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Comprehensive characterization of the overlooked residue generated during roselle calyxes brewing with potential use as functional ingredient

Caracterización integral del residuo generado durante la decocción de cálices de jamaica con uso potencial como ingrediente funcional

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ABSTRACT

A significant quantity *Hibiscus sabdariffa* L. calyxes are generated as by-products during the decoction process commonly used for roselle beverage preparation. We conducted an extensive characterization of polyphenols, organic acids, and antioxidant potential in roselle calyxes, decoction, and their by-product. Roselle calyxes were found to be a rich source of diverse polyphenols, including delphinidin sambubioside, caffeoylquinic acids, hibiscus acid, and citric acid as major components. Importantly, we extracted a significant proportion of these bioactive compounds during the decoction process, resulting in a polyphenol-rich beverage. The used calyces (decoction by-product) retained from 23 % to 140 % of the extractable polyphenols and organic acids found in roselle calyxes. Additionally, due to the leaching of hydrophobic components like soluble dietary fiber and extractable polyphenols and organic acids, the roselle by-products were enriched with non-extractable constituents attached to dietary fiber (126 % - 272 %). Therefore, roselle calyxes and their decoction by-products emerge as promising sources of polyphenols with the potential for use in dietary supplements, alongside the commonly consumed roselle decoction.

Keywords: *Hibiscus sabdariffa* L.; by-product; bioactive compounds; antioxidant capacity; nutraceutical value.

RESUMEN

Durante el proceso de decocción comúnmente utilizado en la preparación de la bebida de jamaica (*Hibiscus sabdariffa* L.), se genera una cantidad significativa de cálices de esta planta como subproducto. En este estudio, realizamos una exhaustiva caracterización de polifenoles, ácidos orgánicos y el potencial antioxidante en los cálices de jamaica, la decocción y su subproducto. Los cálices de jamaica son una fuente rica en diversos polifenoles, incluyendo delfinidina sambubiosido, ácidos cafeoilquínicos, ácido hibisco y ácido cítrico como principales componentes. De manera importante, una proporción significativa de estos compuestos bioactivos se extrajo durante el proceso de decocción, lo que resultó en una bebida rica en polifenoles. Los cálices utilizados (subproducto de la decocción) retuvieron entre el 23 % y el 140 % de los polifenoles y ácidos orgánicos extraíbles totales de la jamaica. Además, dicho subproducto fue enriquecido con componentes no extraíbles unidos a la fibra dietaria (126 % - 272 %) debido a la lixiviación de componentes hidrofóbicos, tales como fibra dietaria soluble y polifenoles y ácidos orgánicos extraíbles. Por lo tanto, los cálices de jamaica y sus subproductos de decocción emergen como fuentes prometedoras de polifenoles con potencial para su uso en suplementos dietéticos, junto con la comúnmente consumida decocción de jamaica.

Palabras clave: *Hibiscus sabdariffa* L.; subproducto; compuestos bioactivos; capacidad antioxidante; valor nutracéutico.

INTRODUCTION

Hibiscus sabdariffa L, commonly known as roselle, is cultivated in numerous tropic and subtropic countries. The calyxes are the main part consumed of this plant, which are consumed fresh, pickled, or dried depending on the local gastronomy. Nevertheless, a common use of roselle calyxes is for the elaboration of infusions or decoctions, leading to a refreshing deep red colored soft drink with a soothing taste (Jamrozik *et al*., 2022) rich in anthocyanins and non-pigmented flavonoids, phenolic acids, and organic acids (Sapian *et al*., 2023). While roselle calyxes have been recognized for their nutraceutical value, a substantial environmental issue arises during their preparation. The significant amount of herb material used for infusions or decoctions is often discarded, contributing to environmental waste. These wastes, as recent studies suggest (Debnath *et al*., 2021), may contain valuable compounds.

Sáyago-Ayerdi *et al*. (2014) reported that roselle residues are rich in dietary fiber with considerable antioxidant capacity. Interestingly, the polyphenols found in roselle decoction by-product were found to be highly bioaccesible in the gastrointestinal tract (Mercado-Mercado *et al*., 2015).

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In a previous study carried out by our research group, we demonstrated that the decoction process increased the porosity in used roselle calyxes (Amaya-Cruz *et al*., 2018).

Therefore, in our study we hypothesized that the modifications of the chemical structure and composition during roselle decoction leads to the leaching of extractable polyphenols and organic acids, which are found as free components within the food matrix; whereas the non-extractable polyphenols and organic acids, which are bound to the dietary fiber of the food matrix can either be released and found as extractable components, or can be concentrated and found in higher amount. Interestingly, these non-extractable components are mainly found in fruit, vegetable, and herbs by-products (Ding *et al*., 2020) and have been reported to exert numerous health beneficial effects.

In this regard, we previously demonstrated that roselle decoction by-product exert similar anti-obesity, hypoglycemic, and hypolipidemic effects than roselle calyx (Amaya-Cruz *et al*., 2019), which could be associated with the high extractable polyphenol content in roselle calyx, and the high non-extractable polyphenol content in roselle by-product. Even though in our study a qualitative polyphenol profile was carried out, we recognized that the quantification of the potential bioactive compounds is essential. Therefore, in our study, we aimed not only to identify, but also quantify, the constituents of the extractable and non-extractable fractions of roselle calyx and its decoction by-product and to determine their impact on their antioxidant capacity, as well as the polyphenol and organic acid composition of roselle decoction through high-resolution mass spectrometry.

MATERIAL AND METHODS

Roselle calyx, decoction, and by-product

Dried roselle (*Hibiscus sabdariffa* L.) calyxes were obtained from local producers from Guerrero, Mexico. Calyxes were disinfected with 1 % Nobac citrus 373 solution for 10 min by immersion, drained, and then dried at 45 ºC for 48 h in a forced circulation oven (BF 400, Binder, Tuttlingen, Germany). Subsequently, 100 g of dried and disinfected roselle calyxes were subjected to a decoction process with 1 L of boiling water for 15 min as reported by Amaya-Cruz *et al*. (2019). The used calyces (by-product) were separated from the liquid (decoction) by decantation. The roselle by-product was dried as previously described, then ground to a size particle < 420 µm. The roselle calyx, decoction, and by-product were stored at -20 ºC until they were further analyzed. Roselle decoction was thawed at 4 ºC for 24 h and subsequently brought to room temperature before undergoing chemical analyses, while roselle calyx and by-product were directly analyzed. The decoction process was performed in triplicate. Within each decoction replicate, the by-product and the calyx were both analyzed in triplicate for each assay.

Polyphenols extraction from roselle calyx and by-product

The roselle decoction was directly utilized to determine the polyphenol composition. The roselle calyx and by-product were subjected to a sequential polyphenol extraction with organic solvents as reported by Hassan *et al*. (2011). Briefly, 500 mg of each sample were extracted with 5 mL of 50:50 (v/v) methanol:water, adjusted to pH 2 using hydrochloric acid. The extraction was performed at room temperature for 60 min under continuous stirring. Then, samples were centrifuged (1500 x g for 10 min). Supernatants were recovered and the residue was further extracted with 5 mL of 70:30 (v/v) acetone:water as previously described. The combination of these supernatants formed the extractable polyphenol (EPP) fraction, utilized to quantify extractable polyphenols, flavonoids, anthocyanins, and proanthocyanidins. The residues were dried at 45 º C for 25 h, forming the non-extractable polyphenol (NEPP) fraction utilized to quantify hydrolysable polyphenols (HPP) and non-extractable proanthocyanidins (NEPA).

Total and extractable phenolic compounds

The polyphenol content was determined as reported by Singleton *et al*. (1999) with minor modifications. Briefly, 10 µL of each sample were mixed with 25 µL of 1 N Folin Ciocalteu, 125 µL of a 20 % (w/v) aqueous solution of sodium carbonate, and 40 µL of distilled water. The reactions were incubated for 30 min under darkness. Then, absorbances were measured at 765 nm. The results were expressed as mg of gallic acid equivalents/mL of roselle decoction or mg of gallic acid equivalents/g of roselle calyx or by-product.

Total and extractable flavonoid content

The flavonoid content was determined as reported by Oomah *et al*. (2005) with minor modifications. Briefly, 100 µL of each sample were mixed with 20 µL of a 1 mg/mL methanolic solution of 2-aminoehtyldiphenylborate, and 130 µL of distilled water. Then, absorbances were immediately measured at 404 nm. The results were expressed as mg of rutin equivalents/mL of roselle decoction or mg of rutin equivalents/g of roselle calyx or by-product.

Total and extractable anthocyanin content

The anthocyanin content was determined as described by Giusti and Wrolstad (2001) with minor modifications. Briefly, 50 µL of each sample were mixed with 175 µL of each buffer: 0.25 M potassium chloride at pH 1 or 0.40 M sodium acetate at pH 4.5. Then, absorbances were immediately measured at 510 and 700 nm. The results were expressed as mg of cyanidin 3-*O*-glycoside equivalents/mL of roselle decoction or mg of cyanidin 3-*O*-glycoside equivalents/g of roselle calyx or by-product.

Extractable proanthocyanidins

The extractable proanthocyanidins (EPA) content was determined as reported by Zurita *et al*. (2012). Briefly, 500 µL of each sample were mixed with 4.5 mL of a 95:5 (v/v) buthanol:hydrochloric acid solution containing 0.07 g/L of iron chloride. The reactions were incubated in a boiling water bath for 1 h. Then, samples were centrifuged at 1500 x g for

10 min and supernatants were recovered. Absorbances were measured at 450 and 550 nm. The results were expressed as mg of proanthocyanidin equivalents/mL of roselle decoction or mg of proanthocyanidin equivalents/g of roselle calyx or by-product.

Hydrolysable polyphenols content

A sequential alkaline and acid hydrolysis was carried out as reported by Quatrin *et al*. (2019). The NEPP residues were incubated with 5 mL of 10 M sodium hydroxide and 12 mL of distilled water under continuous stirring for 16 h. Then, their pH was adjusted to 2 - 3 with 6 M hydrochloric acid. Samples were centrifuged at 2000 x g for 10 min and supernatants were recovered. Afterwards, the residues were re-extracted with 5 mL of distilled water as previously described. The supernatants were mixed to quantify polyphenols as described. Results were expressed as mg of gallic acid equivalents/mL of roselle decoction or mg of gallic acid equivalents/g of roselle calyx or by-product, reflecting the alkaline HPP content. Then, the residue obtained during the alkaline hydrolyses was subjected to acid hydrolysis with 2.5 mL of hydrochloric acid. Samples were incubated at 85 ºC for 30 min. Then, their pH was adjusted to 2 - 3 with 10 M sodium hydroxide. Afterwards, the residues were re-extracted with 5 mL of distilled water as previously described. The supernatants were mixed to quantify polyphenols as described. The results were expressed as mg of gallic acid equivalents/mL of roselle decoction or mg of gallic acid equivalents/g of roselle calyx or by-product, reflecting the acid HPP content.

Non-extractable proanthocyanidins content

The NEPA content was determined as reported by Zurita *et al*. (2012) in the NEPP residue as previously described. The results were expressed as mg of proanthocyanidin equivalents/mL of roselle decoction or mg of proanthocyanidin equivalents/g of roselle calyx or by-product.

Polyphenol profile by UPLC-ESI-QToF MS

The roselle decoction was filtered using a syringe filter with PTFE membrane (13 mm, 0.2 µm). The EPP and HPP extracts obtained from the roselle calyx and by-product were vacuum-dried using a Speedvac system (Savant, Thermo Fisher Scientific, MA, USA), re-suspended in 200 µL of mobile phase, and subsequently filtered. The polyphenol profile was assessed in an Ultra-Performance Liquid Chromatograph coupled to a high-resolution Quadrupole/Time-of-Flight Mass Spectrometer (UPLC-QToF MSE, Vion, Waters Co., MA, USA). The autosampler was maintained at 4 ºC. The chromatographic separation was carried out with 1 µL of each sample injected into a C18 column (2.1 x 100 mm, 1.7 µm, BEH Acquity, Waters Co.) at 35 ºC. The mobile phase was constituted by (a) water with 1 % formic acid and (B) acetonitrile with 1 % formic acid using the gradient conditions throughout 17 min as reported by Reynoso-Camacho *et al*. (2021). An atmospheric pressure electrospray ionization (ESI) source was used at negative and positive mode at 120 ºC using nitrogen as desolvation gas (800 L/h at 450 ºC) and as cone gas (50 L/h). The mass spectra were acquired at 100-1200 m/z at low (6 V) and high (15-45 V) collision energy. Data were mass corrected during acquisition using a 50 pg/mL leucine enkephalin solution at 10 µL/min.

Polyphenol identification was carried out by comparison of the experimental mass with the theorical mass according to their molecular formula, with a mass error <10 ppm cut-off and by the analysis of the fragmentation pattern. For compounds for which a standard was available, quantification was carried out using its corresponding calibration curve. In cases where a commercial standard was unavailable, quantification was conducted using the most similar standard available. The results were expressed as mg/mL of roselle decoction or mg/g of roselle calyx or by-product.

Antioxidant capacity

The antioxidant capacity was determined in the roselle decoction, as well as in the EPP and HPP fractions of the roselle calyx and by-product, using three widely recognized methods. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed following the procedure outlined by Fukumoto and Mazza (2000). The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay was conducted according to the method described by Re *et al*. (1999). The ferric reducing antioxidant power (FRAP) assay was carried out as reported by Firuzi *et al*. (2005). The results were expressed as mmol of Trolox equivalents/mL of roselle decoction or mmol of Trolox equivalents/g of roselle calyx or by-product.

Statistical analysis

Results are presented as mean values ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by means comparison by Tukey's test ($p < 0.05$), using the JMP software (v14.0).

RESULTS AND DISCUSSION

Traditional medicine commonly advocates the preparation of orally suitable beverages, primarily infusions and decoctions, to extract bioactive compounds from various botanical sources (Jaiswal *et al*., 2016). These processes rely on hot water extraction of hydrophilic components such as polyphenols and organic acids found in roselle calyxes. In a beverage like roselle decoction, polyphenols are typically measured as a whole entity, either via spectrophotometry or mass spectrometry techniques. However, in complex matrices like roselle calyxes and by-products, it is crucial to independently analyze the content and composition of extractable and non-extractable components. The former refers to components soluble in the extraction solvents, while the latter can correspond to polymeric constituents or monomeric components bound to macromolecules (Báez-García *et al*., 2023).

In this study, we conducted a comprehensive analysis of the polyphenols, organic acids, and antioxidant potential of roselle calyxes, decoction, and its by-product. We quantified the total, extractable, and non-extractable polyphenols using

spectrophotometric techniques (Table 1). Subsequently, we employed a high-resolution UPLC-QToF MS^E system for an exhaustive identification of antioxidant compounds, with a primary focus on polyphenols and organic acids (Tables 2 - 4), which are major constituents of roselle (Sapian *et al*., 2023). Finally, the antioxidant capacity of each fraction was assessed by three spectrophotometric techniques that assess different mechanisms (Table 5).

Total polyphenol content of roselle calyx decoction

As shown in Table 1, the roselle decoction exhibits a mean polyphenol content of 111 mg/100 mL, significantly higher than other herb-based beverages (Rothwell *et al*., 2013). It is worth noting that the proanthocyanidin content in roselle decoction is 4.4 times higher than its anthocyanin content, despite the latter being traditionally associated with its health benefits. Commonly consumed polyphenol-rich beverages, such as *Camellia sinensis* (tea) infusion and coffee, typically contain a mean total polyphenol content ranging from 62 to 105 mg/100 mL (Rothwell *et al*., 2013). This positions roselle decoction as a competitive antioxidant beverage.

Total extractable and non-extractable polyphenol content of roselle calyx and its decoction by-product

As the global demand for flavorful herb-based beverages grows, there is a concurrent increase in waste generated from the decoction and infusion processes (Debnath *et al*., 2021). As shown in Table 1, used roselle calyxes (decoction by-product) displayed a lower content of extractable polyphenols (1.7-fold) compared to roselle calyxes before the decoction process, attributed to the release of these hydrophilic constituents into the decoction (beverage). This trend was observed in most of the extractable polyphenols identified in used roselle calyxes (Table 2). Notably, the antioxidant capacity assessed by ABTS and DPPH scavenging assays remained similar between roselle calyxes and their by-product, while the FRAP capacity decreased significantly (1.4 - fold; Table 5).

The release of hydrophilic constituents into the decoction led to the concentration of non-extractable constituents in the roselle decoction by-product, resulting in a higher content of both acid and alkali hydrolysable polyphenols (2.3- and 1.3-fold; Table 1) as comparison to the calyx before the decoction process. These hydrolysable polyphenols include low-molecular-weight polyphenols covalently bound to the polysaccharide constituents of dietary fiber through covalent bonds, hydrogen bonds, and/or hydrophobic interactions (Báez-García *et al*., 2023).

Identification and quantification of polyphenols and organic acids in roselle calyx, decoction, and its by-product

Delphinidin sambubioside emerged as the major anthocyanin identified in roselle calyxes, decoction, and its by-product. The MS^E spectra obtained for this component are depicted in Figure 1, revealing a cation radical ($[M]^+$) with a m/z of 597

Table 1. Total, extractable, and non-extractable polyphenol content of roselle decoction, calyx, and by-product.
Tabla 1. Contenido de polifenoles totales, extraíbles y no extraíbles de decocción, calix y subproducto de jamaica.

Polyphenols	Decoction	Calyx	By-product
Polyphenols			
Total content (mg GAE/mL)	1.11 ± 0.05		
Extractable fraction (mg GAE/g)		25.05 ± 0.98 [a]	14.82 ± 0.23 [b]
Acid hydrolysable fraction (mg GAE/g)		5.71 ± 0.20 [b]	13.14 ± 1.10 [a]
Alkaline hydrolysable fraction (mg GAE/g)		8.13 ± 0.59 [b]	10.61 ± 0.95 [a]
Flavonoids			
Total content (mg RE/mL)	0.22 ± 0.02		
Extractable fraction (mg RE/g)		10.87 ± 0.41 [a]	7.91 ± 0.10 [b]
Anthocyanins			
Total content (mg C3GE/mL)	0.13 ± 0.00		
Extractable fraction (mg C3GE/g)		2.24 ± 0.07 [a]	1.29 ± 0.10 [b]
Proanthocyanidins			
Total content (mg CE/mL)	0.57 ± 0.03		
Extractable fraction (mg CA/g)		1.54 ± 0.14 [a]	0.87 ± 0.03 [b]
Non-extractable fraction (mg PA/g)		2.41 ± 0.10 [a]	2.60 ± 0.02 [a]

Different letters indicate significant ($p < 0.05$) differences. GAE: gallic acid equivalents; RE: rutin equivalents; C3GE: cyanidin 3-*O*-glucoside equivalents; CE: (+)-catechin equivalents; PA: proanthocyanidin equivalents.
Letras diferentes indican diferencia significativa ($p < 0.05$). GAE: equivalentes de ácido gálico; RE: equivalentes de rutina; C3GE: equivalentes de cianidina 3-*O*-glucósido; CE: equivalentes de (+)-catequina; PA: equivalentes de proantocianidina.

Table 2. Polyphenol profile of roselle decoction by UPLC-ESI-QToF MS^E.
Tabla 2. Perfil de polifenoles de decocción de jamaica por UPLC-ESI-QToF MS^E.

Compound	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Error (ppm)	Adduct	Fragments	Content (mg/mL)
Anthocyanins								
Delphinidin sambubioside	3.59	C26H29O16	597.1456	597.1436	-3.2458	$[M]^+$	303.05903	10.95 ± 0.02
Delphinidin hexoside	3.68	C21H21O12	465.1033	465.1071	8.1315	$[M]^+$	303.08267	0.02 ± 0.00
Flavonols								
Kaempferol aldo pentosyl-hexoside	4.20	C26H28O15	580.1428	580.1432	0.7137	$[M-H]^-$	284.03284	0.19 ± 0.01
Quercetin aldo pentosyl-rutinoside	6.81	C32H38O20	742.1956	742.1957	0.0777	$[M-H]^-$	300.02781, 178.99754, 151.00338	0.01 ± 0.00
Myricetin hexoside	7.00	C21H20O13	480.0904	480.0912	1.7001	$[M-H]^-$	316.02261, 178.99803, 151.00300	0.04 ± 0.01
Kaempferol rhamnosyl-hexoside-rhamnoside	8.07	C33H40O19	740.2164	740.2165	0.1643	$[M-H]^-$	284.03273	0.01 ± 0.00
Quercetin rutinoside*	8.70	C27H30O16	610.1534	610.1532	-0.2614	$[M-H]^-$	300.02711, 178.99815, 151.00322, 107.01334	0.11 ± 0.00
Quercetin hexoside	9.14	C21H20O12	464.0955	464.0959	0.9470	$[M-H]^-$	300.02711, 178.99800, 151.00321, 107.01259	0.08 ± 0.01
Kaempferol hexoside-rhamnoside	10.74	C27H30O15	594.1585	594.1581	-0.6093	$[M-H]^-$	285.0397	0.02 ± 0.00
Myricetin	11.07	C15H10O8	318.0376	318.0378	0.7452	$[M-H]^-$	179.03454, 151.00331, 137.02392	0.01 ± 0.01
Quercetin*	11.40	C15H10O7	302.0427	302.0427	0.1508	$[M-H]^-$	178.99784, 151.00334, 107.01267	0.02 ± 0.02
Hydroxybenzoic acids								
Gallic acid*	3.01	C7H6O5	170.0215	170.0213	-1.3736	$[M-H]^-$	125.02403	0.01 ± 0.00
Dihydroxybenzoic acid hexoside	3.20	C13H16O9	316.0794	316.0795	0.1541	$[M-H]^-$	153.01887, 136.99077, 109.02902	1.51 ± 0.25
Vanillic acid*	3.24	C8H8O4	168.0423	168.0420	-1.8337	$[M-H]^-$	136.99077, 109.02902	0.03 ± 0.00
Galloylquinic acid isomer I	3.28	C14H16O10	344.0743	344.0748	1.1862	$[M-H]^-$	191.05583, 169.05030	0.01 ± 0.00
3,4-Dihydroxybenzoic acid*	3.37	C7H6O4	154.0266	154.0266	0.0096	$[M-H]^-$	137.02418, 109.02925	0.04 ± 0.00
Hydroxybenzoic acid isomer I	3.96	C7H6O3	138.0317	138.0317	0.1860	$[M-H]^-$	No fragments	0.34 ± 0.02
Dihydroxybenzoic acid isomer II	4.10	C7H6O4	154.0266	154.0266	-0.1822	$[M-H]^-$	136.86270, 109.02951	0.02 ± 0.00
Methylgallic acid	4.47	C8H8O5	184.0372	184.0372	0.1824	$[M-H]^-$	169.01423, 139.04017, 125.02445	0.03 ± 0.02
Hydroxybenzoic acid isomer II	8.91	C7H6O3	138.0317	138.0316	-0.3855	$[M-H]^-$	No fragments	0.06 ± 0.00
Hydroxycinnamic acids								
Caffeoylquinic acid isomer I	3.46	C16H18O9	354.0951	354.0953	0.6817	$[M-H]^-$	191.05586, 179.03485, 135.04501	3.90 ± 0.19
Coumaroylquinic acid isomer I	3.98	C16H18O8	338.1002	338.1007	1.4770	$[M-H]^-$	191.05603, 163.04009, 119.05021	0.63 ± 0.02
Caffeic acid hexoside	4.01	C15H18O9	342.0951	342.0956	1.6240	$[M-H]^-$	178.99889, 135.01643	0.04 ± 0.00
Caffeoylquinic acid isomer II	4.11	C16H18O9	354.0951	354.0956	1.5046	$[M-H]^-$	191.05586, 179.05521, 135.04492	1.26 ± 0.17
Chlorogenic acid*	4.28	C16H18O9	354.0951	354.0958	1.9330	$[M-H]^-$	191.03346, 179.03518, 135.04523	2.41 ± 0.02
Ferulic acid hexoside	4.71	C16H20O9	356.1107	356.1112	1.4254	$[M-H]^-$	193.05060, 178.02642, 135.04506	0.01 ± 0.00
Caffeic acid*	4.72	C9H8O4	180.0423	180.0424	0.8791	$[M-H]^-$	135.04525	0.07 ± 0.00
Feruloylquinic acid isomer I	4.89	C17H20O9	368.1107	368.1116	2.3544	$[M-H]^-$	192.99567, 134.03622	0.12 ± 0.00
Coumaroylquinic acid isomer II	5.31	C16H18O8	338.1002	338.1008	1.9346	$[M-H]^-$	191.03403, 163.04014, 119.05039	0.78 ± 0.01
Feruloylquinic acid isomer II	6.28	C17H20O9	368.1107	368.1114	1.8727	$[M-H]^-$	192.97484, 179.10743, 134.03476	0.07 ± 0.00
p-Coumaric acid*	6.37	C9H8O3	164.0473	164.0474	0.2458	$[M-H]^-$	119.05004	0.01 ± 0.00
Feruloylquinic acid isomer III	7.23	C17H20O9	368.1107	368.1112	1.3841	$[M-H]^-$	191.05562, 179.03476, 135.04494	0.10 ± 0.00
Organic acids								
Citric acid*	1.38	C6H8O7	192.0270	192.0265	-2.7462	$[M-H]^-$	111.00859	2.80 ± 0.06
Hibiscus acid	0.61	C6H8O8	208.0219	208.0215	-1.9499	$[M-H]^-$	189.00344, 127.00330	14.08 ± 0.01
Hydroxycitric acid	1.28	C6H8O8	208.0219	208.0216	-1.3813	$[M-H]^-$	189.00371, 127.00347	0.31 ± 0.04
Quinic acid	0.59	C7H12O6	192.0634	192.0630	-1.9364	$[M-H]^-$	127.00344	0.43 ± 0.01

*Identification confirmed with commercial standards.
*Identificación confirmada con estándares comerciales.

Table 3. Extractable polyphenols and organic acid profiles of roselle calyx and by-product by UPLC-ESI-QToF MS^E.
Tabla 3. Perfil de polifenoles y de ácidos orgánicos extraíbles de calix y subproducto de jamaica por UPLC-ESI-QToF MS^E.

Compound	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Error (ppm)	Adduct	Fragments	Content (mg/g)	
								Calyx	By-product
Anthocyanins									
Delphinidin sambubioside	3.59	C26H29O16	597.1456	597.1436	-3.2458	$[M]^+$	303.05903	1.32 ± 0.14 ^{a}	0.35 ± 0.03 ^{b}
Delphinidin hexoside	3.68	C21H21O12	465.1033	465.1071	8.1315	$[M]^+$	303.08267	0.02 ± 0.00 ^{a}	0.01 ± 0.00 ^{a}
Flavonols									
Kaempferol aldo pentosyl-hexoside	4.20	C26H28O15	580.1428	580.1432	0.7137	$[M-H]^-$	284.03284	0.42 ± 0.01 ^{a}	0.23 ± 0.04 ^{b}
Myricetin rutinoside	6.78	C27H30O17	626.1483	626.1482	-0.1203	$[M-H]^-$	316.02251, 178.99842, 151.00369	0.05 ± 0.01 ^{a}	0.02 ± 0.00 ^{b}
Quercetin aldo pentosyl-rutinoside	6.81	C32H38O20	742.1956	742.1957	0.0777	$[M-H]^-$	300.02781, 178.99754, 151.00338	0.03 ± 0.01 ^{a}	0.01 ± 0.00 ^{a}
Myricetin hexoside	7.00	C21H20O13	480.0904	480.0912	1.7001	$[M-H]^-$	316.02261, 178.99803, 151.00300	0.17 ± 0.04 ^{a}	0.08 ± 0.00 ^{b}
Quercetin rutinoside	8.70	C27H30O16	610.1534	610.1532	-0.2614	$[M-H]^-$	300.02711, 178.99815, 151.00322, 107.01334	0.56 ± 0.12 ^{a}	0.33 ± 0.07 ^{b}
Quercetin hexoside	9.14	C21H20O12	464.0955	464.0959	0.9470	$[M-H]^-$	300.02711, 178.99800, 151.00321, 107.01259	0.44 ± 0.10 ^{a}	0.20 ± 0.05 ^{b}
Kaempferol hexoside-rhamnoside	10.74	C27H30O15	594.1585	594.1581	-0.6093	$[M-H]^-$	285.0397	0.07 ± 0.02 ^{a}	0.06 ± 0.01 ^{a}
Myricetin	11.07	C15H10O8	318.0376	318.0378	0.7452	$[M-H]^-$	179.03454, 151.00331, 137.02392	0.17 ± 0.01 ^{a}	0.14 ± 0.02 ^{a}
Quercetin*	11.40	C15H10O7	302.0427	302.0427	0.1508	$[M-H]^-$	178.99784, 151.00334, 107.01267	0.49 ± 0.02	0.69 ± 0.08 ^{a}
Hydroxybenzoic acids									
Gallic acid*	3.01	C7H6O5	170.0215	170.0213	-1.3736	$[M-H]^-$	125.02403	0.44 ± 0.03 ^{a}	0.34 ± 0.02 ^{b}
Dihydroxybenzoic acid hexoside	3.20	C13H16O9	316.0794	316.0795	0.1541	$[M-H]^-$	153.01887, 136.99077, 109.02902	6.93 ± 0.44 ^{a}	4.83 ± 0.27 ^{b}
Vanillic acid	3.24	C8H8O4	168.0423	168.0420	-1.8337	$[M-H]^-$	136.99077, 109.02902	0.12 ± 0.01 ^{a}	0.10 ± 0.00 ^{a}
3,4-Dihydroxybenzoic acid*	3.37	C7H6O4	154.0266	154.0266	0.0096	$[M-H]^-$	137.02418, 109.02925	0.42 ± 0.01 ^{a}	0.32 ± 0.00 ^{b}
Hydroxybenzoic acid isomer I	3.96	C7H6O3	138.0317	138.0317	0.1860	$[M-H]^-$	No fragments	2.43 ± 0.18 ^{a}	1.78 ± 0.00 ^{b}
Dihydroxybenzoic acid isomer II	4.10	C7H6O4	154.0266	154.0266	-0.1822	$[M-H]^-$	136.86270, 109.02951	0.23 ± 0.01 ^{a}	0.11 ± 0.01 ^{b}
Methylgallic acid	4.47	C8H8O5	184.0372	184.0372	0.1824	$[M-H]^-$	169.01423, 139.04017, 125.02445	0.27 ± 0.05 ^{a}	0.09 ± 0.00 ^{b}
Galloylquinic acid isomer II	7.67	C14H16O10	344.0743	344.0746	0.6374	$[M-H]^-$	191.05583, 169.05030	0.81 ± 0.18 ^{a}	ND
Galloylquinic acid isomer III	7.96	C14H16O10	344.0743	344.0747	0.8987	$[M-H]^-$	190.99818, 169.98481	0.74 ± 0.18 ^{a}	ND
Hydroxycinnamic acids									
Caffeoylquinic acid isomer I	3.46	C16H18O9	354.0951	354.0953	0.6817	$[M-H]^-$	191.05586, 179.03485, 135.04501	15.22 ± 1.29 ^{a}	13.74 ± 0.10 ^{a}
Coumaroylquinic acid isomer I	3.98	C16H18O8	338.1002	338.1007	1.4770	$[M-H]^-$	191.05603, 163.04009, 119.05021	1.60 ± 0.20 ^{a}	1.52 ± 0.01 ^{a}
Caffeic acid hexoside	4.01	C15H18O9	342.0951	342.0956	1.6240	$[M-H]^-$	178.99889, 135.01643	0.16 ± 0.02 ^{a}	0.10 ± 0.00 ^{b}
Chlorogenic acid*	4.28	C16H18O9	354.0951	354.0958	1.9330	$[M-H]^-$	191.03346, 179.03518, 135.04523	10.86 ± 1.27 ^{a}	8.69 ± 0.19 ^{a}
Caffeic acid*	4.72	C9H8O4	180.0423	180.0424	0.8791	$[M-H]^-$	135.04525	0.59 ± 0.08 ^{a}	0.29 ± 0.00 ^{b}
Feruloylquinic acid isomer I	4.89	C17H20O9	368.1107	368.1116	2.3544	$[M-H]^-$	192.99567, 134.03622	1.03 ± 0.13 ^{a}	1.00 ± 0.02 ^{a}
Coumaroylquinic acid isomer II	5.31	C16H18O8	338.1002	338.1008	1.9346	$[M-H]^-$	191.03403, 163.04014, 119.05039	3.24 ± 0.51 ^{a}	2.06 ± 0.01 ^{b}
Feruloylquinic acid isomer II	6.28	C17H20O9	368.1107	368.1114	1.8727	$[M-H]^-$	192.97484, 179.10743, 134.03476	1.01 ± 0.16 ^{a}	0.73 ± 0.02 ^{b}
p-Coumaric acid*	6.37	C9H8O3	164.0473	164.0474	0.2458	$[M-H]^-$	119.05004	0.08 ± 0.01 ^{a}	0.06 ± 0.00 ^{a}
Feruloylquinic acid isomer III	7.23	C17H20O9	368.1107	368.1112	1.3841	$[M-H]^-$	191.05562, 179.03476, 135.04494	1.75 ± 0.28 ^{a}	0.86 ± 0.01 ^{b}
Ferulic acid*	10.74	C10H10O4	194.0579	194.0572	-3.8475	$[M-H]^-$	178.02718, 134.86543	0.04 ± 0.01 ^{a}	0.02 ± 0.00 ^{a}
Organic acids									
Citric acid*	1.38	C6H8O7	192.0270	192.0265	-2.7462	$[M-H]^-$	111.00859	11.17 ± 0.82 ^{a}	11.64 ± 0.88 ^{a}
Hibiscus acid	0.61	C6H8O8	208.0219	208.0215	-1.9499	$[M-H]^-$	189.00344, 127.00330	37.57 ± 2.88 ^{a}	33.38 ± 2.27 ^{a}
Hydroxycitric acid	1.28	C6H8O8	208.0219	208.0216	-1.3813	$[M-H]^-$	189.00371, 127.00347	1.08 ± 0.21 ^{a}	ND
Quinic acid	0.59	C7H12O6	192.0634	192.0630	-1.9364	$[M-H]^-$	127.00344	1.62 ± 0.68 ^{a}	1.00 ± 0.35 ^{b}

Different letters indicate significant ($p < 0.05$) differences. *Identification confirmed with commercial standards. ND: not detected.
Letras diferentes indican diferencia significativa ($p < 0.05$). *Identificación confirmada con estándares comerciales. ND: no detectado.

Table 4. Non-extractable polyphenols and organic acid profiles of roselle calyx and by-product by UPLC-ESI-QToF MS^E.
Tabla 4. Perfil de polifenoles y de ácidos orgánicos no extraíbles de caliz y subproducto de jamaica por UPLC-ESI-QToF MS^E.

Compound	Rt (min)	Molecular formula	Expected mass (Da)	Observed mass (Da)	Error (ppm)	Adduct	Fragments	Content (mg/g)	
								Calyx	By-product
ALKALINE HYDROLYSABLE POLYPHENOLS									
Hydroxybenzoic acids									
3,4-Dihydroxybenzoic acid*	3.37	C7H6O4	154.0266	154.0266	0.0096	$[M-H]^-$	137.02418, 109.02925	0.28 ± 0.02 ^{b}	0.62 ± 0.04 ^{a}
Hydroxybenzoic acid isomer I	3.96	C7H6O3	138.0317	138.0317	0.1860	$[M-H]^-$	No fragments	1.36 ± 0.00 ^{b}	2.63 ± 0.15 ^{a}
Hydroxycinnamic acids									
Ferulic acid*	10.74	C10H10O4	194.0579	194.0572	-3.8475	$[M-H]^-$	178.02718, 134.86543	ND	0.20 ± 0.01 ^{a}
Sinapic acid*	11.17	C11H12O5	224.0685	224.0674	-4.9933	$[M-H]^-$	208.03765	0.14 ± 0.01 ^{b}	0.68 ± 0.13 ^{a}
Organic acids									
Quinic acid	0.59	C7H12O6	192.0634	192.0630	-1.9364	$[M-H]^-$	127.00344	0.26 ± 0.02 ^{b}	0.72 ± 0.4 ^{a}
ACID HYDROLYSABLE POLYPHENOLS									
Hydroxybenzoic acids									
Dihydroxybenzoic acid hexoside	3.20	C13H16O9	316.0794	316.0795	0.1541	$[M-H]^-$	153.01887, 136.99077, 109.02902	2.09 ± 0.14 ^{b}	4.61 ± 0.62 ^{a}
Organic acids									
Citric acid*	1.38	C6H8O7	192.0270	192.0265	-2.7462	$[M-H]^-$	111.00859	ND	0.37 ± 0.01 ^{a}
Hibiscus acid	0.61	C6H8O8	208.0219	208.0215	-1.9499	$[M-H]^-$	189.00344, 127.00330	3.36 ± 0.56 ^{b}	4.25 ± 0.27 ^{a}
Quinic acid	0.59	C7H12O6	192.0634	192.0630	-1.9364	$[M-H]^-$	127.00344	9.47 ± 0.84 ^{b}	12.22 ± 1.7 ^{a}

Different letters indicate significant ($p < 0.05$) differences. *Identification confirmed with commercial standards. ND: not detected.
Letras diferentes indican diferencia significativa ($p < 0.05$). *Identificación confirmada con estándares comerciales. ND: no detectado.

Table 5. Antioxidant capacity of roselle decoction, calyx, and by-product.
Tabla 5. Capacidad antioxidante de decocción, calix y subproducto de jamaica.

Antioxidant capacity	Decoction	Calyx	By-product
ABTS assay			
Total content (mmol TE/mL)	49.41 ± 0.66		
Extractable fraction (mmol TE/g)		394.60 ± 2.55^a	386.96 ± 6.99^a
Acid hydrolysable fraction (mmol TE/g)		34.32 ± 1.42^a	22.11 ± 1.75^b
Alkaline hydrolysable fraction (mmol TE/g)		185.85 ± 1.09^a	18.67 ± 0.31^b
DPPH assay			
Total content (mmol TE/mL)	2.71 ± 0.11		
Extractable fraction (mmol TE/g)		364.76 ± 25.10^a	382.93 ± 6.27^a
Acid hydrolysable fraction (mmol TE/g)		2210.41 ± 47.95^b	2400.05 ± 22.76^a
Alkaline hydrolysable fraction mmol TE/g)		558.72 ± 8.67^b	1886.99 ± 124.78^a
FRAP assay			
Total content (mmol TE/mL)	1.01 ± 0.09		
Extractable fraction (mmol TE/g)		0.23 ± 0.02^a	0.17 ± 0.02^b
Acid hydrolysable fraction (mmol TE/g)		0.14 ± 0.01^a	0.02 ± 0.00^b
Alkaline hydrolysable fraction (mmol TE/g)		0.19 ± 0.01^a	0.08 ± 0.00^b

Different letters indicate significant ($p < 0.05$) differences. TE: trolox equivalents.
Letras diferentes indican diferencia significativa ($p < 0.05$). TE: equivalentes de Trolox.

and a fragment at m/z 303 corresponding to the delphinidin aglycone after the disaccharide moiety is lost. Although delphinidin hexoside, a minor anthocyanin was also identified, our study did not detect cyanidin derivatives, possibly due to the deep red-purple color of the calyxes used, which is attributed to delphinidin derivatives. For instance, Hinojosa-Gómez *et al*. (2020) reported both delphinidin- and cyanidin-sambubioside as major anthocyanins in roselle calyxes from red cultivars. It is noteworthy that delphinidin 3-sambubioside extracted from roselle calyxes decreases hepatic steatosis in both in vivo and in vitro studies by decreasing fatty acid lipogenesis and augmenting fatty acid beta-oxidation (Long *et al*., 2021).

Regarding non-pigmented flavonoids, previous research have identified quercetin and kaempferol aglycones and their derivatives in various roselle varieties and plant parts (Da-Costa-Rocha *et al*., 2014). In our study, these flavonoids were identified as minor components. Some flavones like gossypetin, hibiscitrin, sabdaretin, and hibiscetin, previously reported in roselle flowers (Da-Costa-Rocha *et al*., 2014), were not detected.

In the context of phenolic acids, the major hydroxybenzoic acid was dihydrobenzoic acid hexoside, appearing at 315 m/z as a deprotonated molecular ion. A fragment at 153 m/z resulted from the loss of the hexoside moiety, while another at 109 m/z stemmed from CO_2 detachment (Figure

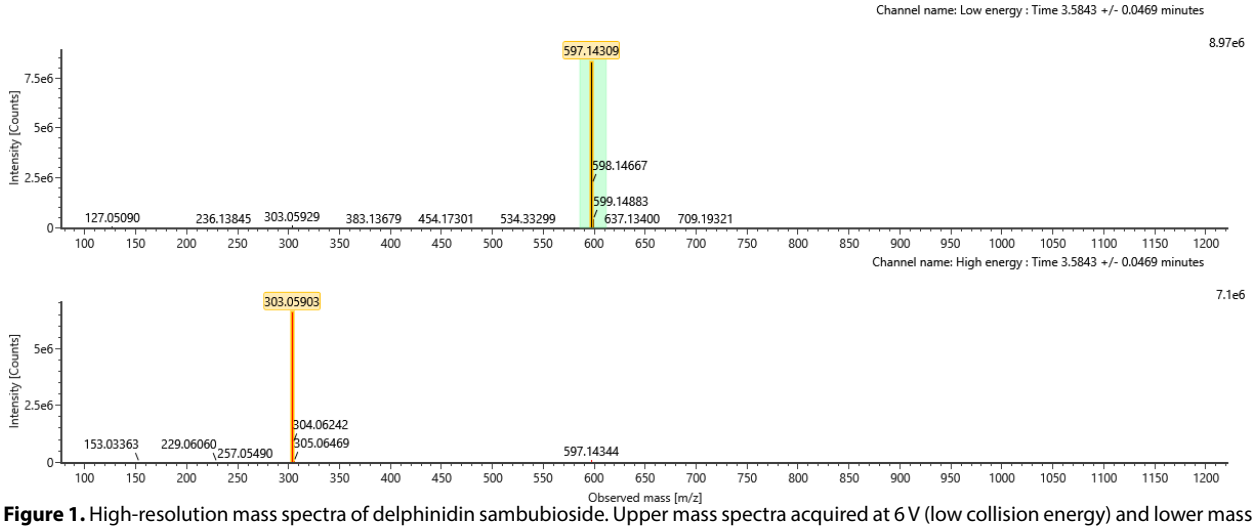

Figure 1. High-resolution mass spectra of delphinidin sambubioside. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 1. Espectro de masas de alta resolución de delfinidina sambubiosida. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

2). Accordingly, protocatechuic acid has been identified as major component in roselle calyx, with a higher content as compared to other Mediterranean plants (Rababah *et al*., 2011) and edible flowers (Trinh *et al*., 2018). Protocatechuic acid (3,4-dihydroxybenzoic acid) and its glucoside derivative are well-known for their antioxidant and anti-inflammatory properties (Kakkar and Bais, 2014); moreover, in silico studies suggest that protocatechuic acid shows a better inhibitory potential of phosphoenolpyruvate carboxikinase, a key enzyme involved in hepatic insulin resistance, than the anti-diabetic drug metformin (Mohanty and Bhadra, 2020).

The major hydroxycinnamic acids were caffeoylquinic acids, including chlorogenic acid, observed at 353 m/z as a deprotonated molecular ion. Fragments corresponding to quinic acid (191 m/z), caffeic acid (179 m/z), and decarboxylated caffeic acid (135 m/z) were also noted (Figures 3 and 4). Interestingly, these compounds have been reported to contribute to the flavor of roselle (Selli *et al*., 2021) and are recognized as primary precursors for developing coffee flavor and aroma (Lin *et al*., 2022). Coumaroulquinic acid was also found as a major phenolic acid, also identified as a deprotonated ion at 337 m/z, with fragments at 191 m/z (quinic acid), 163 m/z (coumaric acid), and 119 m/z (decarboxylated coumaric acid; Figure 5). Accordingly, chlorogenic acids have been identified as the major phenolic acid in roselle extracts, contributing with almost 18 % (Yang *et al*., 2023), which shows high inhibitory activity against the angiotensin converting enzyme, suggesting an anti-hipertensive potential (Salem *et al*., 2020). Conversely to our study, coumaroylquinic acids have been identified as minor components in roselle extracts (Yang *et al*., 2023).

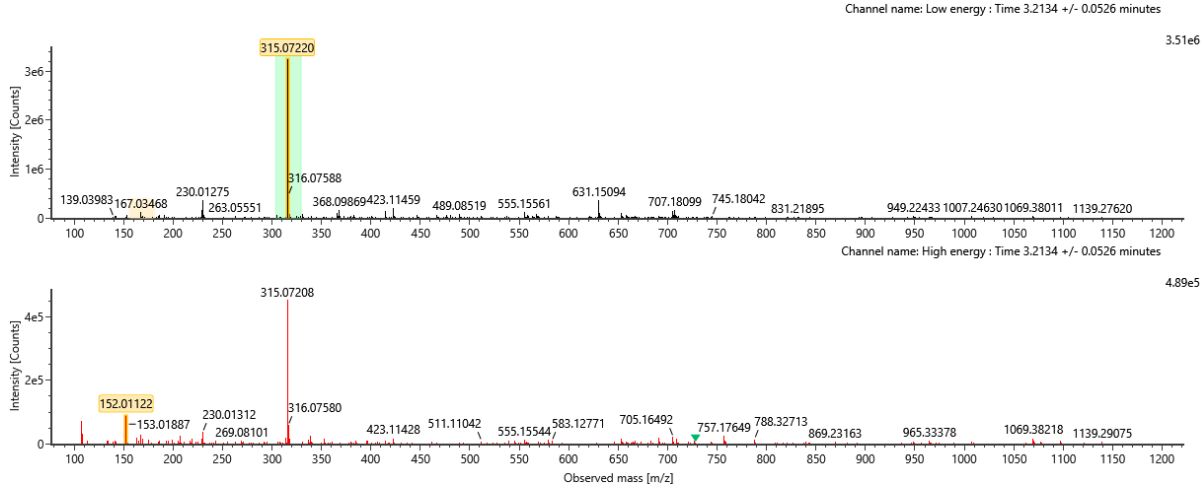

Figure 2. High-resolution mass spectra of dihydroxybenzoic acid hexoside. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 2. Espectro de masas de alta resolución de ácido dihidroxibenzoico hexósido. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

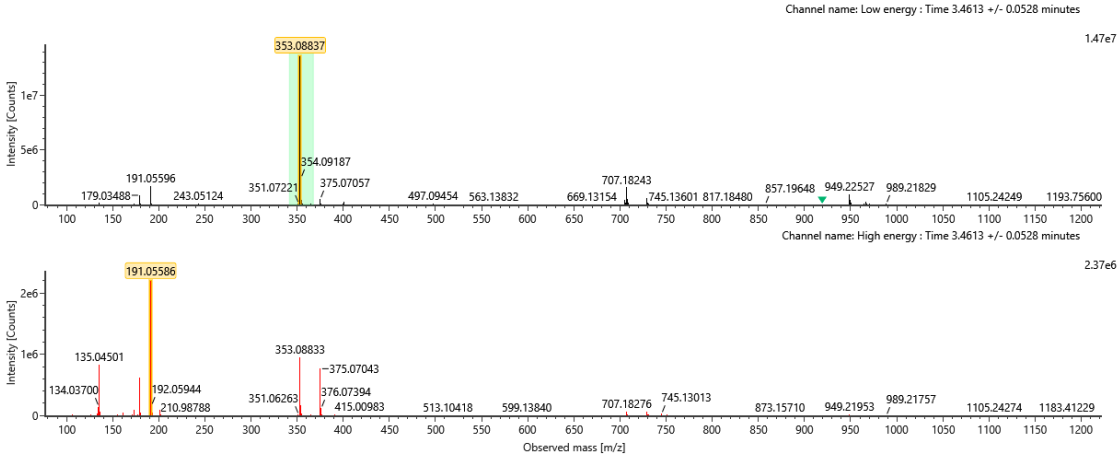

Figure 3. High-resolution mass spectra of caffeoylquinic acid isomer I. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 3. Espectro de masas de alta resolución de ácido cafeoilquínico isómero I. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

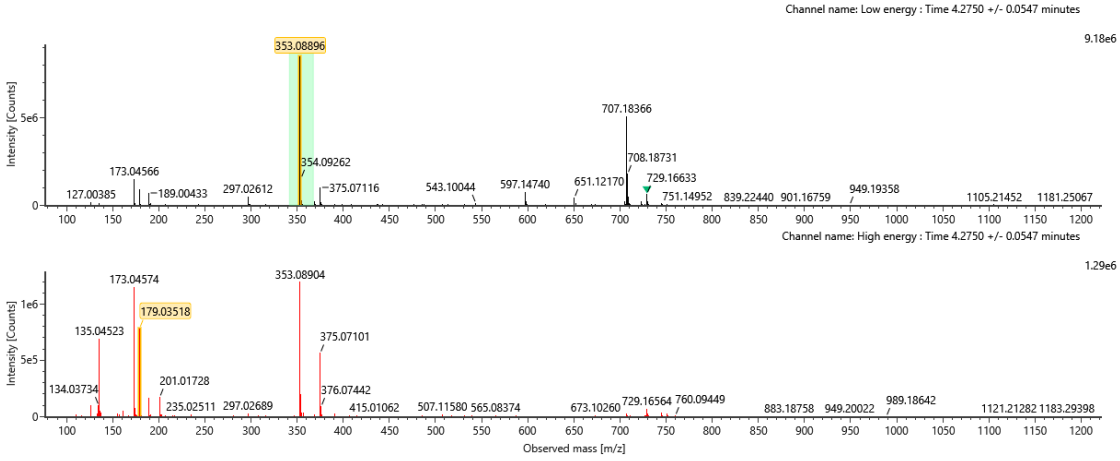

Figure 4. High-resolution mass spectra of caffeoylquinic acid isomer III (chlorogenic acid). Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 4. Espectro de masas de alta resolución de ácido cafeoilquínico isómero III (ácido clorogénico). El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

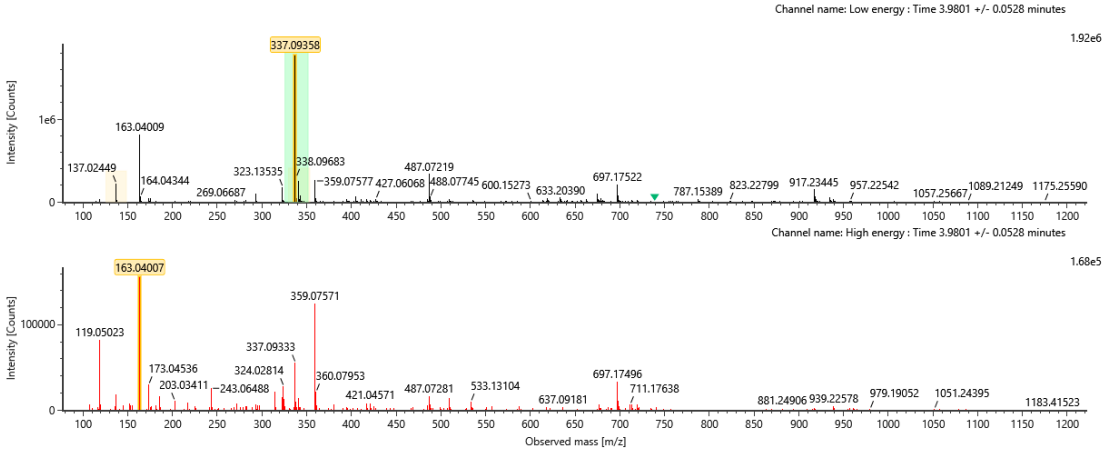

Figure 5. High-resolution mass spectra of coumaroylquinic acid isomer II. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 5. Espectro de masas de alta resolución de ácido cumaroliquínico isómero II. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

Among the organic acids, hibiscus acid highlighted as the major compound, consistent with previous studies (Sapian *et al*., 2023). This organic acid appeared in its deprotonated form ([M-H]-) at 207 m/z, with fragments at 189 and 127 m/z (Figure 6). Additionally, citric and quinic acids were identified as deprotonated ions at 191 m/z with their representative characteristic fragment at 111 and 127 m/z, respectively (Figures 7 and 8). Importantly, the hibiscus acid and citric acid content, the major organic acids identified in this study, remained unaffected by the decoction process (Table 2). It is noteworthy that most studies carried out with roselle have associated its beneficial health effects to their high content of anthocyanins, phenolic acids, and non-pigmented flavonoids, whereas only few studies have focused on the bioactive potential of organic acids (Izquierdo-Vega *et al*., 2020). In this regard, Morales-Luna *et al*. (2018) demonstrated that white roselle calyx extract, with a high organic acid content and no anthocyanins, exert a similar anti-obesogenic effect than red roselle calyx extract with a high anthocyanin content but low organic acid content. Among the composition of organic acids found in roselle, citric acid has been attributed with high antioxidant, anti-inflammatory and anti-coagulant activities, whereas hibiscus acid shows anti-diabetic and anti-hypertensive potential. On the other hand, hydroxycitric acid has been recently proposed as an anti-obesity nutraceutical; however, most studies have been assessed in hydroxycitric acid isolated from *Garcinia cambogia* (Yang *et al*., 2020).

The roselle decoction analyzed in this study was found to have hibiscus acid as the major component, accounting for 79.9 % of the identified organic acids, followed by delphinidin sambubioside (Table 2). Moreover, dihydroxybenzoic acid hexoside represented 73.7 % of all hydroxybenzoic acids, while the three caffeoylquinic acid isomers collectively made up to 80.5 % of the hydroxycinnamic acids. These results are

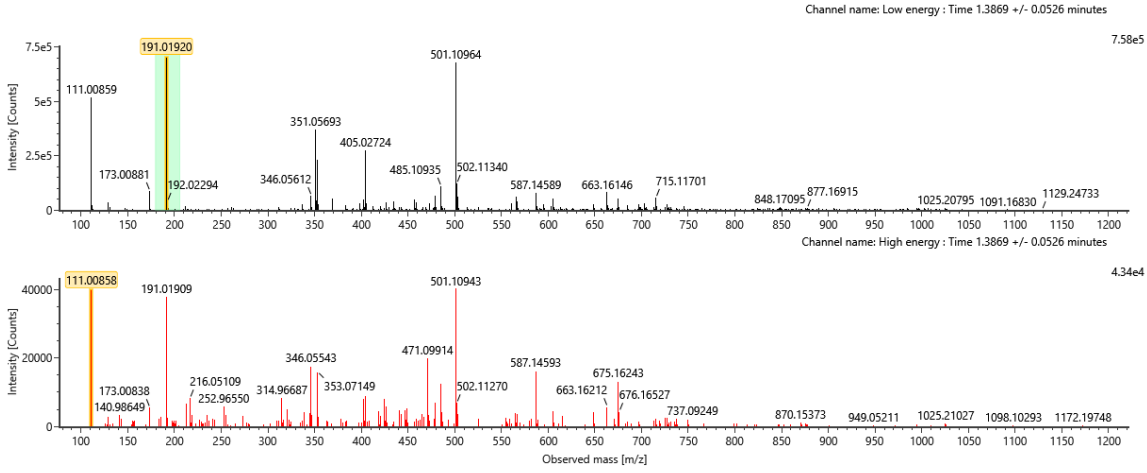

Figure 6. High-resolution mass spectra of citric acid. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 6. Espectro de masas de alta resolución de ácido cítrico. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

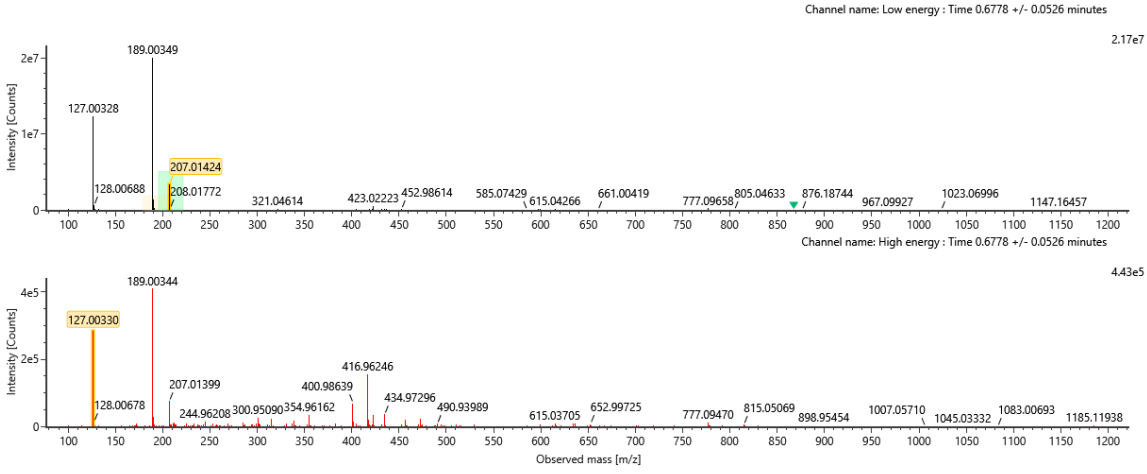

Figure 7. High-resolution mass spectra of hibiscus acid. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 7. Espectro de masas de alta resolución de ácido hibiscus. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

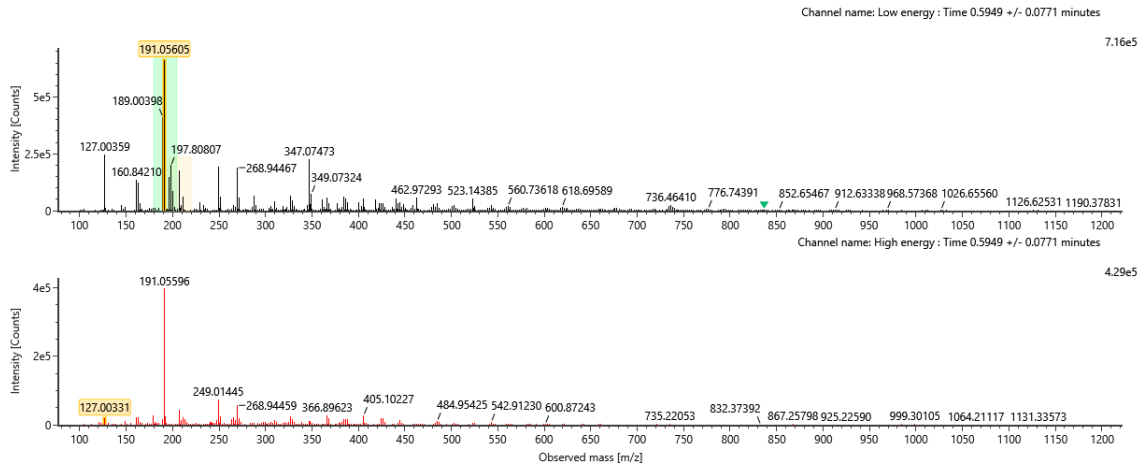

Figure 8. High-resolution mass spectra of quinic acid. Upper mass spectra acquired at 6 V (low collision energy) and lower mass spectra acquired at 15-45 V (high collision energy).
Figura 8. Espectro de masas de alta resolución de ácido quínico. El espectro de masas superior fue adquirido a 6 V (baja energía de colisión) y el espectro de masas inferior fue adquirido a 15-45 V (alta energía de colisión).

consistent with previous studies demonstrating that roselle calyx decoctions are rich in bioactive compounds, known for their numerous health benefits (Da-Costa-Rocha *et al*., 2014; Ríaz and Chopra, 2018; Bedi *et al*., 2020; Sapian *et al*., 2023).

Interestingly, organic acids were identified in both acid and alkali hydrolysable fractions, with higher amounts in the acid hydrolysis fraction. It is noteworthy that quinic acid has been reported to be obtained after the hydrolysis of caffeoylquinic acids and other phenolic acids esterified with quinic acid (Clifford *et al*., 2017). Nevertheless, hibiscus acid and quinic acid were found to be increased by 1.3-fold in the roselle decoction by-product compared to the roselle calyxes. The non-extractable fractions of the roselle decoction by-product primarily consisted of hydroxybenzoic acid derivatives, which were enriched by 1.9 to 2.2-fold after the decoction process (Table 4).

Antioxidant capacity of roselle calyx, decoction, and its by-product

The results indicate that the non-extractable fraction of the roselle decoction by-product exhibits significant antioxidant capacity when assessed using the DPPH method, showing a 1.1- to 3.4-fold increase as compared to roselle calyxes before the decoction process (Table 5). However, the antioxidant capacity, as determined by the ABTS and FRAP assays, is notably lower, with reductions of 1.6- to 9.9-fold and 7.0- to 2.4-fold, respectively. These findings suggest that the non-extractable fraction of the roselle decoction by-product contains antioxidant components, such as polyphenols and organic acids, that are effective in donating protons and scavenging the DPPH radical. On the other hand, it appears to have a limited ability to donate electrons, as indicated by the reduced capacity in scavenging the ABTS radical and reducing Fe^{3+}. This variation in antioxidant activity across different assays highlights the diverse mechanisms by which antioxidants can operate and underscores the importance of considering multiple methods for a comprehensive evaluation of antioxidant potential (Danet, 2021).

Notably, this study presents the first quantitative profile of the non-extractable fraction of roselle calyxes and their decoction by-product. Moreover, we demonstrate that hibiscus acid, the major roselle organic acid, is not only found as a free component but can also be found bound to the matrix by glycosidic linkage, as observed in the composition of the acid hydrolysable fraction. Few studies have determined non-polyphenol constituents in the non-extractable fraction. In this regard, Dong *et al*. (2021) identified eleven organic acids, along with several polyphenols, bound to carrot dietary fiber which were released by alkaline hydrolysis, contributing to its antioxidant capacity. Therefore, further studies must be undertaken to fully characterize the non-extractable fraction of plant sources and wastes.

CONCLUSIONS

This study highlights the substantial content of diverse extractable and non-extractable constituents with significant antioxidant potential in the waste generated during roselle decoction. The extraction and purification of these compounds for use as nutraceuticals, or in the creation of innovative value-added dietary supplements or functional foods, could contribute to a circular economy by reclaiming and repurposing an underappreciated waste stream with potential health benefits.

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CONFLICTS OF INTEREST

The authors declare no actual or potential conflict of interests, including financial, personal or relationship with other organizations.

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