Valorization of Colombian Gulupa (*Passiflora edulis* f. *edulis* Sims) Peel as a Bioactive Agro-Industrial By-product: Antioxidant Activity and Cytotoxic Assessment

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Passiflora edulis f. *edulis* Sims (gulupa) has gained international relevance as one of Colombia's main export fruits. However, its peel, an abundant but underutilized agro-industrial by-product, remains poorly characterized despite its potential as a source of bio-based compounds. This study evaluated the bioactive composition, antioxidant activity, and cytotoxic profile of gulupa peel extract obtained from a single commercial batch, following an initial solvent screening using water, acidified water, ethanol, and acidified ethanol, with analyses performed on independent biological replicates, aimed at supporting its valorization within bioeconomy and circular economy frameworks. The extract exhibited high contents of total polyphenols (2970 mg gallic acid eq/100 g dry weight (d.w.) and flavonoids (595 mg catechin eq/100 g d.w.). Antioxidant activity assessed by ABTS, DPPH, FRAP, and ORAC assays revealed outstanding radical-scavenging capacity, with ORAC presenting the highest value (43,200 µmol Trolox eq/100 g d.w.). Strong positive correlations were observed between polyphenol content and antioxidant activity, particularly with DPPH (r = 0.952). Cytotoxicity assays conducted on CHO-K1 cells (10-200 µg/mL) showed cell viabilities above 72% (neutral red uptake) and 84% (MTT reduction), confirming a low cytotoxic profile. Overall, these findings position gulupa peel as a promising bio-based resource rich in antioxidant compounds.

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Keywords: Passiflora; Gulupa peel; Agro-industrial by-product; Biomass valorization; Antioxidant activity; Bioactive compounds; Polyphenols; Cytotoxicity; Agricultural residues; Circular bioeconomy

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INTRODUCTION

Gulupa (*Passiflora edulis* f. *edulis* Sims), a member of the Passifloraceae family, is a perennial semi-woody plant native to South America. In Colombia, where more than 130 species of this genus have been identified, it is cultivated at elevations of 1800 to 2400 m under temperate climates of 15 to 20 °C and in slightly acidic to neutral soils (pH 6.5 to 7.5) (Rodriguez-Polanco *et al.* 2021). Gulupa has become one of Colombia´s most demanded export fruits, reaching European markets such as the Netherlands, Belgium, Germany, and the United Kingdom (Rodriguez-Polanco *et al.* 2021; Asociación Nacional de Comercio Exterior (ANALDEX) 2024).

However, the industrial processing of gulupa generates large amounts of peel and other residues that are commonly discarded as agro-industrial biomass waste (Zhivkova 2023). Recent studies have revealed that this by-product is rich in polyphenols, flavonoids,

and other secondary metabolites with antioxidant and biological activity, highlighting its potential valorization as a functional ingredient rather than a discarded material (Quirós-Cubillo *et al.* 2025). Importantly, such valorization strategies align with the emerging paradigms of bioeconomy and circular economy, which promote the sustainable management of agricultural residues to create value-added products while reducing environmental impacts (Quirós-Cubillo *et al.* 2025). These approaches are consistent with global sustainability policies that encourage the transformation of agri-food waste into bio-based resources, strengthening the link between environmental stewardship, economic growth, and public health (Pal *et al.* 2024; Quirós-Cubillo *et al.* 2025).

Beyond its economic significance, diverse parts of *Passiflora* species have been traditionally used to treat ailments such as diabetes, hypertension, dysentery and gastritis, underscoring their ethnopharmacological importance (Dhawan *et al.* 2004). In *P. edulis*, several classes of bioactive compounds have been identified, including phenolic derivatives (*e.g.*, 4-hydroxy-β-ionol and 4-oxo-7,8-dihydro-β-ionol), anthocyanins (*e.g.*, cyanidin-3-glucoside and pelargonidine-3-glucoside), and carotenoids (*e.g.*, phytoene, phytofluene, α-carotene, β-carotene and lycopene), with flavonoids recognized as major contributors to antioxidant and therapeutic effects (dos Reis *et al.* 2018).

The biological relevance of these compounds lies in their capacity to counteract oxidative stress. Clinical and epidemiological evidence indicates that diets rich in fruits and vegetables, largely due to their bioactive constituents, can reduce the incidence of chronic diseases related to oxidative imbalance, including type II diabetes, cardiovascular disorders, neurodegenerative diseases, various cancers, and immune dysfunctions (Vauzour *et al*. 2010; dos Reis *et al*. 2018). Among these compounds, polyphenols – particularly flavanols, flavonols, and anthocyanins – stand out for their potent antioxidant capacity and ability to modulate key metabolic processes. They stabilize free radicals, prevent substrate oxidation, and protect essential biomolecules such as lipids, proteins, and DNA from oxidative damage (Rudrapal *et al.* 2022). These mechanisms reinforce endogenous antioxidant defenses and mitigate the harmful effects of reactive oxygen species, ultimately contributing to preventive and therapeutic outcomes in humans (Rudrapal *et al.* 2022). In addition, fruit peels are known to accumulate high levels of phenolic compounds as part of plant adaptive and defense strategies. These metabolites are not produced randomly. Rather, they play essential roles in plant growth and development, including enzymatic regulation, photosynthesis, structural reinforcement, and protection against biotic stresses (*e.g.*, bacteria, fungi, viruses, and herbivores) and abiotic stresses such as drought, ultraviolet radiation, temperature fluctuations, and poor soil fertility (Salunkhe and Chavan 1990). Consequently, peel tissues—being the first physical barrier between the fruit and the environment—often exhibit higher concentrations and greater variability of phenolic compounds than edible tissues, which helps explain their remarkable antioxidant potential.

Despite significant advances in the study of *P. edulis* bioactives, little is known about the phytochemical composition and biological properties of the gulupa peel itself. This knowledge gap limits the full understanding of its potential applications in food, nutraceutical, and therapeutic contexts. Addressing this limitation through a combined chemical and biological evaluation can provide valuable evidence for the safe and effective utilization of this underexplored matrix, thereby integrating scientific discovery with sustainability-oriented innovation.

To assess this potential, it is essential to combine phytochemical quantification and antioxidant assays with cellular safety evaluations. Cytotoxicity analyses, which determine

potential adverse effects on cellular metabolism, are indispensable for evaluating the safety of extracts rich in bioactive compounds (Repetto *et al.* 2008). The CHO-K1 (Chinese hamster ovary) cell line serves as a robust in vitro model owing to its ease of culture, adaptability to both adherent and suspension conditions, capacity for proliferation in serum-free media, and post-translational modifications similar to those of human cells (Tossolini *et al.* 2018). These features make CHO-K1 a well-established platform for cytotoxicity assessment and preliminary safety screening (Tossolini *et al.* 2018).

Given this background, this study evaluated gulupa peel extract from three complementary perspectives: (i) quantification of total polyphenols, flavonoids, and anthocyanins; (ii) determination of antioxidant capacity through ABTS, DPPH, FRAP, and ORAC assays; and (iii) assessment of cytotoxic potential using the CHO-K1 cell line *via* MTT reduction and neutral red uptake tests. This integrative approach not only characterizes the antioxidant and cytoprotective potential of gulupa peel but also establishes a foundation for its inclusion in sustainable bio-based value chains, thereby aligning scientific innovation with the principles of bioeconomy and circular economy.

EXPERIMENTAL

Reagents and Equipment

Diphenyl-1-2,2 picrylhydrazyl (DPPH); 2,2'-azobis (2-amidino-propane) (AAPH); fluorescein; 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxyli acid (Trolox); 2,4,6-tris-(2-pyridyl-s-triazine) (TPTZ); 3,4,5-trihydroxybenzoic acid (gallic acid) and standards of (+)-catechin, (-)-epicatechin and anthocyanins were obtained from Sigma-Aldrich® (St. Louis, MO, USA). Sodium carbonate and Folin reagent were purchased from Merck (Germany). Methanol and other solvents were purchased from Fisher Scientific Co. (Fair Lawn, NJ, USA). Ultraviolet-visible measurements were performed on a Multiskan Spectrum spectrophotometer (Thermo Scientific). The decrease in fluorescence intensity measured in the ORAC assay was performed on a Perkin-Elmer LS-55 Spectrofluorimeter (U.K.). Chromatographic assays were carried out on a Shimadzu® Prominence® UFLC series liquid chromatograph.

Plant Material

Gulupa peels (*Passiflora edulis* f. *edulis* Sims) were obtained from ripe fruits harvested during the main production season (August to September 2024) from a commercial farm located in Jericó, Antioquia (5°47′N, 75°47′W; 106 km from Medellín, Colombia). Approximately 30 fruits at uniform commercial maturity (fully purple-colored peel, firm pulp, and absence of visible mechanical or microbial damage) were selected and pooled to obtain a composite sample for subsequent analyses. At collection, gulupa peels were fresh and had not undergone any prior drying. Based on typical moisture contents reported for fresh *Passiflora edulis* peels (approximately 70 to 80% on a wet basis), the material was considered to have a high initial moisture content. Immediately after collection, the peels were manually separated, gently rinsed with distilled water to remove surface impurities, and stored in perforated polypropylene bags at 4 °C for no longer than 24 h prior to processing. The samples were then frozen at –20 °C and subsequently freeze-dried, ground into a fine powder, and stored in airtight, light-protected containers at –20 °C until extraction and subsequent chemical and antioxidant analyses.

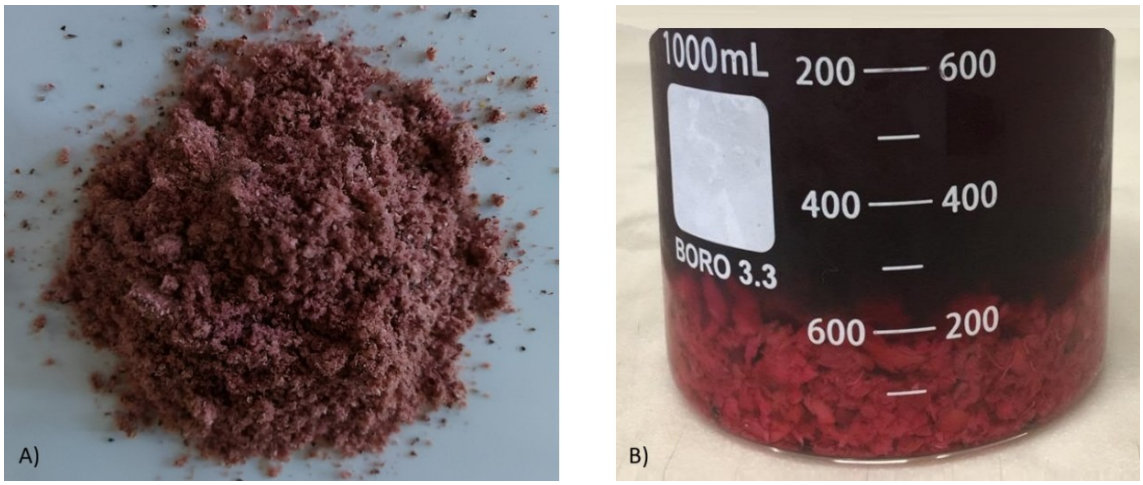

Fig. 1. (A) Freeze-dried gulupa peel and (B) Freeze-dried gulupa peel dissolved in acidified water (HCl 0.01%)

Sample Preparation

Solvent screening was performed using four extraction systems: distilled water, acidified water (HCl 0.01%, v/v), absolute ethanol, and acidified ethanol (HCl 0.01%), in which HCl was also added to a final concentration of 0.02% (v/v). For each condition, 5.0 g of freeze-dried gulupa peel (dry weight basis) (Fig. 1A) were mixed with 100 mL of the corresponding solvent (Fig. 1B). The suspensions were homogenized using an Ultra-Turrax (IKA-WERK©) for 45 s, followed by ultrasound-assisted extraction in a laboratory ultrasonic bath (40 kHz, 150 W; Branson, USA) operated at 50 °C for 20 min. The obtained extracts were centrifugated at 6000 rpm for 5 min at room temperature, and the supernatants were collected and filtered (0.45 µm) prior to analysis. Based on antioxidant capacity and flavonoid recovery, acidified water (HCl 0.01%, v/v) was selected as the extraction solvent for all subsequent chemical and biological assays. All chemical and antioxidant results were normalized to peel dry weight and expressed per 100 g d.w. using the following parameters: initial sample mass (5.0 g d.w.), final extract volume (100 mL), assay-specific dilution factors, and the corresponding calibration curves. Extraction yield (mg dry extract per g peel d.w.) was not determined in the present study and is acknowledged as a limitation for direct process-scale comparison.

Content of Antioxidant Metabolites and Reducing Sugars

Dinitro salicylic acid method (DNS)

The determination of reducing sugars by DNS method was carried out following the protocol of Saqib and Whitney (2011). A total of 500 µL of 1% solution of 3,5-dinitrosalicylic acid in NaOH was mixed with 500 µL of sample. The mixture was heated at 90 °C for 5 min and the absorbance was measured at 540 nm. The results were expressed as mg of glucose equivalent/100 g dry weight (d.w.), using a glucose standard curve.

Total polyphenol content (TPC)

To determine total phenols, the Folin-Ciocalteu colorimetric method was used (Singleton and Rossi 1965). A total of 50 µL of sample was added to 125 µL of Folin's reagent, followed by 400 µL of 7.1% (w/v) sodium carbonate. The mixture was adjusted to 1000 µL with distilled water, and absorbance was measured at 760 nm. Results were expressed as mg of gallic acid equivalent (GAE/100 g dry weight (d.w.).

Flavonoids

Flavonoid quantification was based on the colorimetric method designed by (Marinova *et al.* 2005), with some modifications. A total of 100 µL of sample was mixed with 30 µL of 5% (w/v) $NaNO_2$, 30 µL of 10% (w/v) $AlCl_3$, and 200 µL of 1 M NaOH. The mixture was topped up to 1000 µL with distilled water, and the spectrophotometric reading was performed at 510 nm. The results were expressed as mg of Catechin Equivalent/100 g of dry weight (d.w.), using a (+)-catechin standard curve.

Total anthocyanins

The concentration of total anthocyanins was determined by the differential pH method. Absorbances were measured at 530 and 700 nm using pH 1.0 and 4.5 buffers. Differential absorbance was calculated using the expression A= [(A_{530} - A_{700}) pH1.0 - (A_{530} - A_{700}) pH4.5]. For quantification, a molar extinction coefficient of 26900 was applied, corresponding to cyanidin-3-glucoside (Gaviria-Montoya *et al.* 2009). The results were expressed as mg of cyanidin-3-glucoside equivalents/100 g dry weight (d.w.).

Determination of anthocyanins by HPLC-DAD

Anthocyanins were analyzed by high-performance liquid chromatography with diode-array detection (HPLC-DAD), following the method described by Cordeiro *et al.* 2021, with slight modifications. Analyses were performed using a Shimadzu LC-20AD system (Kyoto, Japan), equipped with a quaternary pump, autosampler, column oven, and photodiode array detector. Chromatographic separation was achieved on a LiChrospher® 100 RP-18 column (5 µm, 250 x 4.6 mm; Merck®), maintained at 35 °C. The mobile phase consisted of solvent A (water/formic acid, 95:5 v/v) and solvent B (acetonitrile/formic acid, 99:1 v/v), using a linear gradient from 5% to 50% solvent B over 40 min. The flow rate was set at 1.0 mL/min, and the injection volume was 20 µL. UV-Vis spectra were recorded over the range of 280 to 800 nm. Under these conditions, native glycosylated anthocyanins are converted into their corresponding aglycones (anthocuanidins), allowing their identification and quantification. Accordingly, cyanidin, pelargonidin, and delphinidin were identified by comparing retention times and UV-Vis spectra with authentic standards. Quantification was carried out using external calibration curves (10 to 150 mg/L) prepared for each anthocyanidin standard, and results were expressed as mg per 100 g of dry weight (d.w.).

Determination of (+)-catechin and (-)-epicatechin by HPLC-DAD

The determination of (+)-catechin and (-)-epicatechin was performed by HPLC with diode array detection (DAD), following the protocol of (Zapata-Vahos *et al.* 2015). The samples were filtered in 0.40 µm vials before being injected into a Shimadzu chromatograph equipped with an autoinjector and a PDA detector set to 280 nm. A C-18 column (5 µm, 250 mm x 4.6 mm) was used for the separation, with a UV-visible spectrum between 20 to 600 nm. Identification and quantification were done with specific calibration curves for each analyte.

Determination of phenolic acids by HPLC-DAD

The extracts were filtered with 0.45 µm membranes and diluted in ultrapure water. The mobile phase consisted of acetonitrile and water acidified with phosphoric acid (pH 2.5) in a 400:600 (v/v) ratio. A constant flow rate of 1 mL/min at 25 ºC in isocratic mode was used. The analyses were performed on a Shimadzu Prominence LC-20AD HPLC

chromatograph equipped with a PDA detector calibrated at 280 nm and a C18 column (4.6 mm, 150 mm, 5 µm). The compounds were identified between 200 to 600 nm and quantified using calibration curves for p-coumaric acid, ferulic acid, and caffeic acid. The assays were performed in triplicate, following the method of Díaz *et al.* (2018).

Evaluation of Antioxidant Capacity

ABTS•+ (2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) assay

The method of (Re *et al.* 1999) was used with modifications. First, 10 µL of the sample was mixed with 990 µL of an $ABTS^{\cdot+}$ solution. The antioxidant capacity was evaluated by measuring the decrease in absorbance at 732 nm after 30 min of reaction. The results were compared with a Trolox standard curve and expressed as TEAC (µmol Trolox equivalents/100 g dry weight, d.w.).

Diphenyl-2-picryl-hydrazyl (DPPH) assay

The antioxidant activity against the DPPH radical was quantified following the protocol of Brand-Williams *et al.* (1995), with some modifications. 10 µL of sample was mixed with 990 µL of DPPH solution, and the absorbance at 517 nm was measured after 30 min at room temperature. The results were expressed in µmol Trolox equivalents/100 g dry weight (d.w.).

Ferric reducing antioxidant power (FRAP) assay

This method measures the ability to reduce ferric iron (Fe^{+3}) to its ferrous form (Fe^{+2}) in a complex with 2,4,6-tris-(2-pyridyl-s-triazine) (TPTZ). First, 50 µL of sample was added to 900 µL of FRAP solution (acetate buffer pH 3.4, TPTZ, and $FeCl_3$ in a 10:1:1 ratio). Absorbance was determined at 593 nm after 30 min of reaction and compared to an ascorbic acid curve, expressing the results as AEAC (mg of ascorbic acid/100 g dry weight, d.w.) (Benzie and Strain 1996).

Oxygen radical absorbance capacity (ORAC) assay

The protocol described by Prior *et al.* (2005) and Romero *et al.* (2010) was used to evaluate peroxyl radicals (ROO*) scavenging capacity. A fluorescein stock solution ($1x10^{-2}$ M) was prepared in phosphate buffer (PBS, 75 mM, pH 7.4) and subsequently diluted to a final working concentration of 70 nM. For the assay, 2,920 µL of the fluorescein working solution were mixed with 30 µL of sample, followed by the addition of 50 µL of AAPH (0.6 M). The reaction was carried out at 37 °C and pH 7.4. Fluorescein was measured with excitation ʎ of 493 nm and emission ʎ of 515 nm. The antioxidant effect was calculated as the difference between the areas under the curve (AUC) of the sample and the blank, comparing it with the Trolox standard curve. The results were expressed as TEAC, µmol Trolox equivalents/100 g dry weight (d.w.) according to Eq. 1,

$$ORAC = \frac{AUC - AUC^{\circ}}{AUC_{Trolox} - AUC^{\circ}} f[Trolox] \quad (1)$$

where AUC is the area under the curve for the sample, AUC° area under the curve for the control, AUC_{Trolox} area under the curve for Trolox, and f is the dilution factor for the extracts.

Evaluation of Cytotoxic Activity

The cytotoxicity assays were aimed at evaluating the cell viability of the CHO-K1 cell line when exposed to different concentrations (10, 50, 100, 150 and 200 µg/mL) of the *Passiflora edulis f. edulis* Sims (gulupa) peel extract.

Cellular Model and Experimental Conditions

The CHO-K1 cell line (ATCC® CCL-61), derived from Chinese hamster ovary, was used. Each assay was performed in duplicate, and each extract concentration was evaluated in triplicate. The culture medium used was RPMI-1640 supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The established controls were: blank (RPMI-1640 supplemented medium), negative control (untreated cells), vehicle control (cells exposed to acidified distilled water, HCl 0.01% v/v, added at the same volume as the extract), and positive control (cells exposed to 10% v/v DMSO).

MTT reduction assay

The assay was carried out following the Mosmann (1983) with some modifications. 6×10^3 CHO-K1 cells were cultured per well in 96-well plates, using RPMI-1640 medium supplemented with 5% FBS. The cells were incubated for 20 h at 37.5 ºC in a 5% CO_2 atmosphere. They were treated with different concentrations of the extract and reincubated under the same conditions for another 20 h. 10 µL of MTT solution (5 mg/mL) was added to each well and incubated for 3.5 h at 37.5 ºC and 5% CO_2 in complete darkness. The formazan crystals formed were solubilized by adding 100 µL of isopropanol-acid, and the absorbance was measured at 570 nm using a Multiskan Spectrum® spectrophotometer.

Neutral red uptake assay

This assay was performed following the protocol of Borenfreund and Puerner (1985) with modifications. The same seeding procedure was used as in the MTT reduction assay. After 20 h of initial incubation, the cells were treated with 10 µL of the extract and reincubated for another 20 h. The culture medium was removed, and the wells were washed with sterile PBS or plain RPMI-1640 medium. 50 µL of a neutral red solution (0.1 mg/mL) was added to each well, and the plates were incubated for 1.5 h at 37.5 °C and 5% CO_2. After removing the neutral red, the wells were washed with PBS and 100 µL of a 50% ethanol/0.1% acetic acid solution was added. The absorbance was measured at 540 nm using a Multiskan Spectrum® spectrophotometer.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD) of three independent biological replicates, defined as separately prepared extracts obtained from independent aliquots of the pooled, freeze-dried peel material, each measured in technical triplicate. Statistical analysis was performed using Statgraphics Centurion XVI software. A one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test was applied where appropriate, with the significance level set at $p < 0.05$. Linear correlations between total phenols, flavonoids and antioxidant capacity (ABTS, DPPH, FRAP and ORAC) were evaluated by calculating the corresponding correlation coefficients (r). For cytotoxicity assays, biological replication was defined as independently prepared extract dilutions evaluated in three separate experiments, each measured in technical triplicate.

RESULTS AND DISCUSSION

Based on previous studies conducted by the research group, the authors have identified solvent polarity and acidification as key factors that influence the efficiency of bioactive extraction, especially anthocyanins, in different matrices derived from plants of Colombia´s biodiversity (Jaimes-Gualdrón *et al*. 2019; Zapata *et al.* 2019). Accordingly, four solvent systems were selected: ethanol, ethanol/HCl, water, and water/HCl. These systems were deliberately chosen to cover a broad polarity range and to compare conventional aqueous and ethanolic extractions with their acidified counterparts, which are commonly employed to enhance the recovery of phenolic compounds from fruit by-products. Because the DPPH method is considered one of the most reliable and sensitive methods for measuring antioxidant capacity in complex matrices (Prior *et al.* 2005; Osman *et al.* 2006), it was used as an initial screening tool to evaluate the antioxidant activity of gulupa peel extracts obtained with each solvent system. In addition to antioxidant capacity, flavonoid concentration was also considered as a complementary criterion to select the solvent with the best overall performance. The solvent that showed the best results was subsequently used to perform chemical analysis and more detailed antioxidant evaluations.

Figure 2A shows the results of the measurement performed with the DPPH method, where the acidified solvents (ethanol and water) presented significantly higher values compared to the non-acidified solvents ($p<0.05$). These results are consistent with those reported by various studies, which suggest that the use of acidified solvents increases the extraction of polyphenolic compounds, such as anthocyanins (Ju and Howard 2003), proanthocyanidins, and flavonoids in general (Azman *et al.* 2022). This increase in the recovery of bioactive compounds is reflected in a greater antioxidant capacity observed in various fruits and vegetables.

Dorta *et al.* (2013), reported that high polarity solvents under acidic conditions (pH close to 3) favor the extraction of polyphenols. Likewise, Oreopoulou *et al.* (2019) observed that the alcoholic extraction of grape compounds is more efficient under acidic conditions (pH 3.0 to 3.5), due to the hydrolysis of the glucosidic bonds, which increases the antioxidant activity. Vatai *et al.* (2009), also supported this mechanism, indicating that acidified solvents with a pH between 3 and 4 allow a 30 to 40% more efficient extraction of polyphenolic compounds in plants. The higher yield in these cases is attributed to the capacity of acidified solvents to destabilize cell membranes, thus releasing most of the hydrolyzable polyphenolic compounds that are trapped or adhered to the plant matrix (Azman *et al.* 2022). On the other hand, Alothman *et al.* (2009) mentioned that ethanol/water mixtures are effective in dissolving a wide range of phenolic compounds. Furthermore, these combinations have characteristics that make them acceptable for use in human consumption models, making them appropriate solvents in the context of this study and its projections.

It is important to note that, in the antioxidant capacity measured through the DPPH assay, the values obtained with water and ethanol were statistically similar ($p>0.05$), suggesting that both solvents are suitable for evaluating the antioxidant capacity of gulupa peel. However, when analyzing the total flavonoid content in the different solvents, it was observed that the highest concentration was obtained with acidified water ($p<0.05$) (Fig. 2B). These results suggest that acidified water is the most efficient solvent not only in terms of antioxidant capacity, but also in the recovery of flavonoids, making it the most suitable option for subsequent analyses.

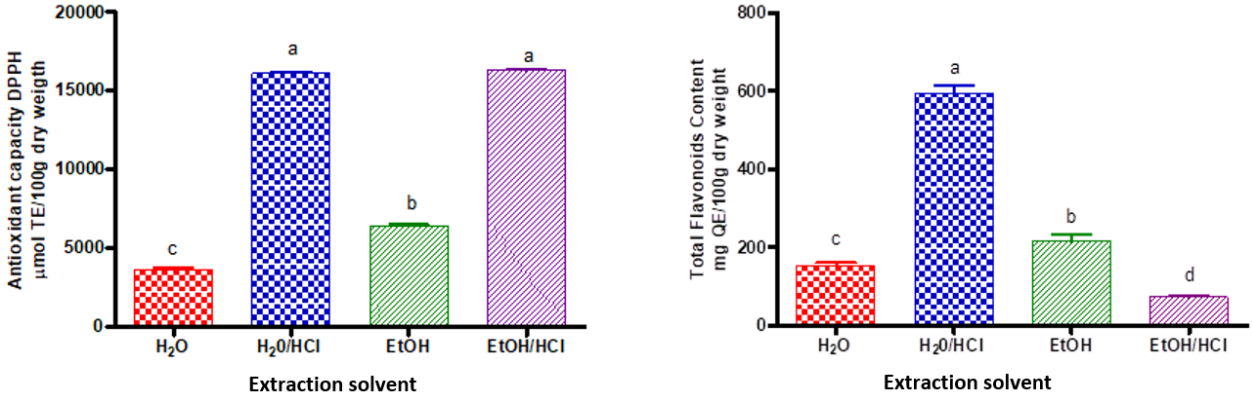

Fig. 2. (A) DPPH values and (B) quantification of total flavonoids in gulupa peel evaluated in 4 different solvents

Bioactive Compounds and Reducing Sugars

Phenolic compounds, a large and complex group of phytochemicals, are widely distributed in nature (Lattanzio 2013). Plant polyphenols, such as anthocyanins, phenolic acids, flavonoids, among others, have attracted considerable interest not only for their antioxidant capacity, which is linked to their potent reactive oxygen species (ROS) scavenging action, but also for their role in various biological mechanisms that modulate the risk of diseases such as cancer, Alzheimer's, Parkinson's, atherosclerosis, diabetes, and cardiovascular disorders (Saad *et al.* 2025). The concentrations of bioactive compounds obtained in this study are presented in Tables 1 and 2.

Table 1. Bioactive Compounds and Reducing Sugars Quantified in Gulupa Peel

Compound	Gulupa peel
Total phenols (mg gallic ac. Eq/100 g)	2971.61±79.28
Flavonoids (mg catechin Eq/100 g)	595.42±19.61
Anthocyanins (mg C3G Eq/100 g)	370.30±10.44
DNS (mg glucose Eq/100 g)	1013.56±66.87

Values are presented as mean ± standard deviation (n=3 independent biological replicates), each measured in technical triplicate. Results expressed on a 100 g dry weight (d.w.).

The main phytochemicals previously identified in gulupa include catechin, epicatechin, and rosmarinic acid (Carmona-Hernandez *et al.* 2019). The polyphenol content recorded in this study (2972.61 mg gallic acid eq/100 g d.w.) exceeds that reported by Domínguez-Rodríguez *et al.* (2019), who found 2496 mg gallic acid eq/100 g d.w. in gulupa peel. It also significantly surpasses the levels found in another *Passiflora* species, granadilla (508 mg gallic acid eq/100 g d.w.). In comparison, methanolic extracts have reported considerably lower values. Carvajal De Pabón *et al.* (2011) detected 137 mg gallic acid eq/100 g d.w., while Carmona-Hernandez *et al.* (2019) reported 162 mg gallic acid eq/100 g d.w. These authors pointed out that methanolic extracts of gulupa may help prevent transepithelial electrical resistance loss in Caco-2 cells exposed to inflammatory agents.

In general, the total polyphenol content in gulupa exceeds that of widely consumed fruits such as blackberry (1864 mg gallic acid eq/100 g d.w.), strawberry (1638 mg gallic acid eq/100 g d.w.), guayaba (1192 mg gallic acid eq/100 g d.w.), passion fruit (665 mg

gallic acid eq/100 g d.w.) and grape (291 mg gallic acid eq/100 g d.w.) (Zapata *et al.* 2014).

Flavonoids, the largest family of polyphenols, comprise more than 9000 different compounds, which can be grouped into seven subclasses based on their molecular structure: flavones, flavanones, isoflavones, flavonols or catechins, flavanols, anthocyanidins, and chalcones (Shen *et al.* 2022). Liu *et al.* (2015) demonstrated that anthocyanin-enriched extracts confer cardiovascular protection in murine models by reducing cardiac dysfunction, ventricular fibrosis and oxidative stress. Likewise, anthocyanin-rich blueberry extracts have shown anticancer activity against murine melanoma (B16-F10), human cervical tumor (HeLa), and human hepatocellular carcinoma (HepG2) cell lines (Wang *et al.* 2017; Pan *et al.* 2019). Anthocyanins have also been linked to antidiabetic, anti-inflammatory, and neuroprotective effects (Saad *et al*. 2025).

In this study, 595 mg of flavonoids (catechin equivalents) and 370 mg of anthocyanins (cyanidin-3-O-glucoside equivalents) were quantified per 100 g d.w. Additionally, concentrations of caffeic, p-coumaric, and ferulic acids, as well as pelargonidin, delphinidin and cyanidin, were identified by HPLC (Table 2).

Table 2. Bioactive Compounds Quantified by HPLC

Compound	Gulupa peel
Catechin (mg/100 g)	889.15±10.64
Epicatechin (mg/100 g)	74.16±5.14
p-coumaric acid (mg/100 g)	114.98±2.62
Caffeic acid (mg/100 g)	115.98±1.99
Ferulic acid (mg/100 g)	103.18±3.29
Pelargonidin (mg/100 g)	33.93±1.99
Delphinidin (mg/100 g)	58.50±3.89
Cyanidin (mg/100 g)	44.71±1.57

Values are presented as mean ± standard deviation (n=3 independent biological replicates), each measured in technical triplicate. Results expressed on a 100 g dry weight (d.w.).

Catechin and epicatechin metabolites have demonstrated antihyperglycemic and antihypoglycemic activities in diabetic murine models (Mechchate *et al.* 2021). Epigallocatechin gallate (EGCG) and epicatechin (EC) have also shown anticancer effects through inhibition of the mammalian *de novo* lipogenesis (DNL) pathway, triggering apoptosis in human hepatocellular carcinoma (HepG2) cells (Khiewkamrop *et al.* 2018). Moreover, catechins have been shown to inhibit cell proliferation in multiple cancer models, such as H1299 and Lu99 (lung) (Takahashi *et al.* 2014), among others.

In this study, catechin and epicatechin were detected at concentrations of 889.15 mg and 74.16 mg per 100 g d.w., respectively and the results are presented in Table 2, while the chromatograms are shown in Fig. 3. The catechin content, in particular, was notably higher than that reported for other fruits known for their antioxidant properties, such as maqui (*Aristotelia chilensis*) (225 mg/100 g d.w.) (Crisóstomo-Ayala *et al.* 2022) and açaí (*Euterpe oleracea*) (5.07 mg/100 g d.w.) (Dantas *et al.* 2019).

In addition, identification of phenolic acids was confirmed through HPLC-PDA chromatographic profiles, further validating the presence and integrity of these bioactive compounds. The chromatogram corresponding to phenolic acids (Fig. 4) revealed well-defined peaks for caffeic acid, ferulic acid, and p-coumaric acid, consistent with their respective retention times and UV-visible absorption spectra, reinforcing the compositional richness of gulupa peel extract.

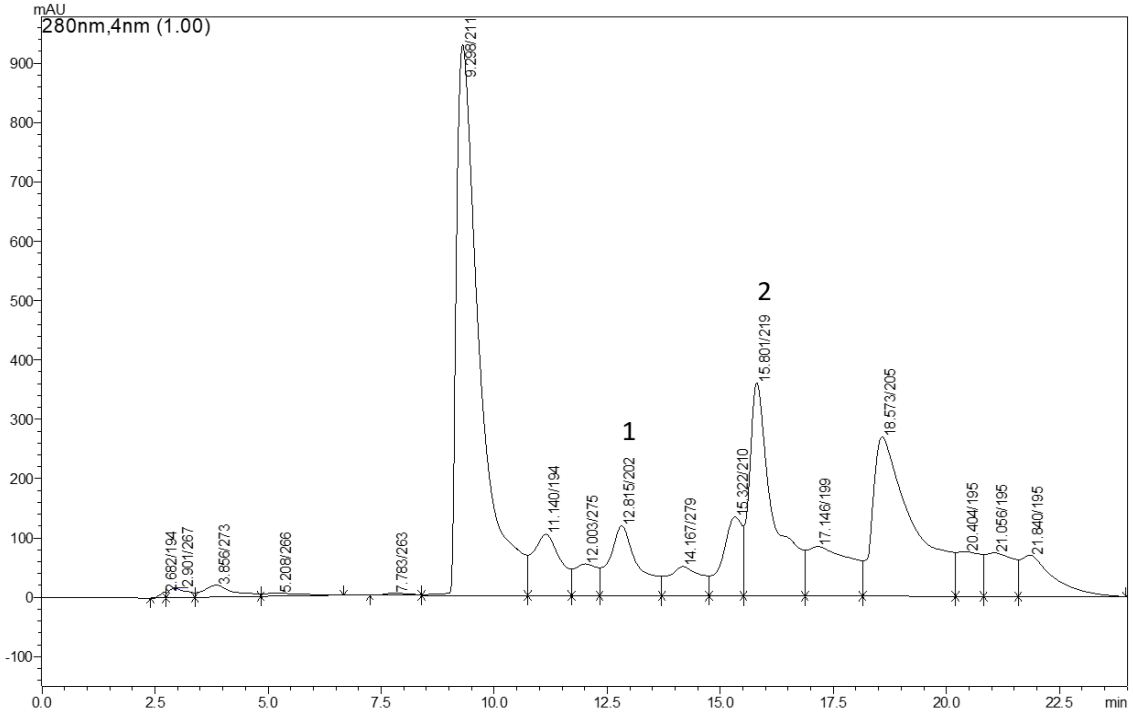

Fig. 3. HPLC-PDA chromatograms at 280 nm of gulupa peel. Peaks are identified as follows: (1) catechin and (2) epicatechin

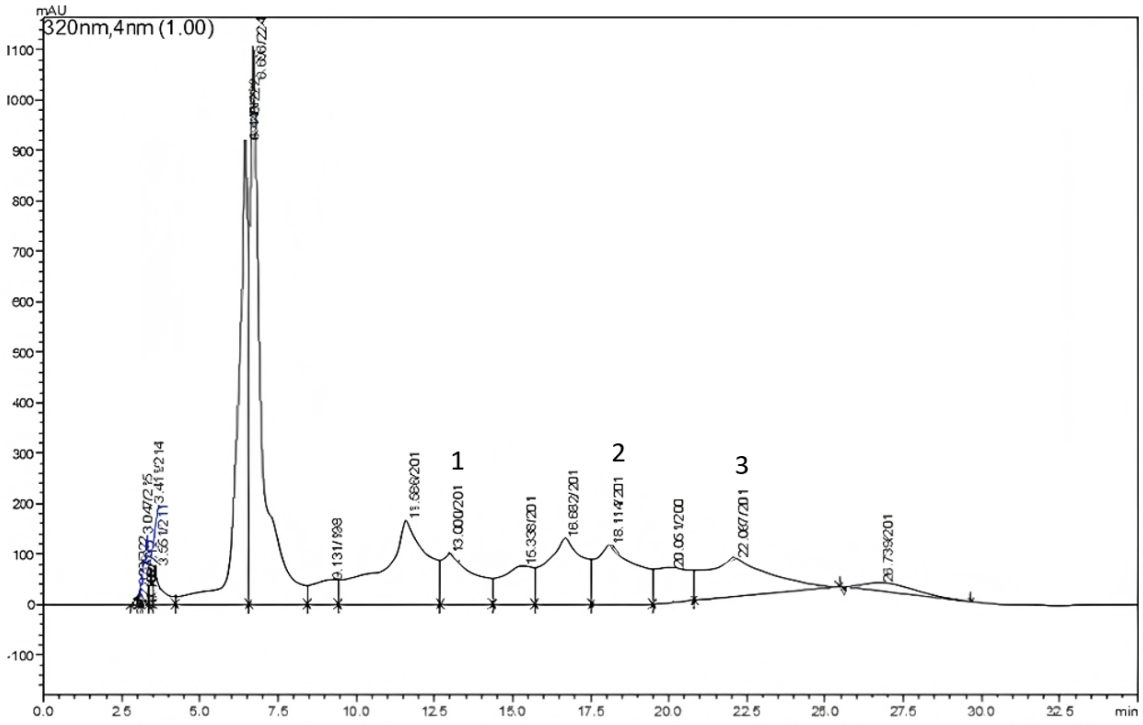

Fig. 4. HPLC-PDA chromatograms at 280 nm of gulupa peel. Peaks are identified as follows: (1) caffeic acid, (2) p-coumaric acid and (3) ferulic acid

It should be noted that, in addition to the identified phenolic compounds, several minor peaks were detected in the HPLC chromatograms, indicating the presence of

additional phenolics or related compounds that were not identified in the present study. These chromatographic fingerprints not only provide analytical validation of the detected metabolites but also establish a reliable chemical profile supporting the potential functional applications of this underutilized agro-industrial byproducts.

Variations in total phenolic content across fruits, and even among gulupa samples, may stem from the common focus on pulp in most studies, due to the limited commercial interest in the peel. However, it is well known that most phenolic compounds, including flavonoids and proanthocyanidins, are concentrated in the peel (Yeddes *et al.* 2013), which acts as a protective barrier against oxidative damage and UV-B radiation (Santin *et al.* 2020). These stress conditions activate genes related to the UVR8 photoreceptor, promoting synthesis and accumulation of bioactive compounds in the peel (Santin *et al.* 2020). It is also important to consider that each plant species exhibit unique behaviors in secondary metabolites synthesis, influenced by their genotype and abiotic factors such as climate, geographic area, photoperiod, solar radiation, temperature, drought, and nutrient availability (Ramakrishna and Ravishankar 2011). These factors help explain why genetically identical fruits or plants may exhibit significant metabolic differences when cultivated and grown under different environmental conditions.

Antioxidant Capacity

In the selected solvent (acidified water), the antioxidant and reducing capacity of gulupa peel extract was characterized using four complementary methodologies (FRAP, DPPH, ABTS, and ORAC) (Table 3). As has been noted, the use of multiple assays is essential given the chemical complexity of plant matrices, which usually comprise mixtures of compounds with diverse structures, functional groups, polarities, chemical behaviors, and mechanisms of action (Petrén *et al.* 2024). As noted by Frankel *et al.* (1994), the outcomes of individual methods may vary depending on the oxidant/antioxidant model applied and the lipophilic/hydrophilic balance. For this reason, Sacchetti *et al.* (2005), recommend the combined application of several techniques, thereby covering a broader spectrum of antioxidant mechanisms and minimizing methodological bias.

Table 3. Antioxidant Capacity in Colombian Gulupa Peel

Methodology	Gulupa peel
FRAP (mg Ascorbic acid/100 g)	1686.73±38.62
DPPH (µmol Trolox Eq/100 g)	16088.16±101.87
ABTS (µmol Trolox Eq/100 g)	27426.48±45.62
ORAC (µmol Trolox Eq/100 g)	43150.82±237.93

Values are presented as mean ± standard deviation (n=3 independent biological replicates), each measured in technical triplicate. Results expressed on a 100 g dry weight (d.w.).

FRAP methodology measures the presence of compounds and/or molecules with the capacity to stabilize free radicals through the electron transfer mechanism (SET). The antioxidant efficacy in this method depends on the redox potentials of the compounds present in the extract. In this study, the antioxidant capacity measured through FRAP showed a value of 1686 mg ascorbic acid/100 g d.w., similar to that reported for fruits such as sugar mango and blackberry (~1500 mg ascorbic acid/100 g d.w.) (Botero *et al.* 2007), although lower than that reported for aqueous extracts of green and black tea from Mortiño (7880 and 15780 mg ascorbic acid/100 g d.w., respectively) (Zapata-Vahos *et al.* 2015). It is important to mention that the FRAP assay not only quantifies the activity of phenolic

compounds, but it can also detect other molecules with reducing properties, which can generate interferences with free reducing sugars and some acids such as ascorbic, citric and folic acids (Hernández-Rodríguez *et al.* 2020). For this reason, the concentration of reducing sugars was quantified using the colorimetric technique with DNS, obtaining a value of 1010 mg of glucose Eq/100 g d.w. Due to the possibility of interference by other components that do not participate in the antioxidant activity, the results were complemented with the ABTS, DPPH and ORAC assays, which exclusively measure antioxidant metabolites.

The DPPH assay is recognized as one of the most reliable methodologies to measure antioxidant capacity, since it focuses on compounds with specific antioxidant properties, and because it presents high selectivity due to its molecular structure (Prior *et al.* 2005; Osman *et al.* 2006). The antioxidant capacity values of gulupa obtained in this study greatly exceeded those reported for fruits such as raspberry, blackcurrant, and blueberry (8667, 6763 and 10999 µmol Trolox Eq/100 g d.w., respectively) (Kim 2018). On the other hand, the ABTS test, unlike DPPH, can be easily dissolved in aqueous media, making it suitable for evaluating hydrophilic antioxidant compounds present in natural extracts (Arnao 2000), which gives it a greater affinity with antioxidants of that nature. Studies such as Cai *et al.* (2004) and Piluzza and Bullitta (2011) have highlighted that the ABTS assay is fast, robust, and accurate in determining total antioxidant capacity. In this study, the results obtained through ABTS were superior to those obtained with DPPH, which can be attributed to differences in the reduction potentials of both methods (ABTS: 0.67; DPPH: 1.2 V) and to the low selectivity of the ABTS radical, which allows it to react with a greater diversity of antioxidants, regardless of its actual antioxidant potential (Mareček *et al.* 2017). Unlike DPPH, which has greater stability and lower reactivity as it does not interact with flavonoids lacking an OH group in the B ring or with aromatic acids with a single OH group (Roginsky and Lissi 2005), ABTS, prepared in hydrophilic phase, showed a greater affinity with antioxidants present in the aqueous extract of gulupa. This coincides with the findings of previous research, which have highlighted the robustness and precision of ABTS to evaluate the total antioxidant capacity in complex matrices, confirming that it is a suitable assay to evaluate extracts rich in hydrophilic compounds (Arnao 2000; Rufino *et al.* 2010). In quantitative terms, gulupa peel extract showed a superior antioxidant capacity compared to other fruits such as blackberry (17835 µmol Trolox Eq/100 g d.w.), raspberry (5148 µmol Trolox Eq/100 g d.w.), and blueberry (10231 µmol Trolox Eq/100 g d.w.) (Kim 2018). Rufino *et al.* (2010) reported for açaí a value of 6450 µmol Trolox Eq/100 g d.w., while for murta a concentration of 16600 µmol Trolox Eq/100 g d.w.

According to Wu *et al*. (2004), the ORAC technique is one of the most widely used to measure the total antioxidant potential of foods and nutritional supplements. This method measures the ability of polyphenolic compounds to capture peroxyl radicals generated *in situ* through the hydrogen atom transfer (HAT) mechanism (Prior *et al.* 2003). In this study, the antioxidant capacity of gulupa peel (43,200 µmol Trolox Eq/100 g d.w.) was found to be higher than that reported for widely consumed fruits with recognized antioxidant potential, such as strawberry (35,700 µmol Trolox Eq/100 g d.w.); blackberry (12,500 µmol Trolox Eq/100 g d.w.), and guava (11,800 µmol Trolox Eq/100 g d.w.) (Zapata *et al.* 2014). This value also exceeds the antioxidant potential of other species with pharmaceutical action, such as blueberry, which has values between 34,500 and 41,800 µmol Trolox Eq/100 g d.w. (Maldonado-Celis *et al.* 2014; Zapata *et al.* 2014). Mortiño, through aqueous extracts, has shown antiproliferative and cytotoxic activity on human

colon adenocarcinoma cells (SW480) and metastatic cells (SW620). Likewise, the antioxidant capacity of gulupa exceeds that reported for curuba (43,200 *vs*. 21,000 µmol Trolox Eq/100 g d.w.), a fruit that has shown promising effects in the inhibition of adenomas in the distal colon of murine animals, suggesting a possible role in reducing the risk of colorectal cancer (Chaparro *et al.* 2015).

The results obtained from the gulupa extract using the different methodologies suggest that its high antioxidant capacity, determined by the ORAC method, is in agreement with the results of the ABTS assay. These findings indicate that the gulupa peel has a high concentration of hydrophilic antioxidants, capable of effectively neutralizing the ROO· peroxide radicals generated in the system (Zamorano-Aguilar *et al.* 2020). Furthermore, the compounds present in the extract showed the ability to neutralize reactive oxygen species (ROS) through the main antioxidant mechanisms: hydrogen atom transfer (HAT), assessed by ORAC, and electron transfer (SET), determined by FRAP and ABTS (Sun and Tanumihardjo 2007). The results of the DPPH assay also suggest the presence of efficient antioxidants and, since most of the compounds in the extract are hydrophilic in nature, it is inferred that a significant part of these compounds could have an amphiphilic behavior, allowing them to interact with both aqueous and lipid phases (Xie and Schaich 2014).

From a sustainability standpoint, the strong antioxidant performance of gulupa peel extract underscores its suitability for inclusion in functional foods, supplements, or natural additives derived from waste valorization. These findings support the view that discarded plant fractions can serve as renewable sources of bioactives, advancing bioeconomy-driven food systems and circular resource flows (Pal *et al.* 2024; Quirós-Cubillo *et al.* 2025).

Correlation

Correlation coefficients (*r*) were calculated to analyze the relationship between total polyphenols, flavonoids, and the antioxidant capacity measured by the ABTS, DPPH, FRAP and ORAC methods (Table 4). Correlations were computed using n = 3 independent biological replicates, defined as separately prepared extracts obtained from independent aliquots of the pooled, freeze-dried peel material. For each variable, values correspond to the mean of technical triplicates per biological replicate.

Table 4. Linear Correlation Coefficients (*r*) between Polyphenol Content and Antioxidant Activity Assays

Methodology	Total polyphenols
ABTS	0.909
DPPH	0.952
FRAP	0.947
ORAC	0.924
Total flavonoids	0.978

The analysis revealed a significant positive correlation between polyphenols and total flavonoids (r: 0.978), suggesting that most of the phenolic content in gulupa peel is associated with flavonoids compounds (Fig. 5).

A positive correlation was also observed between total polyphenols and all the antioxidant assays evaluated (Fig. 6). This is consistent with previous reports, highlighting phenolic compounds as key contributors to the antioxidant capacity of plant matrices (Piluzza and Bullitta 2011).

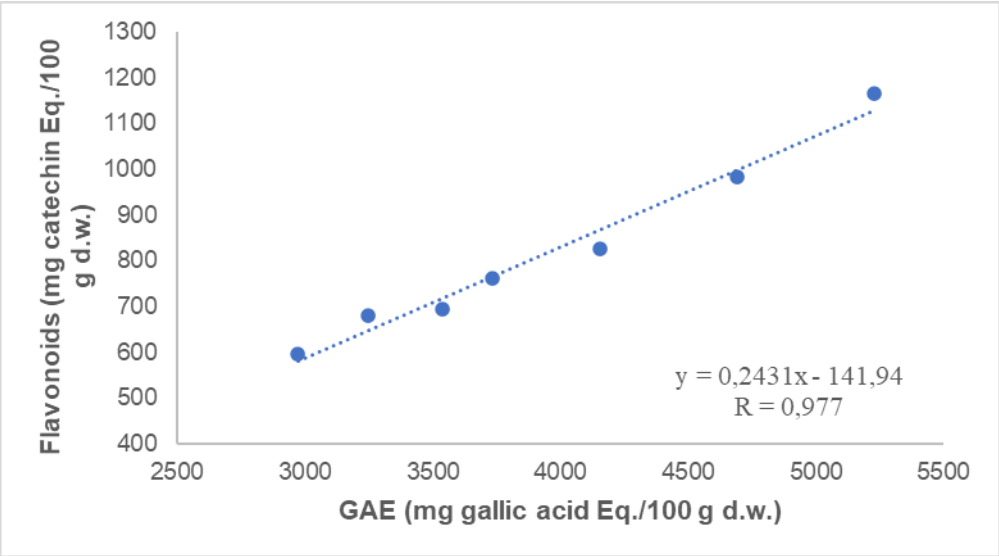

Fig. 5. Correlation between total polyphenols/total flavonoids

Among the methods, the strongest correlation was found between total polyphenols and DPPH assay (r: 0.952), suggesting that less polar antioxidant constituents with higher methanol solubility could contribute more strongly to the DPPH response. However, correlation analysis alone does not allow definitive assignment of compound polarity, and targeted fractionation or partition-based analysis would be suggested to justify this hypothesis (Prior *et al.* 2003). From a mechanistic perspective, this trend can be rationalized by the fact that DPPH is a free radical dissolved in a methanolic medium (Marxen *et al.* 2007), which may favor interactions with relatively less polar molecules. However, another important factor is the acidic conditions of the medium, where many polyphenols can exhibit dual behavior. Under alkaline conditions they tend to be more hydrophilic due to the presence of negatively charged groups in their structure, while under acidic or neutral conditions, such as those of gulupa extract, they can adopt more hydrophobic characteristics due to partial loss of charge (Zhou *et al.* 2021). Moreover, enzymatic lipophilization processes in plant matrices have been reported to further increase the apparent lipophilicity of phenolic compounds (Zhou *et al.* 2021).

It is crucial to clarify that, although the ABTS method showed the highest antioxidant capacity in gulupa extract, this does not contradict the results obtained with DPPH, which revealed the strongest correlation with total polyphenols. This apparent discrepancy can be explained by the structural versatility of polyphenols in response to different environments (acidic, neutral, or alkaline), which allows them to act efficiently in polar *vs.* non-polar media. Therefore, the different behavior of polyphenols in different assays and conditions is an inherent characteristic of their chemistry and not a contradiction in the results. On the other hand, a high correlation was also observed between polyphenols and FRAP methodology (r: 0.947), reinforcing the idea these compounds play a key role in redox reactions, acting as reducing agents and hydrogen donors. This explains its high affinity with FRAP, since this assay is based on the measurement of the capacity of antioxidants to reduce ferric ions. It is noteworthy that both flavonoids and cinnamic acids have demonstrated strong affinity for ferric ions (Fe^{+3}), through chelation and reduction (Vo *et al.* 2022), which supports the high FRAP-polyphenol correlation found in this study.

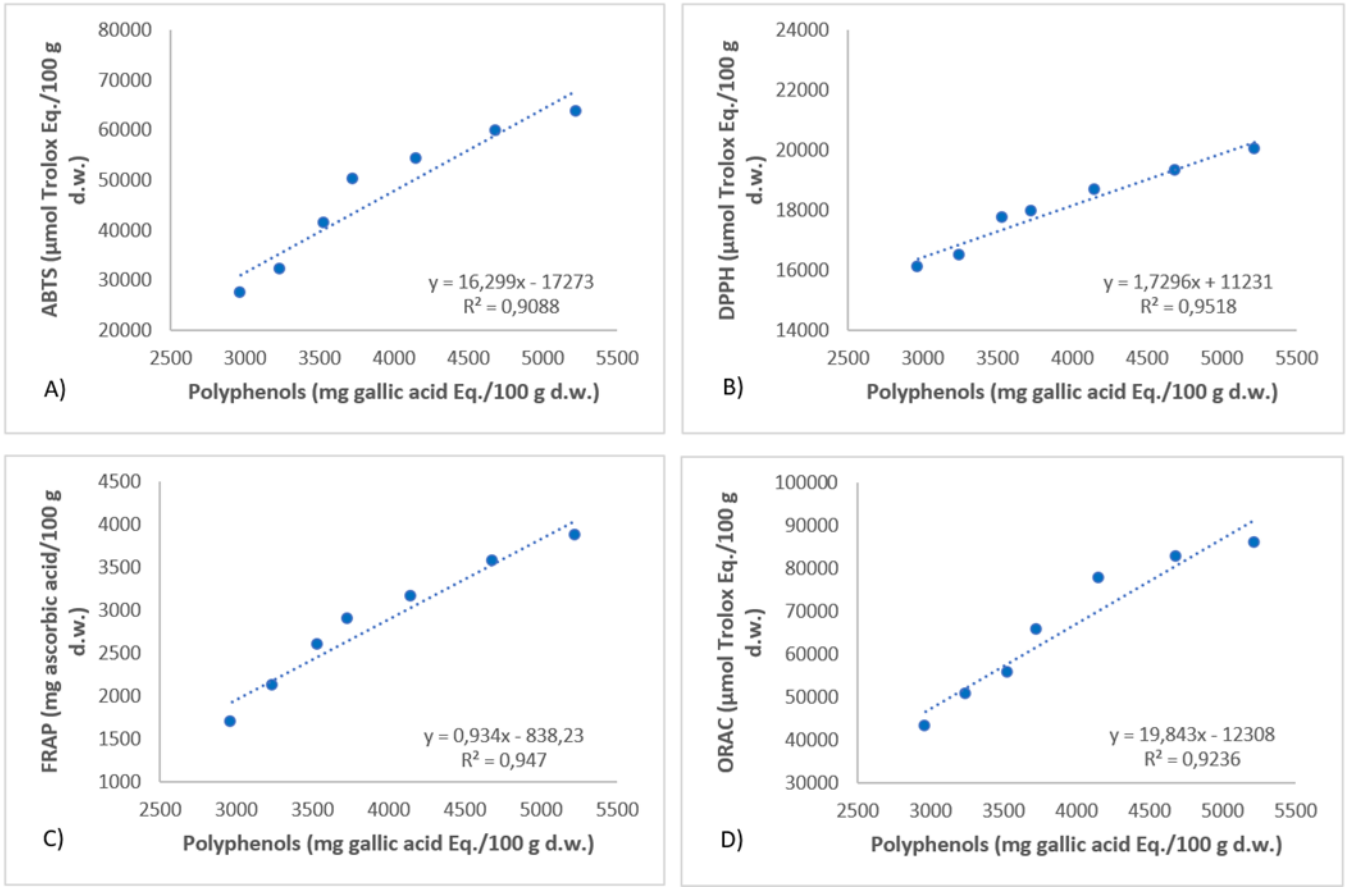

Fig. 6. Correlation between (A) total polyphenols/ABTS, (B) total polyphenols/DPPH, (C) total polyphenols/FRAP, (D) and total polyphenols/ORAC

As previously mentioned, correlations between total polyphenol content and antioxidant activity can vary due to multiple factors. Furthermore, it is important to note that polyphenols are not the only antioxidants present in plant matrices. Other non-polyphenolic compounds, such as carotenoids, tocopherols, tocotrienols, and ascorbic acid, also contribute to the overall antioxidant potential (Derradji-Benmeziane *et al.* 2014). Likewise, it is important to take into account that the synergistic interactions between antioxidants, as well as their concentrations, structures, and stability, are critical factors shaping the total antioxidant activity of plant extracts. In addition, it has been reported that some polyphenols can even enhance antioxidant defenses indirectly, by upregulating enzymes such as ϒ-glutamylcysteine synthetase, which is essential for the synthesis of endogenous antioxidants including glutathione (Moskaug *et al.* 2005). This underlines the importance of not only considering the direct antioxidant activity of polyphenols, but also their ability to modulate the body's antioxidant defense mechanisms.

Cytotoxic Activity

The cytotoxicity of gulupa peel extract (10, 50, 100, 150, and 200 µg/mL) on the CHO-K1 cell line was evaluated using two complementary assays that explore different mechanisms of cellular viability: the MTT reduction assay and the neutral red uptake assay. In this study, cell viability refers to metabolic and lysosomal integrity of CHO-K1 cells as assessed by MTT reduction and neutral red uptake assays, respectively, and does not imply organismal survival or industrial process viability.

The MTT assay, widely recognized for its high sensitivity and reproducibility, evaluates cellular metabolic activity through a redox reaction, in which MTT is reduced to formazan by mitochondrial enzymes present in viable cells. This mechanism makes the assay an effective tool for evaluating cellular metabolic activity. However, its accuracy may be affected by antioxidant compounds with reducing potential, such as phenolics identified in gulupa extract, which may generate formazan non-specifically and thus overestimating cell viability (Zamorano-Aguilar *et al.* 2020). In line with the above, previous studies have documented the presence of phenolic compounds with antioxidant activity in fruits and seeds of the *Passiflora* genus (Medina *et al.* 2017), which coincides with the findings of this research.

To ensure that the measured cell viability reflected only the metabolic activity of the cells, the assay results were corrected considering the direct interaction of the gulupa extract with the MTT reagent. As shown in Fig. 7, the data was adjusted by subtracting the amount of formazan generated by said interaction. After adjustment, the results revealed a directly proportional relationship between extract concentrations and the amount of MTT reduced to formazan, highlighting the importance of this analysis to obtain an accurate assessment of cellular metabolic activity.

In parallel, the neutral red uptake assay was employed to assess cell viability by evaluating the integrity and functional capacity of the lysosomal membrane. This method is based on the ability of viable cells to actively incorporate and retain the neutral red dye within lysosomes through active transport and subsequent binding to anionic sites of the lysosomal matrix (Borenfreund and Puerner 1985; Repetto *et al.* 2008). Thus, any alteration in membrane integrity or lysosomal function reduces the amount of dye accumulated, providing a sensitive and complementary measure of cellular viability that reflects a different biological target compared to mitochondrial activity assessed by the MTT assay. Cell viability data are presented as mean ± standard deviation (SD) of three independent biological replicates, each measured in technical triplicate. Statistical

differences among concentrations were evaluated by one-way ANOVA followed by Fisher´s LSD test ($p < 0.05$).

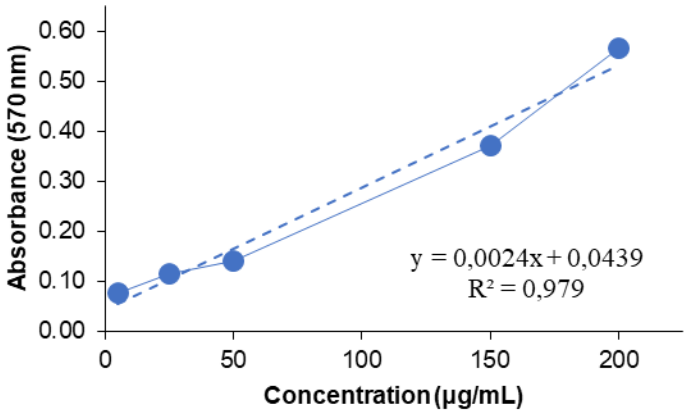

Fig. 7. Interaction between gulupa extract (*Passiflora edulis* f. *edulis* Sims) and MTT

The results indicated cell viability above 72% in the neutral red uptake assay and 84% in the MTT assay (Fig. 8), confirming a low cytotoxicity profile for gulupa peel extract on the CHO-K1 cell line. According to established criteria, which define the cytotoxicity of an extract or compound as a reduction ≥30% in cell viability compared with the untreated control (ISO 2009; Gruber and Nickel 2023), the extract did not present significant cytotoxic effect at any of the tested concentrations.

Although assay-dependent differences in cell viability were observed, these variations likely reflect the distinct sensitivity of their cellular targets. The neutral red uptake assay, which is sensitive to lysosomal integrity, showed a concentration-dependent decrease in viability, whereas the MTT reduction assay, which reflects mitochondrial metabolic activity, exhibited stable or slightly increased responses across the same concentration range (Fotakis and Timbrell 2006). Cell viability data are expressed as mean ± SD of three independent biological replicates, each measured in technical triplicate. The fitted models exhibited moderate to high coefficients of determination ($R^2 \approx 0.78 – 0.84$), indicating that extract concentration explains a substantial proportion of the variability in the neutral red response, while mitochondrial metabolic activity remained largely preserved. (Fig. 8). This finding reinforces the stability of the cellular response across a wide concentration range, confirming a consistent low cytotoxicity profile for all concentrations evaluated. This evidence supports the classification of gulupa peel extract as a matrix with low cytotoxic potential and highlights its safety for future applications.

The results highlight the need for further studies using a broader concentration range to determine the IC_{50} of the extract with greater robustness. In addition, evaluating cytotoxicity under metabolic activation conditions -such as using the rat liver S9 fraction or metabolically active human cell lines (*e.g.*, Hep-G2)- would provide deeper insight into the biological behavior of the extract. These complementary approaches would strengthen the assessment of its safety profile and support its potential applicability in food and nutraceutical contexts.

Overall, this study provides solid scientific and technical evidence supporting the sustainable valorization of gulupa peel as a bio-based resource. The integration of chemical profiling, multi-assay antioxidant evaluation, and low cytotoxicity outcomes establishes a robust foundation for future applications in food, nutraceutical, and related bio-based

fields, fully aligned with bioeconomy and circular economy principles (Pal *et al.* 2024; Quirós-Cubillo *et al.* 2025).

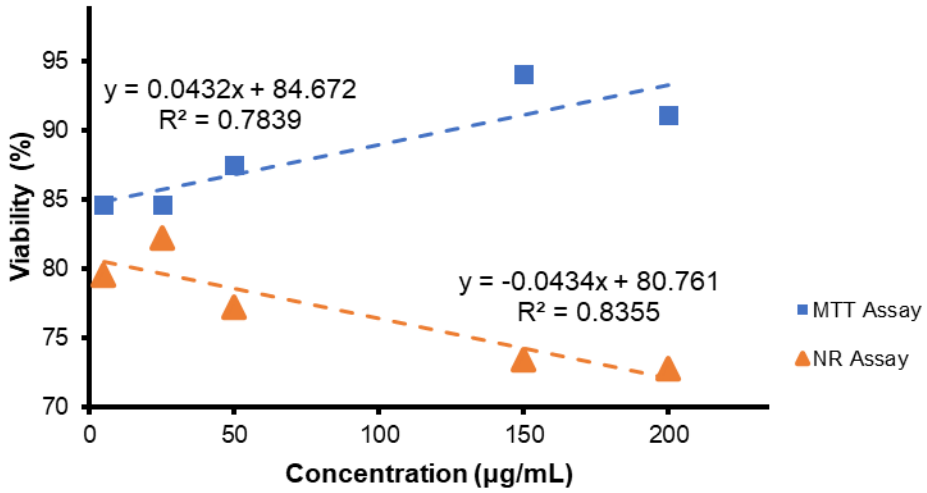

Fig. 8. Effect of *Passiflora edulis* F. Sims extract on the viability of CHO-K1 cell line by MTT reduction and neutral red (NR) assays

The results presented in this study should be interpreted considering certain limitations. First, the gulupa peel material analyzed was obtained from a single commercial batch collected from one production area, and therefore potential variability associated with cultivar, seasonality, or agronomic conditions was not assessed. Second, biological replication was defined as independently prepared extracts obtained from aliquots of pooled, freeze-dried material, while technical replication corresponded to repeated analytical measurements; this design prioritizes analytical robustness but does not capture inter-batch biological variability. Moreover, antioxidant capacity values obtained by ABTS, DPPH, FRAP, and ORAC assays are inherently method-dependent, and comparisons among these assays should be interpreted cautiously, particularly for ORAC, which is sensitive to specific protocol parameters. Finally, future studies could incorporate complementary analytical techniques, such as GC–MS or pyrolysis GC–MS, particularly after fractionation or isolation of specific compounds, to further elucidate the chemical profile and bioactive components of gulupa peel.

CONCLUSIONS

1. This study demonstrated that gulupa peel (*Passiflora edulis* f. *edulis* Sims) is a rich source of bioactive compounds, particularly polyphenols, flavonoids, and anthocyanins, which are directly associated with its high antioxidant activity as determined by multiple complementary assays.
2. The low cytotoxicity profile observed in CHO-K1 cells, with cell viabilities consistently above 72% in the neutral red uptake assay and 84% in the MTT reduction assay across all tested concentrations, supports the biological safety of gulupa peel extract and its suitability for applications in food, nutraceutical, and bio-based product development.

3. The combined chemical characterization, antioxidant performance, and cellular safety evidence confirmed that the valorization of gulupa peel represents a viable strategy for converting agro-industrial residues into high-value bioactive ingredients, fully aligned with the principles of circular economy and bioeconomy-oriented sustainable production systems.
4. The conclusions of this study are based on gulupa peel material obtained from a single commercial production batch and on a specific extraction protocol (acidified aqueous extraction, HCl 0.01%). Therefore, variations associated with cultivation conditions, harvest season, or alternative extraction strategies were not addressed and should be considered when extrapolating these results.

ACKNOWLEDGMENTS

Conflict of Interest

The authors declare no conflict of interest.

Use of Generative AI

During the preparation of this manuscript, the AI tool ChatGPT (OpenAI, USA) was used to polish the English of the translated text and to assist in checking the formatting of the bibliographic references according to the journal's guidelines. The content, data, and scientific interpretation were produced, developed, and verified entirely by the authors, who take full responsibility for the final version of the manuscript.

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