Author contributions

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Data availability

The authors declare that generate or analyzed during this study are available within the paper and its

supplementary information file.

Declarations

Ethics approval and consent to participate

Ethics and Consent to Participate declarations: not applicable.

Consent for publication

Not applicable.

Competing interests

No, I declare that the authors have no competing interests as defined by Nature Portfolio, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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