

Artigo

Electrospray Ionization Mass Spectrometry Fingerprint of the *Byrsonima* Species

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Impressão Digital de Espécies de *Byrsonima* Utilizando Espectrometria de Massas com Ionização por Eletrospray

Resumo: Extratos de plantas apresentam uma grande diversidade química e, geralmente, requerem um tempo excessivo para o isolamento e caracterização de compostos. Análises de amostras complexas utilizando uma fonte de ionização por eletrospray acoplada à espectrometria de massa (ESI-MS) e a espectrometria de massa tandem (ESI-MS/MS) têm-se mostrado úteis na triagem inicial dessas amostras. Assim, é possível fazer a identificação de moléculas conhecidas sem isolamento prévio ou utilizando de outras técnicas analíticas auxiliares. Neste trabalho, os extratos secos de três espécies de *Byrsonima* foram dissolvidos em metanol, analisados através de injeção direta por ESI-MS e cada razão massa-carga (m/z) de interesse foi selecionada para análise de ESI-MS/MS. Impressão digital por ESI-MS mostrou uma distinção entre os constituintes químicos em cada uma das três espécies de *Byrsonima* analisadas e dados de ESI-MS/MS foram utilizados como ferramenta para ajudar na identificação dos principais componentes nos extratos de *B. coccolobifolia*, *B. verbascifolia* e *B. intermedia*. Mais especificamente, ácidos fenólicos, proantocianidinas e flavonoides foram detectados nas três espécies de *Byrsonima* estudadas.

Palavras-chave: Malpighiaceae; *Byrsonima*; ESI-MS; proantocianidinas; flavonoides.

Abstract

Extracts of plants presents a large chemical diversity and usually require extensive time for the isolation and characterization of the compounds. Analysis of complex samples by electrospray ionization source coupled to a mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS) has proven to be useful tools for initial screening of these samples. Therefore, it allows identification of known molecules without isolation or use of other auxiliary analytical techniques. In this work, dried extracts of three *Byrsonima* species were dissolved in methanol, analyzed through direct injection by ESI-MS and each reason mass-to-charge (m/z) of interest was selected to ESI-MS/MS analysis. ESI-MS fingerprint showed a distinction among the chemical constituents in each of the three *Byrsonima* species and ESI-MS/MS data was used as a tool to help identify the main components of *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia* extracts. More specifically, phenolic acids, proanthocyanidins and flavonoids were detected in the three *Byrsonima* species studied.

Keywords: Malpighiaceae; *Byrsonima*; ESI-MS; proanthocyanidins; flavonoids.

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Electrospray Ionization Mass Spectrometry Fingerprint of the *Byrsonima* Species

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1. Introduction

Central region of Brazil is one of the most prominent biogeographic regions in the

world, but it is also the most vulnerable.¹ Many of the 160 families of species found are used as medicine by people.² An ethnopharmacological survey carried out in the savanna regions showed a large number

of plants that are used to treat gastric disorders.³ In the North and Northeastern regions of Brazil, different species of the *Byrsonima* (Malpighiaceae) are used not only in traditional medicine, but also as food in the form of juice, jellies, or liquors.⁴ The *Byrsonima* genus, consisting of approximately 150 species, is widely distributed throughout tropical America.⁵

Byrsonima species present different ethnomedicinal properties. The methanol extract from *B. intermedia* leaves presented gastroprotective, antimicrobial and antidiarrheal activities, supporting the traditional use to treat gastrointestinal disorders.⁶ High antimutagenic effect in the hydroalcoholic leaf extracts from *B. intermedia* and *B. verbascifolia* was related, indicating these plant extracts as potential sources of chemopreventive agents. However, the hydroalcoholic extract from *B. coccolobifolia* leaves showed mutagenic activity, suggesting caution in their use as medicinal plant.⁷

Despite the medicinal use of *Byrsonima* species, there are few reports regarding their chemical constituents.⁸ Among the active principles found in medicinal plants, flavonoids, terpenes, and caffeic acid derivatives have attracted the most notable attention.⁹ Compounds of these classes have been analyzed by gas chromatography coupled to mass spectrometry, high-performance liquid chromatography, or capillary electrophoresis.⁹

Recently, electrospray ionization (ESI) has revolutionized the way that molecules are ionized and transferred to mass spectrometers for mass and structural analysis.¹⁰ Thus, a large applicability of mass spectrometry occurred because of its ability to analyze both small and large molecules of various polarities in a complex biological sample mixture.¹¹ ESI-MS and ESI-MS/MS have gained widespread recognition, mainly due to its successful use in the dereplication analysis of bio-molecules.¹²

ESI-MS has been used as a fast and efficient fingerprinting method involving

direct insertion of complex mixtures, such as those found in wines,¹³ petroleum,¹⁴ beers,¹⁵ propolis,¹⁶ and extracts containing natural compounds.¹⁷ In this study, direct injection of dried extracts of three *Byrsonima* species dissolved in methanol through ESI-MS was applied to quickly generate the fingerprint of the extracts from leaves of *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia* and ESI-MS/MS data were used as a tool to identify the main components of these extracts allowing an initial screening of them.

2. Results and discussion

2.1. ESI-MS and ESI-MS/MS analysis

Methanol extracts from *Byrsonima* species containing complex mixtures of polar compounds were investigated. Using classical phytochemical methods to isolate metabolites from plant extracts is generally time-consuming and sometimes lead to identification of known substances. This study analyzed the compounds present in the extracts from three *Byrsonima* species using ESI-MS methods, which showed to be useful to identify a large number of polar compounds.

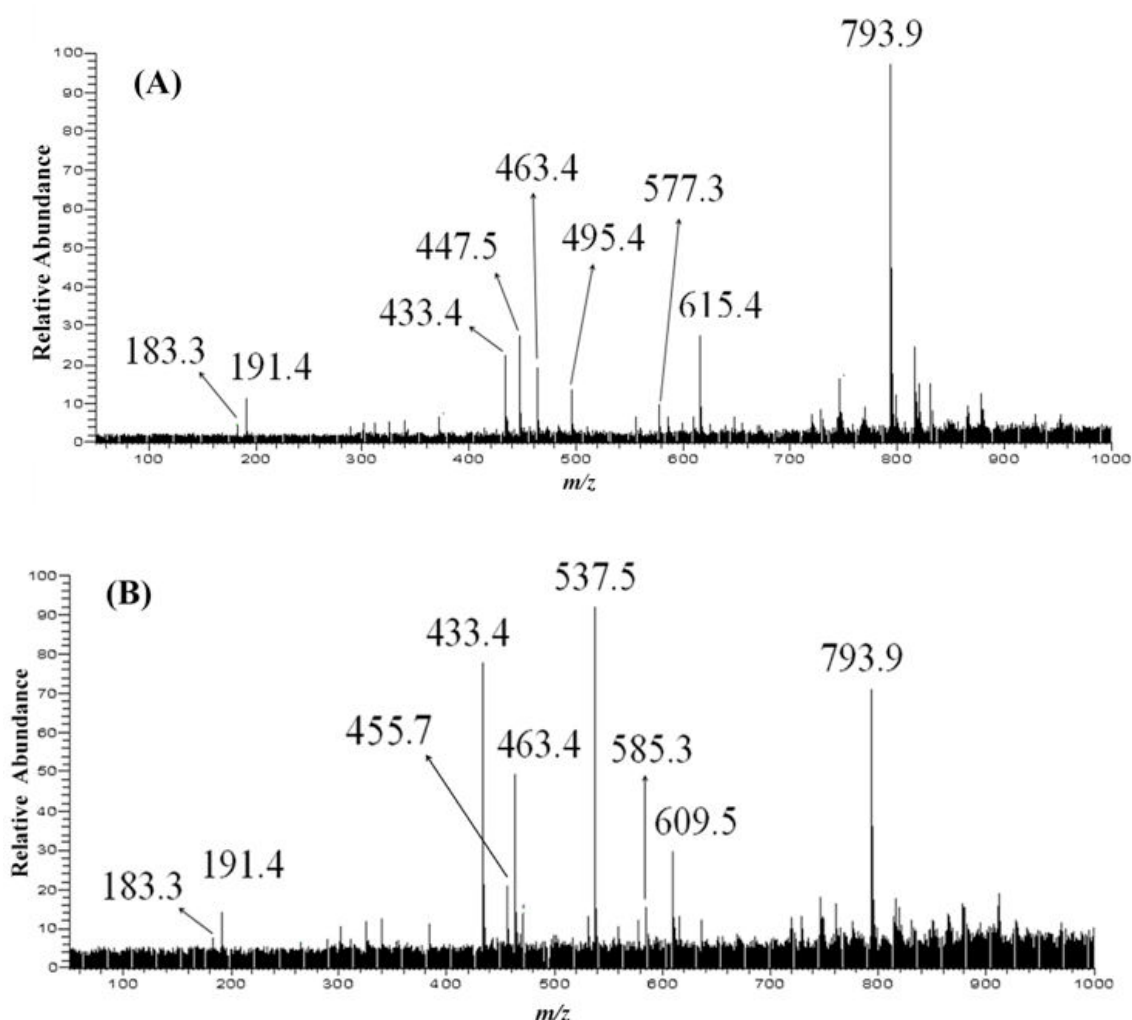
Although experiments using both negative and positive ionization were performed, the negative ion mode of ESI provided the best results and with high sensitivity to the fingerprinting of all *Byrsonima* extracts analyzed. Positive ionization yielded more complex spectra with lower sensitivity and, moreover, the signal of the $[M+H]^+$ ions was too low for MS/MS fragmentation analysis. Moreover, an exhaustive search in the literature about data on fragmentation in the positive ion mode was made and data for comparison were not found. Thus, all the identification of compounds was performed using electrospray ionization in negative ion mode (ESI(-)-MS).

Results of the ESI-MS and ESI-MS/MS suggest that the main constituents of the

methanol extracts from *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia* include phenolic acids, proanthocyanidins and flavonoids (Figure 1 and Table 1). Compounds of these classes are easily deprotonated and efficiently transferred to the gas phase as $[M-H]^-$ ions.

In the ESI(-)-MS of the *B. coccolobifolia* extract (Figure 1-A), relatively intense and characteristic peaks of the extract could be

observed within the m/z range from 433 to 793 corresponding to deprotonated molecules. Anions at m/z 433, 447, 463, 615, and 793 are most likely the $[M-H]^-$ forms of flavonoids, while anions at m/z 495 and 577 are probably the $[M-H]^-$ forms of digalloylquinic acid and proanthocyanidin dimer, respectively, as also indicated by ESI-MS/MS (Table 1) and by comparisons with reported data in literature.¹⁸⁻²⁰



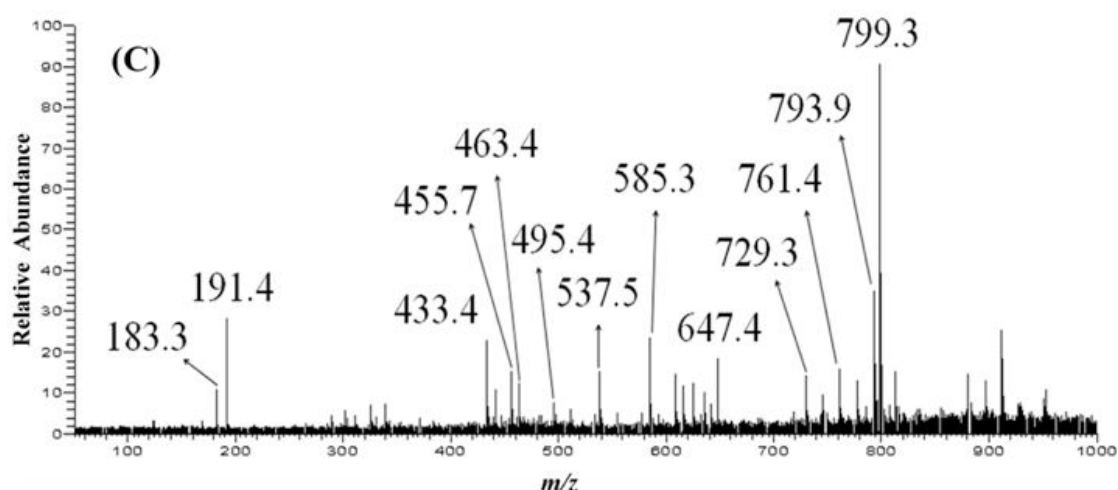


Figure 1. ESI(-)-MS fingerprint spectra representative of methanol extracts from *B. coccolobifolia* (A), *B. verbascifolia* (B) and *B. intermedia* (C)

Table 1. Summary of MS data of the compounds obtained by ESI-MS and ESI-MS/MS from the leaves of *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia*

[M-H] ⁻	MS/MS fragments	Found in*	Compound
Phenolic acids			
183	-	Bco, Bve, Bin	Methyl gallate
191	-	Bco, Bve, Bin	Quinic acid
495	477, 343, 325, 169	Bco	Digalloylquinic acid
647	495, 477, 343, 325	Bin	Trigalloylquinic acid
799	647, 629, 601, 477	Bin	Tetragalloylquinic acid
Proanthocyanidins			
577	559, 451, 425, 407, 289	Bco	Dimer
729	711, 577, 559, 451, 425, 407	Bin	Monogalloyl dimer
Flavonoids			
433	301, 300	Bco, Bve, Bin	Quercetin-O-pentose
447	301, 300	Bco	Quercetin-O-deoxyhexose
455	375, 407	Bve, Bin	Methyl (epi)catechin gallate
463	301, 300	Bco, Bve, Bin	Quercetin-O-hexose
537	375	Bve, Bin	Amentoflavone
585	433, 301	Bve, Bin	Quercetin-O-pentose-galloyl
609	463, 301	Bve	Quercetin-O-disaccharide
615	463, 301	Bco	Quercetin-O-hexose-galloyl
761	609, 463, 301	Bin	Monoacyl diglycosilated quercetin derivatives
793	537	Bco, Bve, Bin	Amentoflavone derivative

*Bco: *B. coccolobifolia*, Bve: *B. verbascifolia* and Bin: *B. intermedia*.

MS/MS fragmentation of many flavonoids showed the formation of the diagnosis ion at m/z 301 and the anion radical at m/z 300, which are characteristics of the deprotonated

quercetin aglycone. For example, the ion at m/z 615 was assigned to the deprotonated molecule of quercetin-O-hexose-galloyl, whose fragmentation led to a peak at m/z 463, $[M-152-H]^-$, and m/z 301, $[M-152-162-H]^-$, corresponding to the sequential loss of galloyl and hexose residues.²¹

MS² analysis of the deprotonated molecule at m/z 495 yields a product ion at m/z 343, $[M-152-H]^-$, due to the loss of a galloyl moiety, indicating the presence of digalloylquinic acid. The formation of a base peak at m/z 343 and the product ion at m/z 191 occur by the sequential loss of galloyl groups, $[M-152-H]^-$ and $[M-2 \times 152-H]^-$, respectively. The product ion corresponding to a peak at m/z 169 was associated with the formation of the galloyl anion.¹⁸

The base peak of proanthocyanidin dimer at m/z 577 produced fragments of m/z 451 ($[M-126-H]^-$), m/z 425 ($[M-152-H]^-$) and m/z 407 ($[M-152-18-H]^-$), which are products from a Retro-Diels-Alder (RDA) cleavage. However, as occurs in other mass spectrometry techniques, no differentiation between stereoisomers was possible and no information about the position and stereochemistry of the interflavonoid linkage was available.¹⁹

Figure 1-B shows the ESI(-)-MS representative of the *B. verbascifolia* extract. The anions of m/z 433, 463 and 793 are also present in the fingerprinting spectra of the *B. coccolobifolia* (Figure 1-A); however, another four anions are clearly present in methanol extract from *B. verbascifolia*. The $[M-H]^-$ anions at m/z 455, 537, 585 and 609 were attributed to methyl (epi)catechin gallate, amentoflavone, quercetin-O-pentose-galloyl and quercetin-O-disaccharide, respectively.^{19,22}

In the spectra of *B. coccolobifolia* and *B. verbascifolia* (Figures 1-A and 1-B), the most intense ion was identified at m/z 793. This anion had not been described in prior literature for any *Byrsonima* species, and its ESI-MS/MS analysis reveals only a product ion at m/z 537, suggesting that it could be a

derivative of the biflavonoid amentoflavone.

Figure 1-C shows the ESI(-)-MS representative of the *B. intermedia* extract. In this spectrum, relatively intense and characteristic anions can be observed in the m/z range from 183 to 799. In fact, the ions at m/z 183 and 191, corresponding respectively to methyl gallate and quinic acid, are present in the three *Byrsonima* species extracts. Furthermore, some anions present in the fingerprint spectrum of *B. intermedia* are also present in the spectra of *B. coccolobifolia* and *B. verbascifolia* (m/z 433, 455, 463, 537, 585 and 793). In addition, other anions at m/z 647, 729, 761, and 799 can easily be detected. Identification of many anions, observed in Figures 1-B and 1-C, was possible by means of a comparison with previously-reported ESI(-)-MS and ESI-MS/MS data.^{18-20,23}

Anions at m/z 647 and 799 were attributed to deprotonated molecules of galloylquinic acid derivatives. MS² fragmentation of the ion at m/z 647 (trigalloylquinic acid) yields product ions at m/z 495, $[M-152-H]^-$, due to the loss of a galloyl moiety, as well as a small quantity of the ion at m/z 477, corresponding to a loss of gallic acid, $[M-170-H]^-$. Moreover, MS/MS spectrum of the precursor ion at m/z 799 (tetragalloylquinic acid) contained product ions at m/z 647 ($[M-(1 \times 152)-H]^-$), reasonably assigned as the loss of a galloyl moiety.^{18,20} In the MS experiments, it was not possible to establish the complete characterization of these compounds, since there are numerous possibilities for the position isomers.

MS² fragmentation of the ion at m/z 761, produced another ion at m/z 609 ($[M-152-H]^-$), indicating the loss of a galloyl unit. Sequential losses of deoxyhexose and hexose yielded product ions at m/z 453 ($[M-152-146-H]^-$) and at m/z 301 ($[M-152-146-162-H]^-$). These data suggest the presence of monoacyl diglycosylated quercetin derivatives, as previously reported in *B. crassa* and *B. fagifolia*.^{5,20}

Previous chemical investigation on

Byrsonima species have resulted in the isolation of steroids, triterpenes, flavonoids, proanthocyanidins, galloylquinic acids and sulphoglycolipids.^{4,19,21-23} Other studies reported the identification of gallic acid, pyrogallol, pyrocatechin and β -amyryn isolated from the roots of *B. intermedia*.²⁵

Although chemical similarity has been observed among species of a genus, studies have shown that each plant contains characteristic substances.²⁶ For example, anions identified at m/z 537 and 585 were previously detected in the ESI(-)-MS of leaf extracts of *B. crassa* and *B. fagifolia*.^{19,20} Conversely, these ions have been identified in the *B. verbascifolia* and *B. intermedia* species. The compounds methyl (epi)catechin gallate and quercetin-O-disaccharide, detected in their deprotonated forms at m/z 455 and 609, respectively, have not been previously reported for both species.²³ In a similar way, the compound detected as an anion of m/z 793 was also first detected in the leaves of *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia*.

Thus, results obtained in this work indicate that *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia* have similar flavonoids and phenolic acids when compared to other species of *Byrsonima*. Moreover, methyl gallate, quinic acid, quercetin-O-pentose and quercetin-O-hexose (detected at m/z 183, 191, 433 and 463 in ESI(-)-MS analysis) were detected in the three *Byrsonima* species studied and could be useful as future chemical markers of these species.

3. Conclusion

ESI(-)-MS fingerprinting ensured a fast and powerful tool for the rapid identification of a complex mixture of known compounds present in the extracts from leaves of the three *Byrsonima* species. Moreover, ESI(-)-MS technique allowed for the simultaneous detection of different constituents present in extract samples and can therefore provide a reliable distinction among the *Byrsonima*

species. Finally, this approach suggests the identification of phenolic acids, proanthocyanidins and flavonoids in the leaves from *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia*.

4. Experimental

4.1. Plant material and extraction

Leaves of *B. coccolobifolia*, *B. verbascifolia* and *B. intermedia* were collected at the Guapuruvu's reserve, located in Itamarandiba city (Minas Gerais, Brazil). This legal reserve is bounded by coordinates 17° 44' 30" to 17° 43' 00" S and 42° 46' 00" to 42° 47' 30" W. Plant materials were authenticated by Dr. Carlos Victor Mendonça Filho from the Biological Sciences Department of the Universidade Federal dos Vale do Jequitinhonha e Mucuri (Minas Gerais, Brazil). A voucher of each specimen [*B. coccolobifolia* (No. 1900), *B. verbascifolia* (No. 1899) and *B. intermedia* (No. 1898)] was deposited at the *Herbarium* of the same university.

After drying at room temperature, leaves of the three *Byrsonima* species were powdered and successively extracted with hexane, ethyl acetate and methanol by maceration, followed by filtration. The extracts were prepared at room temperature and concentrated under vacuum using a rotary evaporator to afford crude extracts from leaves. To this study, dried methanol extracts from *Byrsonima* species were submitted to the ESI-MS analysis.

4.2. Electrospray ionization mass spectrometry (ESI-MS) and tandem mass spectrometry (ESI-MS/MS)

MS analysis were performed on an LCQFleet instrument (ThermoScientific, San Jose, CA, USA) equipped with an ESI source. Mass spectra were acquired and processed

using the software provided by the manufacturer (Xcalibur). Typical MS conditions were as follows: capillary voltage 11 V, spray voltage 4 to 6.5 kV, tube lens offset at 110 V, capillary temperature at 275 °C, sheath gas (N₂) flow rate set to 9 (arbitrary units).

Dried extracts from *Byrsonima* species (approximately 1 mg) were dissolved in 1 mL of methanol, and the analysis were performed by means of direct infusion into the ESI source using a syringe pump at a flow rate of 15 µL min⁻¹. The full-scan range mass spectrum was acquired in *m/z* 50-1000. For ESI-MS/MS analysis, the precursor ions were first isolated inside the ion trap and fragmented via collision-induced dissociation (CID) with gas helium. The relative collision energy was adjusted to yield product ions in quantifiable abundance.

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