Small molecules putative structure elucidation in endemic Colombian fruits: CFM-ID approach

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**ABSTRACT**

The main goal of any structural elucidation process is to achieve the highest level of confidence possible. This is usually attained using identical standards that can confirm the unequivocal structure of a metabolite. Nevertheless, the use of standards is not always possible given the great structural diversity among bioactive compounds. To overcome this problem, informatics tools can be used, such as the open source “CFM-ID” for structure elucidation, along with a meticulous mass fragmentation analysis as an initial step for putative identification. Herein, the “CFM-ID” tool was used on three Colombian fruits especially rich in phenolic compounds: *Bactris gasipaes*, *Borojoa patinoi*, and *Myrciaria dubia*. Thus, we obtained the characterization of new metabolites. To complete this picture, the bioactive content was measured using in-vitro assays (antioxidant, anti-inflammatory, and neuro-protective) with promising results, and was eventually related to the twenty-six new compounds reported here for the first time.

**Introduction**

Structural elucidation is a central and fundamental step in the search for bioactive molecules given the direct relationship between structure and function\textsuperscript{[1–3]} Within this process, mass spectrometry plays an essential role.\textsuperscript{[4,5]} This technique is compatible with several separation systems, such as the different types of chromatography or capillary electrophoresis.\textsuperscript{[6]} Moreover, it has higher accessibility in terms of cost and infrastructure compared to elucidation techniques such as NMR.\textsuperscript{[7]} Thus, mass spectrometry is the initial and often the only tool, especially for the identification of molecules derived from plants or food.\textsuperscript{[8,9]}

In any elucidation process, there are four types of molecules, that will depend on whether the molecule is already known in detail and whether its presence is expected\textsuperscript{[10]} associated with five levels of accuracy.\textsuperscript{[11]} According to the Metabolomics Standards Initiative (MSI),\textsuperscript{[12]} the levels are level zero, for which an unequivocal identification of the molecule has been made in three dimensions; level one, an identification has been made with a high level of confidence in two dimensions; level two, a probable structure has been encountered; level three, a possible structure is assigned; and level four, the structure is unknown. On the other hand, a high structural diversity is also expected. For example, in phenolic compounds, more than 800 structures have been reported in different plants and food matrices\textsuperscript{[13]} where several structural modifications are possible due to variables such as maturity,\textsuperscript{[14]} agricultural techniques,\textsuperscript{[15]} storage conditions,\textsuperscript{[16]} among others,\textsuperscript{[17,18]} which may produce structural changes like different hydroxylation patterns, modifications by methylation,
glycosylation, esterification, or the presence of alkyl groups, modifications that make it necessary to use identical standards to confirm the unequivocal presence of a molecule. Nevertheless, in many cases, it is not possible to acquire all the corresponding standards, either because they are not yet synthesized, they are unstable, or very expensive. Therefore, all these variables can convert the structural elucidation process into a challenging puzzle.

In this regard, several approaches can be used in order to obtain the highest confidence level, such as mass match on databases libraries, adduct formation, neutral loss analysis, isotope ratio analysis, pathway, and network analysis, along with orthogonal information such as UV/Vis data, ion mobility, hydrogen/deuterium exchange, or chemical derivatization. However, the starting point for elucidation is usually made by informatics tools. Thus, the identification process can be enhanced if several approaches are combined with informatics tools to achieve an unequivocal structure assignment. Computational tools allow to predict mass fragmentation patterns based on heuristical approaches for molecules with no MS/MS data available. Among the in-silico approaches for structure analysis based on fragmentation data, we find the CFM (competitive fragmentation modeling) analysis, based on a probabilistic model created from experimental fragmentation data. The method identifies all the possible fragments produced by the breakage of bonds in small molecules, calculates the probabilities for each one, and predicts the spectra. An online open source of this method is the Competitive Fragmentation Modeling for metabolite Identification Software which allows the accurate prediction of the ESI MS/MS spectrum for small molecules and the interpretation of the MS/MS fragmentation patterns by assigning the losses to a possible candidate molecule both in positive and negative mode. This software has proven to be useful for the identification of flavonols, flavones, and flavonoid glycosides. The information obtained is processed by comparison with previously reported fragments (in this case for phenolic compounds), allowing solid assignment identification without the need for an identical standard. Computational tools are an interesting and useful approach for the identification of plant-derived molecules given the profuse presence of bioactive molecules in plants and fruit matrices and the structural diversity that may limit the use of standards for their identification.

Within the great family of bioactive compounds, “polyphenols” are well known as antioxidant agents helpful in relieving oxidative stress, a phenomenon associated with cardiovascular diseases, diabetes, cancer, and neurodegenerative processes and lately related to the prevention and protection against COVID-19. Endemic Amazon fruits have been proven to be a rich source of phenolics as is the case of the fruits considered for this study. The first one, *Borojoa patinoi*, from the family of the *Rubiaceae*, genus *Albertia*, is grown in the Pacific region of Colombia and is highly consumed as a juice. *Borojoa patinoi* has shown moderate antioxidant, antibacterial, and antimicrobial activities. Extracts from this fruit have been studied by common extraction methods and only a few compounds have been identified. However, the present elucidation approach allowed us to identify several additional new phenolics. The second fruit, *Bactris gasipaes*, is an Areceae family member; genus *bactris* is native to the Cauca’s Valley district and grows as a large cluster composed of 50–100 fruits with a green, yellow, or red endocarp (depending on the maturation state), a starchy mesocarp, and a hard seed. The oil obtained from the seeds has shown a high antioxidant capacity, moreover the acetic extract of the flour produced from the fruit has been studied. Nevertheless, the antioxidant, anti-inflammatory, or neuroprotective activities have not yet been investigated in whole fruit extracts. Additionally, there is no study of the structural elucidation of phenolic structures in this fruit. Finally, *Myrciaria dubia* is an exotic fruit that belongs to the family *Myrtaceae*, genus *Myrciaria*, with a strong acid taste, and a berry-like shape. Despite low local production, it shows a high potential for commercialization and distribution. Several studies have shown the high antioxidant capacity of this fruit with different extraction approaches. There is no anti-inflammatory or neuroprotective activity reported.

The search for bioactive molecules is a process in which mass spectrometry plays a preponderant role. The main problem with this process is the lack of precise standards that help to confirm the mass fragment analysis. In this study, we used a diverse set of available phenolic standards and the CFM-ID
(Competitive Fragmentation Modeling for metabolite Identification) software in the elucidation analysis. This was carried out according to the pseudo-molecular ion peaks and the fragmentation patterns. Specifically, using the pseudo-molecular ion peak, several candidates were selected based on the chromatographic retention time. Finally, the assignment was made using the fragments obtained in each case, fitting the mass fragmentation pattern with the CFM tool, and comparing them with previously reported fragments. Additionally, we quantified the total phenolic content as well as the antioxidant, neuroprotective, and anti-inflammatory assessments of methanolic extracts of the aforementioned fruits.

Materials and methods

Chemical and reagents

The reagents, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), linolenic acid, lipoxidase from glycine max (soybean), xylene orange disodium salt, acetylcholinesterase (AchE), acetylthiocholine iodide (ATCI), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), 5-chlorogenic acid (5-CGA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), 5-chlorogenic acid (5-CGA), quercetin hydrate, gallic acid, apigenin, 7-hydroxicoumarin, pelargonidin and physostigmine were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The water used in the experiments (type I ultra-pure) was obtained from a smart RO Healforce water purification system (Bio-meditech Holdings Limited, Shanghai-China). The LC-MS grade solvents used were methanol (Burdik & Jackson, Muskegon, MI, USA) and Formic Acid (Sigma-Aldrich, St. Louis, MO, USA).

Fruit material

The samples of Bactris gasipaes and Borojoa patinoi were acquired in the Buenaventura’s City market located in Cauca’s Valley district of Colombia. The Myrciaria dubia sample was obtained in the Leticia’s city market in the Amazona’s district. Fifty grams of each fruit were cut into small pieces and then macerated with liquid nitrogen. The processed material was stored at −80°C until further use.

Polyphenol extracts preparation

Ten grams of the freeze-grind powder were suspended in 50 mL of MeOH-HCOOH (99:1) and shaken for 30 min in an ice bath in the dark. [36] The mixture was centrifuged for 15 min at 4800 rpm; the insoluble residue was re-extracted three times with a fresh aliquot of the same mixture. The yellow extracts were pooled and concentrated to dryness under rotary evaporation (Buchi Rotavapor V-850, New Castle, DE, USA) at 35°C. For the identification analysis the extract was reconstituted with 2 mL of MeOH and filtered through a nylon membrane of 0.22 μm (Whatman®, Sigma- Aldrich, St. Louis, MO, USA), for the in-vitro analysis the extract was reconstituted with 50 mL of MeOH and filtered through a nylon membrane of 0.45 μm (Whatman®, Sigma- Aldrich, St. Louis, MO, USA).

Total phenolics (Folin-Ciocalteu method)

The total phenolic content was quantified using the Folin-Ciocalteu assay. [37] Based on a redox reaction in which the pH is previously increased to favor the production of phenolate ions, which reduce Mo (VI) to Mo (V), readily measurable at 725 nm. Using gallic acid as reference standard, appropriate volumes were taken to obtain final concentrations over the range 120 ppm to 440 ppm. The solution standard/extract was mixed with 1 mL of Na2CO3 20% for 10 min, then 200 μL of Folin-Ciocalteu reagent were added. After 30 minutes of reaction, the absorbance was measured at 720 nm against the blank. Results were expressed as equivalent mg of gallic acid (GAEs) per 100 g of dried
weight (mg of GAEs/100 g of DW). The same procedure was performed on the extracts and on the standard solutions.

**Liquid chromatography-tandem mass spectrometry**

The HPLC-ESI-MSn analyses were carried out using a UHPLC/Focused - Dionex UltiMate 3000, equipped with a binary pump, coupled to a LCQ FleetTM Ion Trap Mass Spectrometer (all from Thermo Fisher Scientific, Walthman, MA, USA) through an ESI source. The separation was achieved on a Kinetex Luna C18 column (150 x 3.0 mm, 3 μm) (Phenomenex, Torrance, CA, USA) with a mobile phase consisting of 0.1% formic acid in water (elucent A) and methanol (elucent B) at a flow rate of 0.2 mL/min. The gradient elution went from initial 2% of B to 5% in 10 min, followed by an increase from 5% to 40% in 50 min, then to 60% in 10 min and to 100% in 10 min. This was hold for 10 more min giving a total run time of 90 min, followed by a reconditioning . The column oven and the sample tray were set at 4°C. The injection volume was 10 μL. The ion trap operated in full scan (150–1000 m/z), and ion tree mode to obtain fragment ions with a collision energy of 35% and an insolation width of 2 m/z. The data dependent scan mode was used for the acquisition of data. The positive ion mode ESI source parameters were optimized by direct infusion of 10 ppm of 5-CGA in 0.1 formic acid methanolic solution, to an ionization voltage of +6 kV, capillary temperature of 260°C, sheath gas flow rate of 10 arbitrary units and auxiliary gas flow rate of 5 arbitrary units. The Thermo Fisher Scientific Excalibur 3.0 software was used for data processing.

**In vitro activities**

**DPPH+ assay**

The DPPH antioxidant activity of the extract was determined according to a previously described method with a few modifications. Dilutions of the extract were prepared over the concentration range of 4 μg/mL to 30 μg/mL. The diluted solutions were mixed with the necessary volume of a DPPH solution 0.2 mg/mL to get the desired final volume. The mixture was shaken and allowed to react. After 45 min, the absorbance was measured in a UV spectrophotometer (Cary Varian 100) at 515 nm. Quercetin solution was used as a positive control.

**ABTS+ assay**

The antioxidant activity was also measured by the ABTS+ method with a few modifications. A 1.5 mM aqueous solution of the ABTS cationic radical was prepared from an oxidation reaction of ABTS with K2S2O8 (1:0.5). This mixture was left isolated from the light from 12 to 16 hours under an inert atmosphere. The ABTS solution was then added to different concentrations of the extract, ranging from 2 μg/mL to 12 μg/mL, and allowed to stand for 45 min at room temperature. Then, the absorbance was measured in a UV spectrophotometer (Cary Varian 100) at 745 nm. Quercetin was used as a positive control.

**Anti-inflammatory assay**

The assay was performed according to the ferrous oxidation–xylenol orange (FOX) method with some modifications. Initially, the highest possible amount of lipoperoxides is generated in such a way that can oxidize Fe^{2+} to Fe^{3+}. Then the ferric ions will form a complex with xylenol that can be quantified at 590 nm. A volume of 8 μL of lipoxygenase (20000 U/mL) solution was incubated with different extract concentrations at 5°C for 5 min. Then 40 μL of Linoleic acid (0.6 mM) in borate buffer (50 mm, pH 9.0) were added and the mixtures kept at 5°C for 30 minutes in the dark. Subsequently, 200 μL of FOX reagent, [consisting of sulfuric acid (30 mm), xylenol orange (100 mm), and iron sulfate (II) (100 mm) in methanol/water (9:1)] were added and the absorbance measured at 590 nm. Quercetin was used as positive control.
**Neuroprotective assay**

This method is based on the ability of the extract to inhibit the enzyme acetylcholinesterase, which hydrolyzes acetylcholine in the synapsis. The choline can react with DTNB (5,5'-Dithiobis (2-nitrobenzoic acid) to produce a molecule with an absorbance between 400–420 nm.\(^{[41]}\) This assay was carried out following the procedure described by Konrath\(^{[42]}\) with some modifications. AChE solution (20 µL) prepared in 50 mm Tris-HCl pH 8 with 0.1% of bovine serum albumin was mixed with 750 µL of DTNB solution prepared in the same buffer with NaCl 0.1 M. The sample was added into the AChE-DNTB mixture stirring vigorously and allowed to stand for 5 min. Then, 60 µL of ATC 1.5 mM was added and after 45 min the absorbance was measured at 415 nm. Eserine was used as positive control.

**Statistical analysis**

All the experiments were performed in triplicated, and the results are expressed as the means ± standard deviation. The experimental data were analyzed with the program RStudio. Version (2015) performing one-way analysis of variance (ANOVA) followed by a Fisher test. Only p-values lower than 0.05 were considered significant.

**Results and discussion**

**Total phenolic content and in-vitro bioassays**

The values found in the extracts for TPC were 31.58 mg GAE/100 g DW for Borojoa patinoi, 55.12 mg GAE/100 g DW for Bactris gasipaes and 137.34 mg GAE/100 g DW for Myrciaria dubia. According to previous data, similar values of GAE/100 g DW were reported for Borojoa patinoi,\(^{[24]}\) 65.7 mg GAE/100 g DW for Bactris gasipaes,\(^{[24]}\) 155.0 mg GAE/100 g DW for Myrciaria dubia.\(^{[43]}\) The slight differences in these values can be explained according to several variables, such as the stages of fruit maturation\(^{[44]}\) and the sample treatment, which in our case was carried out considering both the pulp and the skin.\(^{[45]}\) Additionally, the procedures used during the extraction, such as the acidification, the concentration of acid, the volume of solvent, the extraction times, temperatures, etc. may cause further differences.\(^{[46]}\) Moreover, Myrciaria dubia showed the highest TPC and the lowest EC50 values, revealing once again the potential of phenolic compounds as antioxidants for the anti-inflammatory and the neuroprotective assays (the last one first reported) for this fruit. In Bactris gasipaes, the TPC is significantly lower compared to Myrciaria dubia, despite its the IC50 in the neuroprotective assay being relatively analogous to Myrciaria dubia (Table 1). Therefore, it is possible that the inhibition of AChE in Bactris gasipaes, is not necessarily due to phenolic compounds, showing a promising potential of bioactive molecules in this fruit. Now, when we compare the TPC values for Bactris gasipaes and Borojoa patinoi a trend between the TCP and IC50 values is found. With a slightly higher TPC for Bactris gasipaes the lowest IC50 values are found, except for the anti-inflammatory test. Thus, the ability of Borojoa patinoi extract to inhibit lipoygenase may be due to other metabolites rather than phenolic showing again a promising potential for bioactive molecules on this fruit. Finally,

| Table 1. IC50/EC50 values and quantification of total phenolics in methanolic extracts of Borojoa patinoi, Bactris gasipaes and Myrciaria dubia. |
|---------------------------------------------------------------|
| Assay                              | Value ± SD | R²  | Value ± SD | R²  | Value ± SD | R²  |
|------------------------------------|------------|-----|------------|-----|------------|-----|
| Anti-inflammatory (IC50 mg/mL)     | 8.12 ± 0.48| 0.993| 11.60 ± 0.44| 0.994| 1.88 ± 0.08| 0.991|
| Neurprotective (IC50 50 mg/mL)    | 28.27 ± 1.10| 0.991| 2.91 ± 0.17| 0.998| 1.57 ± 0.30| 0.991|
| ABTS (EC 50 mg/mL)                | 3.05 ± 0.12| 0.992| 9.32 ± 0.31| 0.994| 1.63 ± 0.20| 0.998|
| DPPH (EC 50 mg/mL)                | 8.39 ± 0.64| 0.998| 20.32 ± 1.22| 0.998| 3.92 ± 0.80| 0.995|
| Total phenolics (mg gallic acid equivalents/100 g of dried fruit) | 31.58 ± 0.31| 0.998| 37.25 ± 0.57| 0.998| 137.34 ± 2.04| 0.998|

Values are means of triplicate analyses ± standard deviation (* P < 0.05, ** P < 0.01 and *** P < 0.001)
a correlation between all the assays with the TPC values was made, and they showed a moderate relative correlation between the antioxidant activities; ABTS and DPPH ($r = 0.61$ p < .05, and $r = 0.67$ p < .05 respectively). In the case of the neuroprotective activity, a poor correlation was found ($r = 0.57$ p < .05). For the anti-inflammatory assay, a strong correlation was found ($r = 0.91$ p < .05).[47] This can be explained due to the nature of the ABTS and the DPPH radicals since they are not found in living organisms; therefore, a greater affinity for lipoperoxides can be expected by the phenolics.[48]

**Phenolic acids and small phenolics**

We used three standards, namely Gallic Acid, Chlorogenic Acid, and 7-Hydroxy Coumarin, in order to confirm the ability of the CFM-tool to assign the mass fragments values to the assumed molecule (Table S1, Supplementary Information). A pseudo-molecular ion peak at m/z 171 was found for Gallic Acid, with a base peak ion at m/z 125 in the MS² spectrum. This fragment can be explained as the loss of formic acid (Figure 1-I).[49] For Chlorogenic Acid, a mass-to-charge ratio of 355 was found for the pseudo-molecular ion with a loss of 192 uma, corresponding to Quinic Acid (Figure 1-II),[50] giving a linear non-aromatic fragment that can rearrange to the reported structure for this loss by the CFM tool. For Hydroxycoumarin, a pseudo-molecular peak ion at m/z 163 was found and the fragmentation yielded a base peak ion at m/z 190, which is consistent with the ones previously reported.[51,52] This fragmentation can be understood as the loss of CO by a retro Diels-Alder reaction.[53,54] The CFM tool proposes a linear non-aromatic structure obtained by a hydride shift and a cycling process (Figure 1-III). As far as the fruit’s extracts are concerned, *Borjooa patinoi* analysis presents two peaks, 1 and 2 (Table S2, S.I), which have the same mass-to-charge ratio values, suggesting that these two compounds might be isomers of Ferulaldehyde (m/z 179)[55] with successive losses of H₂O (18 uma) and CO (28 uma) (Fig S2, S.I). Peak 13 has a pseudomolecular ion at m/z 123 and MS² fragments ions at m/z 81 and m/z 95 and was therefore assigned to Benzoic Acid[56] (Fig S3, S.I). Peak 44 (m/z 149), for its fragmentation pattern, was confirmed as Cinnamic Acid[57] (Fig S4, S.I). The large peak number 51, with a pseudomolecular ion at m/z 339 and successive losses of H₂O, was assigned to p-Coumaroylquinic acid.[58] The same compound was also found in *Bactris gasipaes* (Table S2, S.I), confirmed through the retention time, mass spectrum, and mass

![Figure 1](https://example.com/figure1.png) **Figure 1.** Mass fragmentation mechanism proposed by CFM-tool (PS) and reported (RP) for the phenolic standards in positive mode. I) Gallic acid II) Cholorgenic acid III) 7-hydroxy coumarin IV) Pelargonidin V) Quercetin VI) Apigenin.
fragmentation. In *Bactris gasipaes*, the three phenolic acids found correspond to 4-Aminobenzoic Acid,[48,59] Acetylsalicylic Acid,[60] and p-Comaroyl-quinic Acid[58] (Table S2, S.I). They were all identified by comparing the fragmentation patterns to the reported literature. For 4-Aminobenzoic Acid, the fragmentation produced a base peak at m/z 92, which can be explained as the loss of water followed by the loss of C₂H₄ (Fig S5, S.I). The fragmentation spectrum of Acetylsalicylic Acid shows a base peak at m/z 165 and another at m/z 136, produced by the loss of a methyl group and a carbon dioxide respectively (Fig S6, S.I). For p-Comaroyl-quinic Acid, several adducts and dimers are present: [M+ Na]⁺ at m/z 361, [2 M+ H]⁺ at m/z 678 and [2 M+ Na]⁺ at m/z 700. The MS² spectrum of this peak yielded two fragments corresponding to the consecutive loss of two H₂O molecules. The MS³ spectrum of the fragment ion at m/z 321 (produced in the MS² fragmentation) shows all the characteristics ions expected for the fragmentation of such ion (Fig S7, S.I) and was therefore useful in confirming the identification. In the base-peak chromatogram of *Myricaria dubia* (Table S2, S.I), peak 1 corresponds to a pseudo-molecular ion at m/z 175 which fragmented to m/z 157 and 143 (due to the loss of H₂O and CH₃OH, respectively) and was assigned to Shikimic Acid[61,62] (Fig S8, S.I). Peak 3 with an m/z of 193 was assigned to Quinic Acid[63,64] with losses of CO₂ and H₂O in the fragmentation spectrum (Fig S9, S.I). Peak 4 has an m/z of 355 corresponds to Chlorogenic Acid, with fragment ion at 163, which corresponds to the loss of Quinic Acid moiety (Fig S10, S.I).

**Flavonols and flavonones**

Standards of Quercetin and Apigenin were used to prove the capacity of the CFM-Tool in this class of compounds (Table S1, S.I). Quercetin showed a pseudo-molecular ion at 303 m/z with ion fragments at 229 and 257 m/z in the MS² spectrum obtaining them for the loss of a formic acid fragment (Figure 1 -V). The molecular ion mass for Apigenin was found at m/z 271 and yielded a fragment ion at m/z 153, corresponding to the 1,3 A⁺ fragment through the previously reported mechanism[2] also assigned by the CFM-Tool (Figure 1-VI). As far as the analyses of the fruits are concerned, for *Bactris gasipaes* (Table S2, S.I), peak 6 showed the molecular ion as the base peak at m/z 565, together with 587 and 1151 m/z ([M+ Na]⁺ and [2 M+ Na]⁺ respectively) and it was identified as Isorhamnetin 3-(6”-malonylglucoside)[66] for the peak at m/z 547 observed in the MS² spectrum, corresponding to loss of H₂O (Fig S11, S.I). Peak 8 had a base peak at m/z 318 and for its MS² fragments corresponding to 1,3 A⁺ and 1,4 B⁺ and 0,4 B⁺ (Fig. S12, SI) could therefore be assigned to Myricetin,[36,67] Quercetin was assigned as peak 9 with a m/z 303 and a MS² m/z 284 [M-H₂O]⁺[67,68] together with the fragments 1,3 A⁺ and 0,3 A⁺ (Fig S13, S.I). Peak 12 was assigned to Rhamnetin for the m/z at 317, with the m/z at 633 corresponding to the dimer[69] (Fig S14, S.I). In the

![Figure 2. Proposed mass pattern fragmentation for Malvidin.](image-url)
chromatogram of Myrciaria dubia (Table S2, S.I), peak 3 was assigned to Kampferide\(^{[70]}\) with the pseudo molecular ion at m/z 300 and the MS\(^2\) fragmentation ion at m/z 147, corresponding to the ion \(^{1,3}\)A\(^+\) (Fig S15, S.I). The same fragmentation pattern already encountered in Bactris gasipaes for Myricetin, with the pseudo-molecular ion at m/z 318 producing the fragment ion at m/z 300 due to [M-H\(_2\)O]\(^+\), was also found for peak 5 of Myrciaria dubia (Fig S16, S.I). Peak 6 was discovered to contained a pseudo-molecular ion at m/z 317, an adduct [M+Na]\(^+\) at m/z 339, as well as dimers [2 M+H]\(^+\) at m/z 633 and [2 M+Na]\(^+\) m/z 655. A loss of H\(_2\)O produces an ion at m/z 299 in the MS\(^2\) spectrum; therefore, the compound was assigned as Rhamnetin (Fig S14, S.I).\(^{[69]}\) Lastly, a flavonone was found in peak 7 of Myrciaria dubia, namely Naringenin, with a pseudo-molecular ion at m/z 273 and an MS\(^2\) m/z at 153 corresponding to the fragment \(^{1,3}\)A\(^+\) (Fig S17, S.I).\(^{[71]}\)

**Anthocyanin and (epi)catechins**

The CFM-tool was confirmed by using Pelargonidin as a standard. In the mass spectrum of this compound, a pseudo-molecular ion at m/z 271 (Table S1, S.I) was found with a fragment at 253 due to the loss of H\(_2\)O. The reported fragmentation mechanism\(^{[72]}\) starts with the protonation of the hydroxyl in ring C, followed by the loss of a water molecule, which causes the formation of a triple bond, then a structure with higher stability is generated by the ring rupture. Although the CFM-ID tool does not generate the structure proposed by the reported fragmentation mechanism (Figure 1-IV), an equivalent structure was obtained, in which the fragment ions are produced by a rupture in the C ring, the weakest spot of the molecule, due to the lack of aromaticity.\(^{[53]}\) In Bactris gasipaes (Table S2, S.I), peak 10 was assigned as Malvidin with a molecular ion at m/z 330 and MS2 base peak at m/z 312 (Fig S18, S.I), additionally, a fragmentation pattern was proposed in which there are two types of bond breaks in the C-ring generating several possible fragmentations for the B-ring: the \(^{0,2}\)B\(^+\) fragment and the \(^{0,1}\)B\(^+\) fragment (Figure 2).\(^{[73-75]}\) This fruit also contained (Epi)-Catechin glucoside\(^{[76]}\) and Methyl-(Epi)-Catechin\(^{[77]}\) corresponding to peaks 5 and 11. The first one was identified by the loss of sugar moiety (m/z 299) and other characteristic MS2 fragment ions at m/z 333 (loss of C\(_2\)H\(_2\)O) and at m/z 435 (loss of H\(_2\)O), from the pseudo molecular ion at m/z 453 (Fig S19, SI). The second, Methyl-(Epi)-Cate was identified using the pseudo-molecular ion at m/z 305 with MS2 fragment ions at m/z 273 (loss of CH4), 259 (loss of CH2O), 241 (successive loss of H2O and CH3OH), and 227 (successive loss of H2O and CH3H8O). Neither anthocyanins nor catechins were found in Myrciaria dubia or Borojoa patinoi.

**Proanthocyanidins**

In Bactris gasipaes (Table S2, S.I), peak 14 with m/z 695 was tentatively assigned as Protocyanindin A5 glucoside \(^{[78]}\). In the MS\(^2\) spectrum, the peak at m/z 695 can be explained as the loss of H\(_2\)O followed by the loss of C\(_2\)H\(_2\); the peak at m/z 678 is due to a loss of 1,2-ethanediol and m/z 675 produced by the loss of H\(_2\)O followed by loss of ethanol. The m/z 575 corresponds to the aglycone moiety, whereas m/z 453 is due to the loss of the Gallic Acid fragment followed by the loss of the glucoside moiety. The m/z 417 is caused by an initial loss of the glucoside moiety, followed by C ring fragmentation that results in a 1,3B+ fragment, whereas the m/z 288.84 is produced by the (Epi) catechin moiety (Fig S21, SI). Peak 16, with an m/z of 915, was assigned as Prodelphinidin B5 3,3’-digallate\(^{[79]}\); in the MS\(^2\) a fragment at m/z 657 was found, which can be explained as the loss of Shikimic Acid followed by the loss of Butadione. The fragment at m/z 633.25 is due to the loss of the Gallic Acid moiety and, successively, the loss of the ring B of one of the Gallo catechins in the structures. In addition, the fragment m/z 453 can be understood as the product of the rupture of the C-C bond between the gallo catechin moieties, whereas the fragment at m/z 355 is given by the fragmentation in the C ring (0–4) of one of the Gallo catechin moieties. Another fragment at m/z 339 is also due to the fragmentation in the C ring of the Gallo catechin moiety but with a different kind of rupture (1–4) and the fragment at m/z 319 comes from the fragmentation in (1–3) of the C ring in a Gallo catechin moiety (Fig S22, S.I).
Nitrogen compounds

In Bactris gasipaes (Table S2, S.I), two amino acids were found. Phenylalanine\(^{[80]}\) was assigned peak 3, with m/z 166 and MS2 fragments at m/z 149, 139, and 120, which can be explained as NH3, 2NH3, and CO2 losses, respectively\(^{[80]}\) (Fig. S23, S.I). Peak 4 was identified as Tryptophan, with m/z 205 and an MS\(^2\) peak at m/z 188 due to NH3\(^{[81]}\) (Fig S24, S.I). These two amino acids were also found in Myrciaria dubia (Table S2, S.I) where the same fragmentation pattern was observed for Phenylalanine (Fig S25, S.I) and for Tryptophan (Fig S26, S.I). In this extract, an alkaloid was found with a pseudo molecular ion at m/z 232 and MS\(^2\) fragment ions at m/z 214 (loss of H\(_2\)O), m/z 188 (loss of C\(_2\)H\(_4\)O), and m/z 158 (loss of C\(_4\)H\(_{10}\)O) and was assigned as Rotundine A\(^{[82]}\) (Fig S27, S.I).

Other compounds

In Myrciaria dubia (Table S2, S.I), peak 2 with a base peak ion at m/z of 219 was assigned tentatively as Tyrosol Sulfate,\(^{[83]}\) being the MS\(^2\) fragment ions found at m/z 203 (loss of H\(_2\)O), m/z 200 (loss of CH\(_4\)) and m/z 143 (loss of C\(_3\)H\(_6\)O) (Fig 28, S.I).

Conclusion

The CFM-ID fragmentation analysis tool was assessed through a wide range of phenolics and equivalent or equal structures to the previously reported mass fragmentation mechanisms were obtained, providing a further means of identification for 26 phytochemicals not previously reported on these fruits. Among these, twelve phenolic acids, nine flavonoids, two proanthocyanins, two amino acids, and one alkaloid were encountered, contributing to the chemical information on these three fruits. Furthermore, a complete study of the bioactive content of three Amazonic fruits was carried out. The Folin–Ciocalteu quantitation, the assessment of antioxidant capacities by the DPPH and ABTS methods, the neuroprotective and anti-inflammatory activities, together with their correlation values, were determined. There were positive correlations between the Total Phenolic Content and both antioxidant activity and anti-inflammatory activities. A noteworthy content of potentially interesting metabolites was found in the Myrciaria dubia extract, showing the highest values of all the assays.

Acknowledgments

This research was funded by the FAPA project of Chiara Carazzone from the Faculty of Science at University of los Andes, by the grant No. 120465741393.

Author contributions

Conceptualization, Chiara Carazzone; Methodology, Chiara Carazzone, Daniel Arias Ramirez; Investigation, Daniel Arias Ramirez; Formal analysis, Daniel Arias Ramirez; Writing – Original Draft, Daniel Arias Ramirez; Writing – Review & Editing, Daniel Arias Ramirez, Chiara Carazzone; Resources, Daniel Arias Ramirez; Funding Acquisition, Chiara Carazzone; Supervision, Chiara Carazzone. All authors read and approved the final manuscript.

Data availability statement

All datasets generated for this study are included in the article/Supplementary Material.

Disclosure statement

No potential conflict of interest was reported by the author(s).
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