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Myrica faya: A New Source of Antioxidant Phytochemicals

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ABSTRACT: *Myrica faya* is a fruit tree endemic of the Macaronesia (Azores, Madeira, and Canary Island), and its edible fruits are known as "amorinhos" (little loves), bright red to purple berries, used fresh and in jams and liquors. The phenolic composition and antioxidant capacity of leaves and berries from *M. faya* are presented here for the first time. The screening of phytochemical compounds was carried out using high-performance liquid chromatography with online UV and electrospray ionization mass spectrometric detection (HPLC-DAD-ESI-MS^n). There were 55 compounds characterized, mostly galloyl esters of flavonoids and phenolic acids; 26 of the identified compounds (anthocyanins, isoflavonoids, lignans, terpenes, fatty acids, and phenylethanoids) have not been reported in Myrica *genus* so far. From the data presented here, it can be concluded that *faya* berries represent a rich source of cyanidin-3-glucoside, flavonoids, and vitamin C. In fact, higher antioxidant activity than that of the well-known *Myrica rubra* berries (Chinese bayberry) has been observed.

KEYWORDS: *Myrica faya, phenolic compounds, HPLC-DAD-ESI/MS^n, vitamin C, antioxidant activity*

INTRODUCTION

Under oxidative stress, the human body produces more reactive oxygen species than enzymatic and nonenzymatic antioxidants. This imbalance leads to cell damage and facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, and Alzheimer's disease.[1] Fruits and vegetables provide a variety of phytochemicals, including phenolic compounds, a class of secondary metabolites, synthesized by the plants during normal development, and in response to stress conditions. Polyphenols (such as phenolic acids and flavonoids) present high antioxidant activity and, therefore, many health promoting effects (anti-inflammatory, antiallergic, antiaging, and anticarcinogenic activities), serving as a type of preventive medicine.[2,3] Hence, research on the chemical composition of already-known medicinal plants and on new plants with potential antioxidant value is currently being performed throughout the world.

Laurisilva, the Madeira (Portugal) laurel forest, is a subtropical forest with a very rich flora and is considered the most important remnants of the evergreen laurel forest from the Tertiary period. It was declared a biogenetic reserve of the European Council and world natural patrimony under the protection of UNESCO in 1999. The plants present in this forest are endemic to Macaronesia, and are protected species. They are well-studied and characterized from the botanical point of view, but their phytochemical composition remains unknown, despite the use of leaves and fruits of many species in folk medicine. Due to the absence of bibliographic data, the study of their polyphenolic composition is relevant and can provide information about new plants with important medicinal applications.

Myrica faya Aiton (syn. *Morella faya* Ait.), commonly called "fire tree", is one of the plants associated with Laurisilva. *M. faya* is a species of *Myrica*, belonging to the genus *Myrica* in the family Myricaceae, native to Macaronesia (the Azores and Madeira Archipelagos and the Canary islands). It is a common evergreen shrub or small tree that usually grows around 8 m tall. Leaves are coriaceous, oblanceolate, 4−11 cm long, 1−2.5 cm wide; they are dark green, shiny, smooth, aromatic, and alternate along the stem. Fruits are small, red to purple when ripe, and are edible. They can be directly consumed, although they have very low sugar contents and present a bitter taste.[4] Eaten raw, the berries have some astringency that limits their palatability. As a result, they are underutilized, and they are mainly used to produce jams and liquors and to add color to homemade wine. The waxy fruits were also used in the Canary Islands for skin care.[5] *M. faya* grows abundantly in Hawaii, where it was introduced by Portuguese immigrants from Madeira and Azores in the XIX century. There, the tree is considered an invasive species, since it competes vigorously with Hawaiian native trees by its nitrogen-fixing capacity in the poor volcanic soils. In the European islands it is considered a valuable species while in Hawaii all efforts are made to eradicate it since no use is found for it. Therefore, it is important to find valuable applications for *M. faya*, especially taking into account that it is a protected species in Madeira Archipelago, and new applications for this plant would result in a higher concern for its current situation.

Studies on the chemical composition and antioxidant capacity of *Myrica* species have usually focused on *Myrica rubra* due to its economic importance in Asia, mainly in China.[6−13] Its polyphenolic composition has been determined by HPLC-DAD-ESI-MS^n methods;[7,8,11,12] its radical scavenging

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capacity has been studied using different assays,[6,9] and high amounts of phenolic compounds and high antioxidant activities were observed. In addition, research on other *Myrica* species has been performed. *Myrica esculenta* (syn. *Myrica nagi*) has also been reported to be rich in antioxidant compounds and to present several medicinal applications and satisfactory antioxidant and anticancer activities.[14−16] However, no studies have been published regarding the chemical composition or antioxidant capacity of *Myrica faya*. Considering the high antioxidant activity reported in previous studies regarding other *Myrica* species, special attention should be paid to the chemical composition of *M. faya* and other underutilized plants.

In this work we present, for the first time, a report on the phytochemical content and antioxidant activity of *Myrica faya*. The methanolic extracts of its fruits and leaves were characterized by HPLC-DAD/ESI-MS^n, putting special emphasis on the phenolic composition. In addition, its antioxidant capacity was evaluated using radical scavenging methods (ABTS and DPPH) and analyzing its L-ascorbic acid (L-AA) content. The obtained results were compared to the previous ones reported for other *Myrica* species, the main goal of this work being to find out if the chemical composition of *M. faya* makes it a valuable plant from the health and economic points of view.

MATERIAL AND METHODS

Chemicals and Instruments. All reagents and standards were of analytical reagent (AR) grade. L-Ascorbic acid (L-AA) (purity: 99%), quercetin hydrated (99%), potassium iodate (99%), Folin−Ciocalteu's phenol reagent (FCR), gallic acid (99%), rutin (≥98%), and potassium acetate (>99.5%) were purchased from Panreac (Madrid, Spain). Ellagic acid (≥96%), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (>99.8%), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (≥99%), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (>95%) were obtained from Fluka (Lisbon, Portugal). Apigenin (≥99%) and (+)catechin hydrated (>99%) were purchased from Extrasynthese (Genay, France). Caffeic acid (≥98%), potassium persulfate (99%), sodium carbonate (p.a.), metaphosphoric acid (MPA) (33.5−36.5%), and formic acid (98%) were obtained from Sigma-Aldrich (St. Louis, MO); aluminum chloride hexahydrated (98%) and potassium iodine (98%) were from Riedel-de Haën (Hanover, Germany). Acetic acid (99.8%), ethylenediaminetetraacetic acid disodium salt (EDTA) (98%), and starch (98%) were supplied by Merck (Darmstadt, Germany). Cyanidin-3-glucoside (C3G) chloride (>98%) was obtained from Biopurify phytochemicals LTD (Chengdu, China). The methanol (99.9%) used for the extraction of *M. faya* was purchased from Fisher (Lisbon, Portugal). LC−MS grade acetonitrile (CH_3CN) (99%) (LabScan; Dublin, Ireland) and ultrapure water (Milli-Q Waters purification system; Millipore; Milford, MA) were used for analysis.

Sample Preparation. Samples of *Myrica faya* were collected in the wild in Machico (Madeira Island) in July 2013 and identified by taxonomist Fátima Rocha. Voucher specimens have been stored at Madeira Botanical Garden Herbarium (Funchal, Madeira) (voucher: MADJ 13165). For analysis, plant material was separated into leaves and berries (fully ripe), destemmed, and washed. Then, samples were lyophilized to dryness (Savant vapor trap RVT400; Thermo Scientific Inc.; Waltham, MA), ground to powder, and stored at −20 °C.

Extraction of Phenolic Compounds. Before the samples were subjected to evaluation, an extraction procedure was optimized. Acetone and methanol were tested as extraction solvents. Briefly, 5 g of leaf powder and 100 mL of solvent were submitted to ultrasound sonication (Bandelin Sonorex; Germany) at 35 Hz and 200 W for 60 min (room temperature). Then, extracts were filtered and concentrated to dryness under reduced pressure in a rotary evaporator (Buchi Rotavapor R-114) at 40 °C. The efficiency of the different extraction conditions was determined by means of total phenolic content assay (described below). On the basis of the results, the concentration of solvent in water (%, v/v) and influence of extraction duration were also tested (60, 30, and 15 min). Finally, the optimal conditions found were applied to the target plant material, and the resulting extracts were stored at 4 °C until further analysis.

Chromatographic Conditions. The HPLC analysis was performed on a Dionex ultimate 3000 series instrument (Thermo Scientific Inc.) coupled to a binary pump, a diode-array detector (DAD), an autosampler, and a column compartment (kept at 20 °C). Separation was achieved on a Phenomenex Gemini C_{18} column (5 μm, 250 mm × 3.0 mm i.d.) using a mobile phase composed by CH_3CN (A) and water/formic acid (0.1%, v/v) at a flow rate of 0.4 mL min^{-1}. The following gradient program was used: 20% A (0 min), 25% A (10 min), 25% A (20 min), 50% A (40 min), 100% A (42−47 min), and 20% A (49−55 min). Spectral data for all peaks were accumulated in the range 190−520 nm. A solution with concentration (w/v) of 5 mg mL^{-1} was prepared by dissolving the dried extract in the initial HPLC mobile phase, filtered through 0.45 μm PTFE membrane filters, and 10 μL was injected. The chromatographic analysis was performed in triplicate (n = 3) for each sample.

For HPLC-DAD/ESI-MS^n analysis, a Bruker Esquire model 6000 ion trap mass spectrometer (Bremen, Germany) with an ESI source was used. MS^n analysis worked in negative and positive mode, and scan range was set at m/z 100−1000 with speed of 13 000 Das^{-1}. The conditions of ESI were as follows: drying and nebulizer gas (N_2) flow rate and pressure, 10 mL min^{-1} and 50 psi; capillary temperature, 325 °C; capillary voltage, 4.5 keV; collision gas (He) pressure and energy, 1 × 10^{-5} mbar and 40 eV. The acquisition of MS^n data was made in auto MS^n mode, with isolation width of 4.0 m/z, and fragmentation amplitude of 1.0 V (MS^n up to MS^4). Esquire control software was used for the data acquisition and Data Analysis for processing.

Quantification of Phenolic Compounds. For this quantitative analysis, one polyphenol was selected as the standard for each group, and was used to calculate individual concentrations by HPLC-DAD. Caffeic and gallic acids were used for hydroxycinnamic and hydroxybenzoic acids, respectively. Anthocyanins standard was cyanidin 3-*O*-glucoside. Quercetin and apigenin were the standards used for the flavonols and flavones, respectively. (+)-Catechin hydrate and ellagic acid were used as standards for quantification of flavanols and ellagitannins. Stock standard solutions (1000 mg/L) were prepared in methanol, and calibration curves were prepared by diluting the stock solutions with the initial mobile phase. Six concentrations (5−100 mg/L) were used for the calibration, plotting peak area versus concentration, obtaining $R^2 \geq 0.967$ in all cases. Peak area was used as the analytical signal for polyphenol quantification. Total individual phenolic contents (TIPC) was defined as the sum of the quantified phenolic compounds.

Analysis of L-AA Content and Sugars. Fresh berries were homogenized in a blender, and the pH was measured directly in the pulp using a Metrohm 7444 pH-meter (calibrated with standard buffer solutions of pH 7 and pH 9, respectively). The total soluble solids (TSS) were determined using an Atago RX-1000 refractometer, and the results were reported as Brix degrees (°Brix).

L-AA determination was carried out using the procedure indicated in our previous work.[17] Briefly, 10 mL of extraction solution (30 g L^{-1} MPA−80 mL L^{-1} acetic acid−1 mmol L^{-1} EDTA) was added to 3 mL of pulp, and the mixture was centrifuged (4000 rpm; 20 min; 4 °C). The resulting extract was immediately analyzed by iodometric titration: 1 mL of 10 g L^{-1} starch solution and 1 mL of 100 g L^{-1} potassium iodide solution were added to fruit extract (diluted 1:10 with deionized water). Then, the samples were titrated with 0.002 mol L^{-1} potassium iodate solution, until the mixture became dark blue and the color persisted for more than 60 s. This procedure was repeated in triplicate.

TPC, TFC, and Antioxidant Capacities Assays. *Total Phenolic Content (TPC).* The total phenolic content was determined by the Folin−Ciocalteu method.[18] Briefly, 50 μL aliquots (5 mg mL^{-1} of dried extract dissolved in methanol) were mixed with 1.25 mL of FCR (diluted 1:10) and 1 mL of 7.5% Na_2CO_3 solution. After 30 min in darkness and room temperature, the absorbance was measured at 765

nm (n = 3) in a PerkinElmer UV−vis Lambda 2 spectrophotometer. The amounts of total phenolics were expressed as mg gallic acid equivalents (GAE)/100 g of dried extract (DE).

Total Flavonoid Content (TFC). The total flavonoid content was evaluated using the aluminum chloride colorimetric method:[18] 0.5 mL of methanolic extract (2.5 mg mL^{-1}) was mixed with 1.5 mL of methanol, 2.8 mL of distilled water, 0.1 mL of CH_3COOK (1 mol L^{-1}), and 0.1 mL of $AlCl_3{\cdot}6H_2O$. The absorbance was measured at 415 nm after 30 min of reaction. The final results were expressed as mg of rutin equivalent (RUE)/100 g DE.

ABTS Radical Scavenging Activity. The $ABTS^{\bullet+}$ assay was performed according to Gouveia et al.[18]For each analysis, 40 μL of methanolic solution was added to 1.96 mL of $ABTS^{\bullet+}$ solution (diluted in phosphate buffered saline, PBS; absorbance 0.700 ± 0.021). The reduction of absorbance at 734 nm was measured during 10 min, and the results were expressed as μmol Trolox equivalent (TE)/100 g DE.

DPPH Radical Scavenging Activity. The DPPH assay followed a method previously reported:[18] 100 μL of methanolic solution (5 mg mL^{-1}) were added to 3.5 mL of DPPH radical solution (0.06 mol L^{-1}). The decrease in absorbance at 516 nm was measured every minute during 30 min. The DPPH results were expressed as μmol Trolox equivalent (TE)/100 g DE.

Statistical Analysis. Analysis of variance (ANOVA) was used to evaluate the results obtained in L-AA, TPC, TFC, and antioxidant assays determinations (IBM SPSS Statistics 20, SPSS, Inc.).

RESULTS AND DISCUSSION

In this study, we aimed to establish, for the first time, the phenolic profile from different morphological parts of *Myrica faya*. Prior to the phenolic characterization, the influence of different experimental variables on the extraction procedure (solvent type, concentration, and duration of extraction) was investigated to increase the extraction efficiency of phenolics.

The results from the extraction experiments are shown in Figure 1.

A significant difference (p < 0.05) was found between extraction with pure methanol or pure acetone, with a higher extraction yield using pure methanol (Figure 1a). Our results contradict those of Saini et al., who reported that acetone was more efficient than methanol for the extraction of phenolics from *M. esculenta*.[16] On the basis of our data, methanol was chosen for further investigations, and results showed that an increase in the percentage of this solvent influenced positively the extraction efficiency (Figure 1b). Significant differences (p < 0.05) were observed between the different concentrations of methanol, except for 90% and 80%. Moreover, the yields of phenolic content were equal (p > 0.05) when using aqueous methanol (80%) and acetone as the extraction solvents. Taking this fact into account, pure methanol was used to evaluate the influence of extraction time, and the results indicated that increasing the extraction duration had a positive effect on the extraction efficiency (p < 0.05) (Figure 1c). Thus, an extraction time of 60 min with 100% methanol was considered as optimum.

HPLC-DAD-ESI/MS^n Screening. Figure 2 shows the chromatogram obtained during the analysis of the methanolic extracts from *Myrica faya* by HPLC-DAD-ESI/MS^n. The identification of compounds was carried out by comparison of their UV−vis spectra and mass spectrometric data obtained under negative electrospray ionization (ESI^-) conditions with the data available in scientific literature.

The method achieved a good separation, and no relevant variation was observed in the three determinations performed for each sample. In general, in the MS^1 spectrum the most

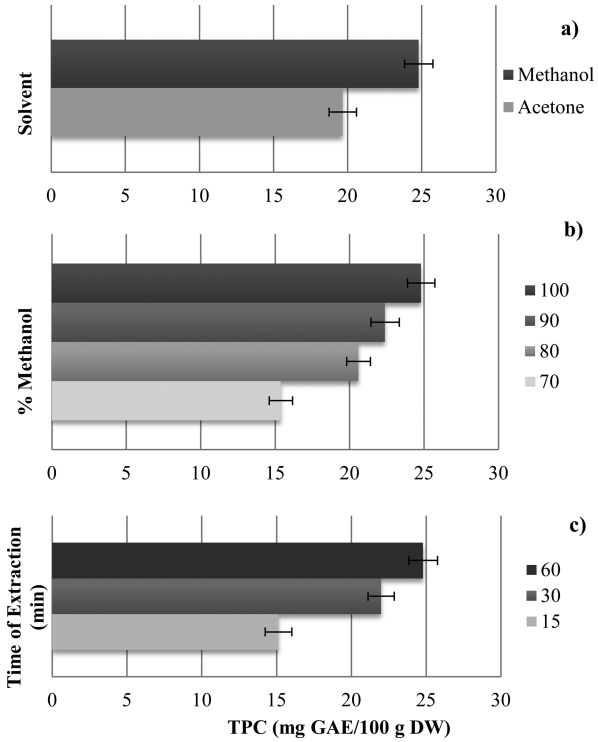

Figure 1. Extraction efficiency of different extraction conditions determined by TPC (mg/100 g DW) in *Myrica faya* leaves: (a) effect of solvent (methanol versus acetone); (b) effect of methanol concentration (v/v); (c) effect of extraction time. All extractions procedures were repeated three times (n = 3).

intense peak corresponded to the deprotonated molecular ion $[M - H]^-$. The mass spectra of the conjugated form of the phenolic compounds showed the aglycone ion as a result of the loss of moieties like hexosyl, deoxyhexosyl, pentosyl, rutinosyl, caffeoyl, and glucuronyl (−162, −146, −132, −308, −162, and −176 Da, respectively). The identification of the compounds detected in leaf and berries extracts is presented in Tables 1 and 2, respectively, and their chemical structures are shown in Figure 3.

Compounds were numbered by their elution order, since most of them were not found in both samples (leaves and berries). More than 50 different compounds were detected and classified into two main groups: flavonoids (flavan-3-ols, flavones, isoflavones, and flavonols) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids). Quinic acid and derivatives were also relevant in leaves. Additionally, mass spectra data from the positive ionization mode (ESI^+) was used for confirmation of the anthocyanidin compounds, namely cyanidin-3-glucoside and delphinidin-*O*-hexoside, in berries. A characteristic esterification with gallic acid was found in the majority of the compounds, representing the dominant group bound to polyphenols of leaves and berries.

The phenolic profiles obtained by our HPLC-UV/DAD-MS^n analysis were similar to previous reports on *Myrica*.[6−8,10−13,15] In addition, we were still able to identify for the first time in this genus 26 compounds, namely flavones, ellagitannins, lignans, terpenoids, among others. The analysis showed that leaves of *M. faya* were significantly more complex when compared to berries, most of the identified compounds exclusively being detected in the leaf extracts. Nevertheless, some compounds were only detected in berries (**2**, **4**, **6**, **9**, **13**, **16**, **19**, **24**, and **26**).

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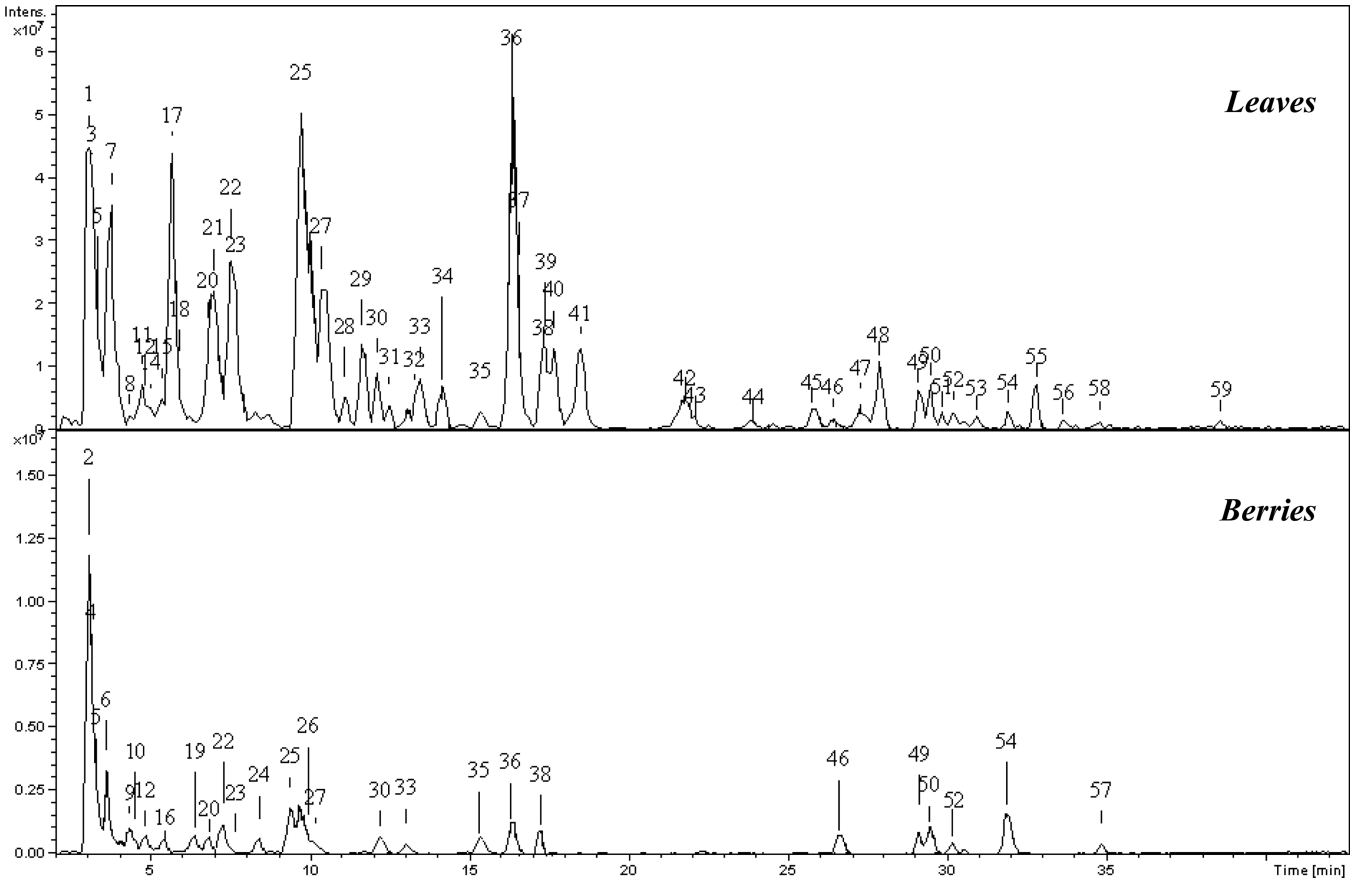

Figure 2. HPLC-DAD-ESI/MS^n base peak chromatograms (BPC) of the methanolic extracts from *Myrica faya*: leaves and berries.

Negative Mode Ionization. For the analysis of the phenolic composition of *M. faya*, both the positive and negative ionization modes were used. However, the majority of the information was obtained using the negative mode, and the positive mode was mainly used for confirmation purposes.

Identification of Phenolic Acids. Compound **4** presented $[M - H]^-$ ion at m/z 341. It suffered the neutral loss of 162 Da (hexoside), producing a fragment ion at m/z 179. This ion suffered further fragmentation, producing fragment ions at m/z 161 and 135, which are typical from caffeic acid, so the compound was identified as caffeic acid *O*-hexoside.[19]

Compound **29** exhibited a $[M - H]^-$ ion at m/z 415 and was characterized as a caffeic acid derivative. Its MS^n spectrum was identical to that described previously in *H. obconicum*[19] by our group. To our best knowledge, the presence of caffeic acid derivatives has not been reported, so far, in *Myrica*.

Compound **17** displayed a $[M - H]^-$ ion at m/z 421, which gave origin to an ion at m/z 385 (by loss of 36 Da). Further fragmentation led to sinapic acid aglycone at m/z 223 (by loss of 162 Da),[10] being characterized as sinapic acid-*O*-hexoside derivative.

Compounds **37** and **39**, showing $[M - H]^-$ ions at m/z 511 and 481, were identified, for the first time in *Myrica*, as derivatives of sinapic acid-*O*-hexoside and ferulic acid-*O*-hexoside, respectively. Both showed identical neutral losses at MS^2 (126 + 162 Da), but the presence of sinapic and ferulic acids led to different characterizations. While the 162 Da loss are attributed to hexoside units attached to the aglycones, the 126 Da loss could not be identified.

Compound **6** with $[M - H]^-$ at m/z 331 was plausibly identified as galloyl-*O*-hexoside, according to previous studies in pomegranate.[20,21]

Compound **8** exhibited $[M - H]^-$ ion at m/z 467 and fragmented into ion at m/z 169 [gallic acid $- H]^-$ due to loss of 298 Da. In the absence of more specific data, **8** was assigned as a gallic acid derivative.

Compound **18**, with $[M - H]^-$ at m/z 285, was identified as protocatechuic acid-*O*-pentoside based on bibliographic data.[22] The presence of this hydroxybenzoic acid in *Myrica* species is consistent with previous reports.[13]

Flavonoids. In this study, flavonoids (flavones, flavonols, and flavan-3-ols) were detected in their glycosylated form and/or esterified with acyl groups and were the most abundant components identified.

Compound **11** had an $[M - H]^-$ ion at m/z 761 and displayed typical product ions for galloyl-di(epi)gallocatechin at m/z 609 $[M - 152 - H]^-$, 591 $[M - 170 - H]^-$, and 423 $[M - 170 - 168 - H]^-$, which corresponded to losses of galloyl moieties (170 and 152 Da) and retro-Diels–Alder reaction product ion (168 Da), respectively. This fragmentation behavior is congruent with the previously published[11] for this compound in *Myrica rubra*.

Compound **12** was assigned as gallo(epi)catechin with characteristic $[M - H]^-$ ion at m/z 305, based on previous characterization on pomegranate.[21]

Compound **13** displayed $[M - H]^-$ ion at m/z 483 and gave origin to a product ion at m/z 447 (by loss of 36 Da). Sequential loss of a hexoside moiety produced luteolin aglycone

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Table 1. Characterization of Phenolic and Organic Compounds of the Methanolic Extracts of Leaves from *Myrica faya*

no.	t_R (min)	λ_{max}[a] (nm)	$[M - H]^-$ (m/z)	HPLC-DAD-ESI/MS^n m/z (% base peak)	assigned identity
1	3.0	234, 273	683 $[2M - H]^-$	MS^2 [683]: 341 (100), 342 (10.3)	unknown
				MS^3 [683→341]: 179 (100), 161 (24.1), 143 (17.7), 119 (15.5), 113 (18.0)	
				MS^4 [683→341→179]: 161 (29.7), 149 (22.7), 143 (87.7), 113 (48.8), 101 (30.1), 89 (100)	
3	3.1		533	MS^2 [533]: 191 (100)	quinic acid derivative
				MS^3 [533→191]: 173 (100), 127 (64.9), 109 (32.8), 99 (50.1), 93 (59.0), 85 (42.1)	
				MS^4 [533→191→173]: 109 (100)	
5	3.3		191	MS^2 [191]: 173 (58.2), 127 (100), 111 (40.5), 109 (23.8), 93 (41.7), 85 (37.1), 109 (23.8)	quinic acid
				MS^3 [191→127]: 109 (100), 99 (53.9), 85 (39.8)	
7	3.8		383 $[2M - H]^-$	MS^2 [383]: 191 (100)	quinic acid dimer
				MS^3 [383→191]: 127 (100), 85 (69.8), 93 (58.4), 109 (60.4), 111 (43.0), 173 (24.5)	
8	4.3	213, 273	467	MS^2 [467]: 436 (36.6), 391 (52.4), 301 (42.1), 275 (71.9), 169 (100)	gallic acid derivative
				MS^3 [467→169]: 125 (100), 123 (41.5)	
11	4.7	207, 276	761	MS^2 [761]: 635 (17.8), 609 (69.0), 575 (36.6), 591 (51.2), 593 (33.8), 423 (100), 305 (38.4)	galloyl(epi)gallocatechin dimer
				MS^3 [761→423]: 305 (51.2), 297 (61.7), 283 (100), 255 (77.4), 243 (36.5)	
				MS^4 [761→423→283]: 255 (33.5), 241 (100)	
12	4.8		305	MS^2 [305]: 261 (54.6), 221 (34.0), 219 (85.6), 204 (21.7), 179 (100), 166 (17.0), 139 (16.5), 137 (63.1)	gallo(epi)catechin[b]
				MS^3 [305→179]: 163 (100), 152 (45.5), 151 (77.3), 135 (32.1)	
14	5.0		935	MS^2 [935]: 917 (20.9), 659 (21.2), 633 (100), 615 (36.7), 571 (18.5), 329 (25.4), 301 (21.9), 299 (49.4)	galloyl-bis-HHDP-*O*-hexoside (Casuarinin)[b]
				MS^3 [935→633]: 615 (76.5), 571 (100), 481 (44.3), 383 (31.7), 329 (76.1), 301 (28.7), 299 (97.9), 275 (26.5)	
15	5.3		447	MS^2 [447]: 401 (100)	benzyl alcohol hexose pentose (formate adduct)[b]
				MS^3 [447→401]: 269 (100), 179 (48.2), 161 (38.9), 159 (14.7)	
				MS^4 [447→401→269]: 161 (100), 143 (32.5), 99 (17.5)	
17	5.6		421	MS^2 [421]: 386 (68.4), 385 (100), 305 (12), 205 (90.2), 153 (14.1)	sinapic acid-*O*-hexoside derivative
				MS^3 [421→385]: 326 (22.4), 265 (23.5), 224 (13.6), 223 (100), 205 (23.1)	
				MS^4 [421→385→223]: 208 (46.4), 179 (79), 164 (100)	
18	5.9	209, 277	285	MS^2 [285]: 154 (11.8), 153 (100), 152 (21.2), 109 (12.0)	protocathechuic acid-*O*-pentoside
				MS^3 [285→153]: 109 (77.0), 108 (100)	
20	6.8		457	MS^2 [457]: 331 (19.8), 319 (14.8), 305 (12.4), 193 (16.3), 169 (100)	gallo(epi)catechin-*O*-gallate
				MS^3 [457→169]: 125 (100)	
21	7.0	209, 275	915 $[2M - H]^-$	MS^2 [915]: 458 (14.6), 457 (100)	gallo(epi)catechin-*O*-gallatedimer
				MS^2 [915→457]: 331 (27.4), 305 (33.1), 193 (16.2), 169 (100)	
				MS^3 [915→457→169]: 125 (100)	
22	7.5	209, 268, 359	479	MS^2 [479]: 317 (100), 316 (92.3), 179 (16.0)	myricetin-*O*-hexoside
				MS^3 [479→317]: 287 (30.6), 271 (81.6), 193 (40.6), 179 (100)	
23	7.7		631	MS^2 [631]: 479 (39.7), 318 (12.7), 317 (100)	myricetin-*O*-(*O*-galloyl)hexoside
				MS^3 [631→317]: 179 (100), 151 (39.1)	
25	9.7	209, 262, 349	463	MS^2 [463]: 318 (10.1), 317 (100), 316 (64.5)	myricetin-*O*-deoxyhexoside
				MS^3 [463→317]: 288 (11.4), 287 (24.7), 272 (27.2), 271 (59.7), 270 (35.9), 179 (100), 151 (10.7)	
27	10.3	207, 358	615	MS^2 [615]: 302 (14.7), 301 (100), 313 (16.6), 463	galloylquercetin-*O*- hexoside[b]
				MS^3 [615→301]: 179 (100), 193 (15.5), 151 (63.4)	
				MS^4 [615→301→179]: 257 (11.5), 151 (100), 169 (64.3)	
28	11.0		593	MS^2 [593]: 285 (100), 286 (18.8)	kaempferol-*O*-rutinoside[b]
				MS^3 [593→285]: 257 (100), 241 (58.8), 229 (35.9), 197 (17.6), 169 (23.2), 163 (39.6), 93 (30.3)	
				MS^4 [593→285→257]: 255 (22.1), 151 (100)	
29	11.6		415	MS^2 [415]: 369 (65.9), 225 (30.0), 179 (100), 161 (11.4), 149 (10.7), 143 (12.2)	caffeic acid derivative (formate adduct)
				MS^3 [415→179]: 161 (100), 135 (48.4), 89 (49.6)	
30	12.1	208, 267, 343	447	MS^2 [447]: 285 (91.8), 284 (100), 255 (22.0), 256 (16.8)	kaempferol-*O*-hexoside[b]

Table 1. continued

no.	t_R (min)	λ_{max}[a] (nm)	$[M - H]^-$ (m/z)	HPLC-DAD-ESI/MS^n m/z (% base peak)	assigned identity
				MS^3 [447→285]: 255 (100), 227 (42.3), 256 (22.7), 257 (17.4), 239 (16.1), 223 (103.), 151 (10.4)	
				MS^4 [447→284→255]: 255 (16.2), 229 (100), 227 (25.7), 211 (38.7), 167 (46.0)	
31	12.5		579	MS^2 [579]: 534 (16.7), 533 (100), 372 (22.5), 371 (99.5)	phylligenin-*O*-hexoside[b] (formate adduct)
				MS^3 [579→533]: 372 (15.9), 371 (100)	
				MS^3 [579→371]: 342 (18.1), 341 (100), 340 (61.9), 297 (13.1)	
				MS^4 [579→533→371]: 357 (29.2), 356 (100), 342 (18.3), 341 (68.7), 297 (13.1)	
32	13.2	208, 265, 347	599	MS^2 [599]: 313 (100), 285 (98.5), 314 (22.6), 286 (12.3)	kaempferol-*O*-(*O*-galloyl)hexoside[b]
				MS^3 [599→313]: 169 (100), 125 (36.0), 152 (34.0), 211 (29.3)	
33	13.4		447	MS^2 [447]: 301 (100), 300 (24.9), 302 (14.8)	quercetin-*O*-deoxyhexoside
				MS^3 [447→301]: 273 (22.8), 271 (14.8), 255 (10.7), 211 (21.3), 179 (76.6), 169 (32.5), 151 (100)	
				MS^4 [447→301→179]: 169 (28.3), 151 (100)	
34	14.1		539	MS^2 [539]: 378 (13.5), 377 (64.0), 308 (29.3), 307 (100), 275 (71.0)	oleuropein[b]
				MS^3 [539→307]: 276 (13.2), 275 (100), 223 (56.8), 149 (39.8), 139 (13.8)	
35	15.3		633	MS^2 [633]: 488 (16.5), 487 (12.2), 470 (18.7), 469 (100), 347 (50.7)	benzoyl-*p*-dicoumaryl- 2,7-anhydro-3-deoxy-2-octulopyranosonic acid[b]
				MS^3 [633→469]: 347 (76.0), 323 (56.3), 303 (20.4), 259 (33.1), 235 (15.7), 163 (100), 145 (50.9)	
				MS^4 [633→469→163]: 119 (100)	
36	16.3	211, 267, 345	615	MS^2 [615]: 318 (16.8), 317 (100), 463 (41.8)	myricetin-*O*-(*O*-galloyl)deoxyhexoside
				MS^3 [615→317]: 227 (11.6), 193 (16.2), 191 (12.6), 180 (12.4), 179 (100), 151 (33.3), 137 (16.7)	
				MS^4 [615→317→179]: 151 (100)	
37	16.5		511	MS^2 [511]: 385 (31.4), 287 (29.3), 269 (12.8), 224 (13.2), 223 (100), 163 (13.5)	sinapic acid-*O*-hexoside derivative[b]
				MS^3 [511→223]: 208 (69.4), 179 (100), 178 (43.3), 209 (50.0), 164 (32.3)	
				MS^4 [511→223→179]: 164 (100)	
38	17.3	211, 267, 345	615	MS^2 [615]: 463 (31.2) 318 (13.2), 317 (100)	myricetin-*O*-(*O*-galloyl)deoxyhexoside
				MS^3 [615→317]: 271 (10.7), 255 (10.9), 193 (16.3), 192 (15.5), 179 (100), 151 (60.7), 137 (25.0)	
				MS^4 [615→317→179]: 169 (22.6), 151 (100)	
39	17.4	209, 329	481	MS^2 [481]: 463 (15.2), 355 (15.5), 287 (24.2), 193 (100), 161 (13.5)	ferulic acid-*O*-hexoside derivative[b]
				MS^3 [481→193]: 178 (93.3), 149 (100), 134 (44.8)	
40	17.6		431	MS^2 [431]: 286 (16.7), 285 (100), 284 (28.4), 255 (10.5)	kaempferol-*O*-rhamnoside[b]
				MS^3 [431→285]: 257 (61.7), 255 (100), 239 (32.5), 229 (52.5), 197 (30.7), 163 (19.4)	
				MS^4 [431→285→255]: 229 (46.7), 213 (20.3), 189 (13.3), 185 (10.5), 151 (100), 93 (38.7)	
41	18.5		549	MS^2 [549]: 505 (23.7), 504 (24.9), 503 (100), 324 (15.4), 323 (89.2)	unknown
				MS^3 [549→503]: 413 (81.0), 324 (10.9) 323 (100), 161 (33.0)	
				MS^4 [549→503→323]: 308 (34.6), 295 (48.8), 292 (38.0), 279 (47.2), 265 (48.5), 263 (71.8), 247 (68.9), 235 (47.8), 233 (100)	
42	21.7		489	MS^2 [489]: 454 (32.3), 447 (36.5), 445 (39.0), 403 (38.6), 301 (100), 300 (25.8)	quercetin-*O*-acetylrhamnoside
				MS^3 [489→301]: 151 (100), 243 (63.0), 271 (84.2)	
43	22.0		565	MS^2 [565]: 358 (22.3), 357 (100)	dichotomitin-*O*-hexoside[b]
				MS^3 [565→357]: 343 (20.4), 342 (100), 327 (41.9), 299 (21.6)	
				MS^3 [565→357]: 343(16.6), 342 (60.5), 327 (100)	
				MS^4 [565→357→342]: 328 (22.3), 327 (100), 299 (34.5)	
				MS^4 [565→357→327]: 299 (100), 268 (87.9), 228 (48.1), 227 (87.1)	
44	23.8		599	MS^2 [599]: 553 (28.3), 485 (25.8), 447 (45.6), 301 (100), 299 (11.1), 297 (18.7)	quercetin-*O*-(*O*-galloyl)deoxyhexoside
				MS^3 [599→301]: 273 (15.5), 187 (12.9), 179 (57.7), 151 (100)	
				MS^4 [599→301→179]: 151 (100)	
45	25.7		507	MS^2 [507]: 461 (100), 443 (38.5), 294 (10.5), 293 (64.0)	lactiflorin[b] (formate adduct)
				MS^3 [507→461]: 443 (29.5), 293 (100), 149 (58.9), 131 (23.9)	
				MS^4 [507→461→293]: 149 (100), 122 (47.9)	

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Table 1. continued

no.	t_R (min)	λ_{max}[a] (nm)	$[M - H]^-$ (m/z)	HPLC-DAD-ESI/MS^n m/z (% base peak)	assigned identity
46	26.6		491	MS^2 [491]: 371 (12.9), 330 (28.5), 330 (20.3), 329 (100), 314 (10.7)	tricin-*O*-hexoside[b]
				MS^3 [491→329]: 315 (53.0), 314 (100)	
				MS^3 [491→329→314]: 300 (40.3), 299 (100)	
47	27.3		491	MS^2 [491]: 330 (26.9), 329 (100)	tricin-*O*-hexoside[b]
				MS^3 [491→329]: 315 (14.3), 314 (100), 136 (38.6), 135 (40.1)	
				MS^4 [491→329→314]: 300 (43.1), 299 (100)	
48	27.8		555	MS^2 [555]: 417 (21.7), 305 (32.9), 287 (27.1), 269 (100), 267 (22.4), 223 (54.3), 161 (22.2)	baicalein derivative[b]
				MS^3 [555→269]: 251 (20.5), 241 (38.8), 227 (45.9), 226 (51.3), 225 (32.3), 223 (100), 197 (48.9), 195 (33.5), 179 (17.1)	
				MS^4 [555→269→223]: 197 (100)	
49	29.1		491	MS^2 [491]: 473 (20.8), 330 (15.2), 329 (100)	tricin-*O*-hexoside[b]
				MS^3 [491→329]: 314 (100), 299 (70.5), 271 (52.1), 193 (48.3), 181 (62.0), 135 (51.6)	
50	29.4		563	MS^2 [563]: 356 (11.0), 355 (100)	conidendrin-*O*-hexoside[b]
				MS^3 [563→355]: 341 (12.8), 340 (100), 325 (43.4)	
				MS^4 [563→355→340]: 326 (15.2), 325 (100), 296 (79.8), 281 (36.7), 212 (27.6)	
				MS^4 [563→355→325]: 296 (100), 281 (79.8), 212 (27.6)	
51	29.8		583	MS^2 [583]: 286 (17.7), 285 (100)	kaempferol derivative
				MS^3 [583→285]: 267 (55.6), 257 (100), 241 (43.8), 151 (93.5), 169 (48.8)	
52	30.4		535	MS^2 [535]: 490 (31.6), 489 (100)	5,7-dihydroxy-6,8-dimethoxyflavone-7-*O*-glucuronide[b]
				MS^3 [535→489]: 327 (46.0), 313 (100), 298 (55.5), 283 (33.6)	
				MS^4 [535→489→313]: 298 (100), 283 (22.7), 269 (32.4), 254 (29.4)	
53	30.9		677	MS^2 [677]: 593 (75.0), 575 (61.3), 285 (100), 284 (99.2), 268 (17.7), 255 (22.3), 229 (19.3)	kaempferol-*O*-rutinoside
				MS^3 [677→285]: 283 (51.6), 257 (100), 255 (65.6), 241 (74.3), 229 (38.5), 197 (49.1)	derivative[b]
54	31.9		779	MS^2 [779]: 634 (26.5), 633 (100), 616 (26.2), 615 (74.3), 469 (38.7)	benzoyl-*p*-tricoumaryl- 2,7-anhydro-3-deoxy-2-octulopyranosonic acid[b]
				MS^3 [779→633]: 488 (18.7), 487 (17.9), 470 (35.0), 469 (100), 325 (17.3), 265 (11.1)	
				MS^4 [779→633→469]: 307 (88.9), 163 (100), 145 (49.5)	
55	32.8		695	MS^2 [695]: 488 (25.6), 487 (100)	unknown
				MS^3 [695→487]: 421 (31.1), 410 (48.3), 409 (100), 401 (35.3), 391 (59.4), 390 (16.2)	
				MS^4 [695→487→409]: 392 (15.7), 391 (35.2), 380 (34.7), 379 (100), 377 (25.2), 359 (24.2)	
56	33.6		673	MS^2 [673]: 638 (23.4), 637 (100), 655 (22.6), 619 (15.2), 611 (15.7), 595 (16.7)	unknown
				MS^3 [673→637]: 609 (54.4), 401 (26.0), 365 (37.7), 332 (28.9), 209 (100), 203 (64.2)	
58	34.8		515	MS^2 [515]: 454 (55.9), 269 (100), 243 (18.2), 241 (28.1), 227 (71.6), 183 (40.5)	unknown
				MS^3 [515→269]: 228 (100), 213 (80.2), 149 (14.8)	
59	38.5		515	MS^2 [515]: 285 (43.9), 284 (100), 255 (23.0)	kaempferol derivative
				MS^3 [515→284]: 257 (19.2), 256 (25.0), 255 (100), 242 (34.0), 195 (21.1)	

[a]Wavelengths not provided when the UV spectrum was not properly observed due to low intensity. [b]Compound identified for the first time in *Myrica* genus.

(m/z 285). Thus, **13** was identified as a luteolin-*O*-hexoside derivative, reported for the first time in *Myrica*.

(epi)Catechin monomer and (epi)catechin-*O*-gallate (compounds **19** and **26**) displayed $[M - H]^-$ ions at m/z 289 and 441, respectively, and were assigned according to previous characterizations[23] in grape pomace. Catechin has been previously detected in *M. esculenta* by an HPLC-PDA method.[15]

Compound **20** exhibited a $[M - H]^-$ ion at m/z 457 and was assigned as gallocatechin-*O*-gallate, on the basis of previous studies in *M. rubra*.[11] Although compound **20** was detected before in leaves of bayberry, we report here for the first time its presence in fruits of this genus. With $[M - H]^-$ ion at m/z 915 and MS^2 product ion at m/z 457, compound **21** was plausibly identified as a dimer of gallo(epi)catechin-*O*-gallate.

Conjugates of myricetin (compounds **22**, **23**, **25**, **36**, and **38**), quercetin (compounds **33**, **42**, and **44**), and kaempferol (compounds **28**, **30**, **32**, and **40**) were characterized according to the sugar moieties attached to their aglycones (at m/z 317, 301, and 285, respectively). The characterizations of these compounds in *Myrica faya* were supported by previous reports in *M. rubra*[6−8,11,13] and pomegranate.[20] Myricetin derivatives were reported as the major flavonoids in extracts of *M. rubra*'s leaves and berries. Besides kaempferol-*O*-hexoside (**30**), all

Table 2. Characterization of Phenolic and Organic Compounds of the Methanolic Extracts of Berries from *Myrica faya*

no.	t_R (min)	λ_{max}[a] (nm)	$[M - H]^-$ (m/z)	HPLC-DAD-ESI/MS^n m/z (% base peak)	assigned identity
2	3.0	219, 280, 516	449 (+)	MS^2 [449]: 288 (14.1), 287 (100)	cyanidin-3-glucoside
				MS^3 [449→287]: 241 (87.2), 213 (100), 193 (58.2), 185 (47.6), 175 (57.5), 169 (50.1) 165 (24.1), 161 (31.3), 137 (69.0)	
				MS^4 [449→287→213]: 167 (100)	
4	3.2	234, 274	341	MS^2 [341]: 179 (100), 135 (22.7)	caffeic acid-*O*-hexoside[b]
				MS^3 [341→179]: 161 (11.7), 135 (100)	
5	3.3		191	MS^2 [191]: 173 (58.2), 127 (100), 111 (40.5), 109 (23.8), 93 (41.7), 85 (37.1), 109 (23.8)	quinic acid
				MS^3 [191→127]: 109 (100), 99 (53.9), 85 (39.8)	
6	3.4		331	MS^2 [331]: 271 (30.5), 169 (100), 125 (29.7)	galloyl-*O*-hexoside[b]
				MS^3 [331→169]: 125 (100)	
9	4.3	280, 519	465 (+)	MS^2 [465]: 304 (17.7), 303 (100)	delphinidin-*O*-hexoside[b]
				MS^3 [465→303]: 258 (22.3), 257 (100), 247 (11.8), 229 (80.5), 163 (10.3), 135 (31.4)	
				MS^4 [465→303→257]: 229 (56.4), 213 (100), 173 (63.4)	
10	4.5		405	MS^2 [405]: 388 (11.6), 387 (16.7), 191 (100)	quinic acid derivative
				MS^3 [405→191]: 173 (23.3), 127 (100), 111 (14.0), 93 (10.2)	
13	4.8		483	MS^2 [483]: 448 (20.6), 447 (100)	luteolin-*O*-hexoside derivative[b]
				MS^3 [483→447]: 286 (17.8), 285 (100), 284 (20.5)	
				MS^4 [483→447→285]: 243 (100), 241 (55.0), 217 (29.2), 199 (16.8), 167 (41.9), 125 (37.7)	
16	5.4		431	MS^2 [431]: 386 (41.1), 385 (100), 384 (22.1), 175 (17.4)	roseoside[b] (formate adduct)
				MS^3 [431→385]: 223 (100), 153 (30.1), 138 (11.4)	
				MS^4 [431→385→223]: 206 (47.0), 153 (100), 147 (39.9)	
19	6.4		289	MS^2 [289]: 247 (19.3), 245 (100), 205 (48.0), 203 (16.2), 179 (14.6), 161 (15.7)	(epi)catechin[b]
				MS^3 [289→245]: 205 (40.1), 204 (85.6), 203 (100), 202 (47.2), 187 (53.4), 162 (17.2), 121 (56.3)	
20	6.8		457	MS^2 [457]: 331 (19.8), 319 (14.8), 305 (12.4), 193 (16.3), 169 (100)	gallo(epi)catechin-*O*-gallate[b]
				MS^3 [457→169]: 125 (100)	
22	7.5	209, 268, 359	479	MS^2 [479]: 317 (100), 316 (92.3), 179 (16.0)	myricetin-*O*-hexoside[b]
				MS^3 [479→317]: 287 (30.6), 271 (81.6), 193 (40.6), 179 (100)	
				MS^4 [479→317→179]: 169 (100)	
23	7.7		631	MS^2 [631]: 479 (39.7), 318 (12.7), 317 (100)	myricetin-*O*-(*O*-galloyl)hexoside
				MS^3 [631→317]: 179 (100), 151 (39.1)	
				MS^4 [631→317→179]: 169 (28.6), 151 (100)	
24	8.4		597	MS^2 [597]: 477 (45.0), 459 (15.3), 417 (25.2), 388 (17.3), 387 (53.6), 358 (20.0), 357 (100)	glucaric acid derivative[b]
				MS^3 [597→357]: 300 (15.3), 209 (100), 123 (12.1),121 (25.4)	
				MS^4 [597→357→209]: 191 (70.5), 165 (48.0), 147 (100)	
26	10.2	207, 265, 352	441	MS^2 [441]: 289 (100), 290 (21.5), 169 (17.5), 331 (11.6)	(epi)catechin-*O*-gallate[b]
				MS^2 [441→289]: 245 (100), 179 (28.3), 161 (27.4), 135 (17.6), 85 (13.0)	
				MS^3 [441→289→245]: 203 (100), 202 (39.7), 204 (65.1), 205 (43.4), 188 (53.4)	
27	10.3	207, 358	615	MS^2 [615]: 302 (14.7), 301 (100), 313 (16.6), 463	galloylquercetin-*O*- hexoside[b]
				MS^3 [615→301]: 179 (100), 193 (15.5), 151 (63.4)	
				MS^4 [615→301→179]: 257 (11.5), 151 (100), 169 (64.3)	
30	12.1	208, 267, 343	447	MS^2 [447]: 285 (91.8), 284 (100), 255 (22.0), 256 (16.8)	kaempferol-*O*-hexoside
				MS^3 [447→285]: 255 (100), 227 (42.3), 256 (22.7), 257 (17.4), 239 (16.1), 223 (103.), 151 (10.4)	
				MS^4 [447→284→255]: 255 (16.2), 229 (100), 227 (25.7), 211 (38.7), 167 (46.0)	
33	13.4		447	MS^2 [447]: 301 (100), 300 (24.9), 302 (14.8)	quercetin-*O*-deoxyhexoside
				MS^3 [447→301]: 273 (22.8), 271 (14.8), 255 (10.7), 211 (21.3), 179 (76.6), 169 (32.5), 151 (100)	
				MS^4 [447→301→179]: 169 (28.3), 151 (100),	
35	15.3		633	MS^2 [633]: 488 (16.5), 487 (12.2), 470 (18.7), 469 (100), 347 (50.7)	benzoyl-*p*-dicoumaryl- 2,7-anhydro-3-deoxy-2-octulopyranosonic acid[b]
				MS^3 [633→469]: 347 (76.0), 323 (56.3), 303 (20.4), 259 (33.1), 235 (15.7), 163 (100), 145 (50.9)	

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Table 2. continued

no.	t_R (min)	λ_{max}[a] (nm)	$[M - H]^-$ (m/z)	HPLC-DAD-ESI/MS^n m/z (% base peak)	assigned identity
				MS^4 [633→469→163]: 119 (100)	
36	16.3	211, 267, 345	615	MS^2 [615]: 318 (16.8), 317 (100), 463 (41.8)	myricetin-*O*-(*O*-galloyl)deoxyhexoside
				MS^3 [615→317]: 227 (11.6), 193 (16.2), 191 (12.6), 180 (12.4), 179 (100), 151 (33.3), 137 (16.7)	
				MS^4 [615→317→179]: 151 (100)	
38	17.3	211, 267, 345	615	MS^2 [615]: 463 (31.2) 318 (13.2), 317 (100)	myricetin-*O*-(*O*-galloyl)deoxyhexoside
				MS^3 [615→317]: 271 (10.7), 255 (10.9), 193 (16.3), 192 (15.5), 179 (100), 151 (60.7), 137 (25.0)	
				MS^4 [615→317→179]: 169 (22.6), 151 (100)	
40	17.6		431	MS^2 [431]: 286 (16.7), 285 (100), 284 (28.4), 255 (10.5)	kaempferol-*O*-rhamnoside[b]
				MS^3 [431→285]: 257 (61.7), 255 (100), 239 (32.5), 229 (52.5), 197 (30.7), 163 (19.4)	
				MS^4 [431→285→255]: 229 (46.7), 213 (20.3), 189 (13.3), 185 (10.5), 151 (100), 93 (38.7)	
46	26.6		491	MS^2 [491]: 371 (12.9), 330 (28.5), 330 (20.3), 329 (100), 314 (10.7)	tricin-*O*-hexoside[b]
				MS^3 [491→329]: 315 (53.0), 314 (100)	
				MS^3 [491→329→314]: 300 (40.3), 299 (100)	
49	29.1		491	MS^2 [491]: 473 (20.8), 330 (15.2), 329 (100)	tricin-*O*-hexoside[b]
				MS^3 [491→329]: 314 (100), 299 (70.5), 271 (52.1), 193 (48.3), 181 (62.0), 135 (51.6)	
50	29.4		563	MS^2 [563]: 356 (11.0), 355 (100)	conidendrin-*O*-hexoside[b]
				MS^3 [563→355]: 341 (12.8), 340 (100), 325 (43.4)	
				MS^4 [563→355→340]: 326 (15.2), 325 (100), 296 (79.8), 281 (36.7) , 212 (27.6)	
				MS^4 [563→355→325]: 296 (100), 281 (79.8), 212 (27.6)	
52	30.4		535	MS^2 [535]: 490 (31.6), 489 (100)	5,7-dihydroxy-6,8-dimethoxyflavone-7-*O*-glucuronide[b] (formate adduct)
				MS^3 [535→489]: 327 (46.0), 313 (100), 298 (55.5), 283 (33.6)	
				MS^4 [535→489→313]: 298 (100), 283 (22.7), 269 (32.4), 254 (29.4)	
54	31.9		779	MS^2 [779]: 634 (26.5), 633 (100), 616 (26.2), 615 (74.3), 469 (38.7)	benzoyl-*p*-tricoumaryl- 2,7-anhydro-3-deoxy-2-octulopyranosonic acid[b]
				MS^3 [779→633]: 488 (18.7), 487 (17.9), 470 (35.0), 469 (100), 325 (17.3), 265 (11.1)	
				MS^4 [779→633→469]: 307 (88.9), 163 (100), 145 (49.5)	
57	34.7		327	MS^2 [327]: 311 (27.9), 294 (13.1), 293 (18.7), 229 (100), 211 (72.1), 183 (14.5), 171 (12.4)	oxo-dihydroxy-octadecenoic acid[b]
				MS^3 [327→229]: 211 (100), 209 (32.3), 165 (16.9), 127 (32.2), 125 (67.3)	

[a]Wavelengths not provided when the UV spectrum was not properly observed due to low intensity. [b]Compound identified for the first time in *Myrica* genus.

other kaempferol conjugates have never been characterized in *Myrica* species.

Compound **27**, with $[M - H]^-$ at m/z 615, was plausibly identified as galloylquercetin-*O*-hexoside after sequential loss of 152 and 162 Da.[24] This compound has been previously characterized in tropical fruits, but not in *Myrica*.

Compound **51** exhibited $[M - H]^-$ at m/z 583, and its MS^2 fragmentation revealed kaempferol aglycone at m/z 285 (by loss of 298 Da). However, complete identification of **51** was not achieved, being characterized as a kaempferol derivative.

Compound **53** displayed $[M - H]^-$ at m/z 677 and MS^2 base peak at m/z 285, due to the loss of 392 Da. Product ions at m/z 593 and 575 corroborated kaempferol-*O*-rutinoside and dehydrated kaempferol-*O*-rutinoside, with **53** being identified as a kaempferol-*O*-rutinoside derivative.

Compound **43** exhibited a $[M - H]^-$ ion at m/z 565 and a direct loss of 208 Da (46 + 162 Da) that suggested a formate adduct plus hexoside moiety. Further fragmentation showed identical behavior as dichtomitin, an isoflavone, with product ions at m/z 342 $[M - H - CH_3]^-$, 327 $[M - H - CH_3 \times 2]^-$, and 299 $[M - H - CH_3 \times 2 - CO]^-$.[25] Thus, **43** was plausibly classified as dichtomitin-*O*-hexoside, for the first time in *Myrica*.

Compounds **46** and **52** exhibited $[M - H]^-$ ions at m/z 491 and 535 and showed neutral losses of 162 and 208 Da, respectively. The sequential fragmentation allowed for the identification of two losses of 15 Da each in both compounds, due to two methoxyl groups. Their fragmentation behaviors were consistent with those described before in herbs[18] for tricin-*O*-hexoside (dihydroxy-dimethoxy-*O*-hexoside flavones) (**46**) and 5,7-dihydroxy-6,8-dimethoxy-7-*O*-glucuronide flavone (**52**).[26] With longer retention times, compounds **47** (t_R = 27.3 min) and **49** (t_R = 29.1 min) exhibited similar fragmentation pattern as **46**, being also assigned as tricin-*O*-hexoside. On the basis of only MS^n data, the stereochemical structures of the sugar moieties could not be elucidated.

Compound **48** exhibited $[M - H]^-$ ion at m/z 555 and product ion at m/z 269, due to loss of 286 Da. Further fragmentation produced characteristic ions of baicalein: at m/z 251 $[M - H_2O - H]^-$, 241 $[M - CO - H]^-$, and 223 $[M - H_2O - CO - H]$, according to Han et al.[27] Thus, **48** was

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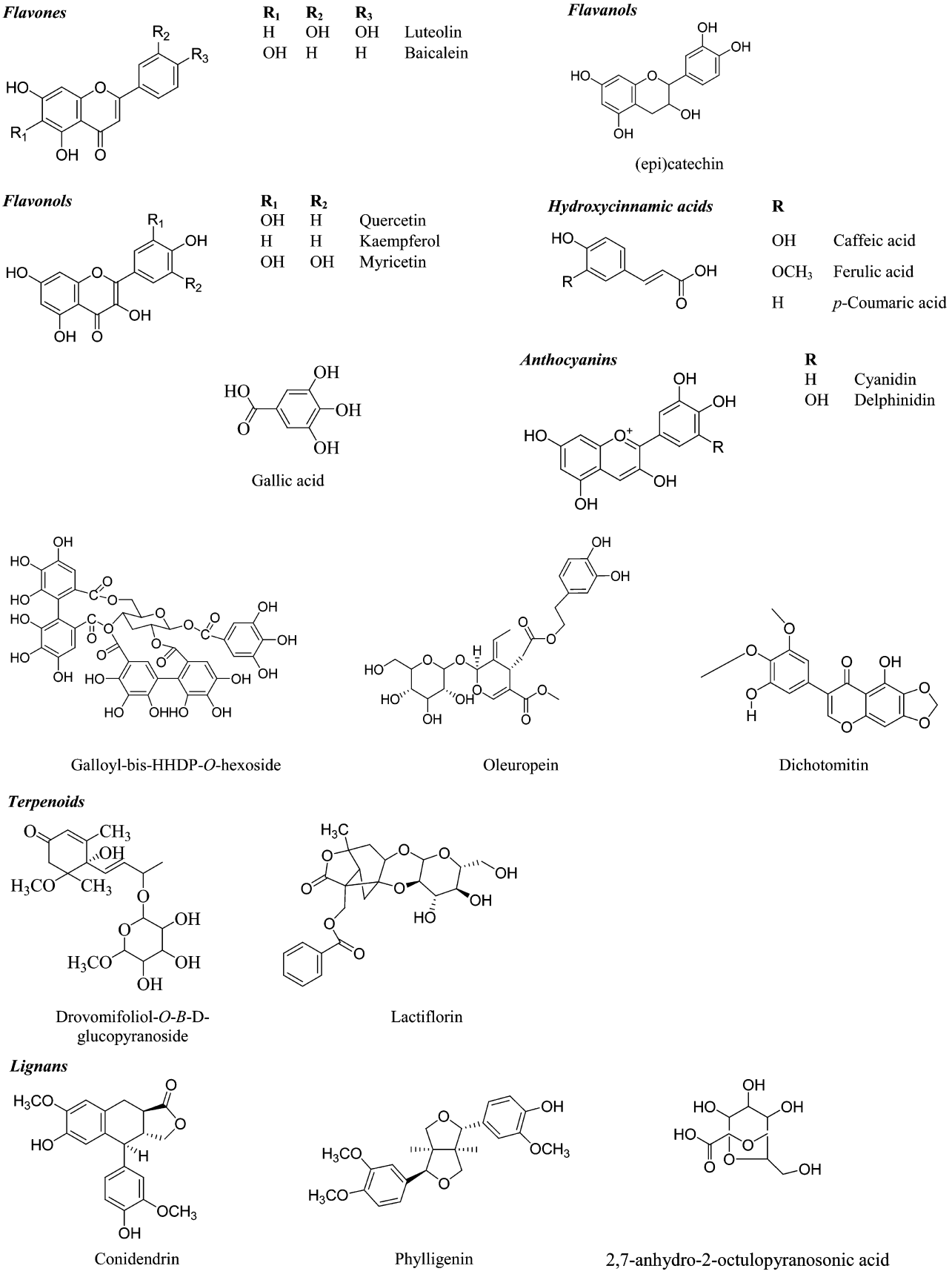

Figure 3. Chemical structures of the main phytochemicals detected in the methanolic extracts from *Myrica faya* (leaves and berries).

identified as a baicalein derivative, detected for the first time in *Myrica*.

Compound **59** displayed $[M - H]^-$ ion at m/z 515, and produced kaempferol aglycone at m/z 284 (by loss of 231 Da).

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In the absence of more specific data, **59** was characterized as a kaempferol derivative.

Lignans. Compounds **31** and **50** displayed $[M - H]^-$ ions at m/z 579 and 563. In MS^2 both compounds showed a loss of 208 Da (possibly formic acid plus hexoside moieties). Further MS^n data were in accordance with those previously described in pomegranate for pylligenin and conidendrin.[28] Thus, **31** and **50** were characterized as phylligenin-*O*-hexoside and conidendrin-*O*-hexoside, respectively. To our best knowledge, we report here for the first time the presence of lignans in *Myrica*.

Other Compounds. Galloyl-bis-hexahydroxydiphenoyl-(HHDP)-*O*-hexoside (compound **14**) was plausibly identified in leaves according to previous findings.[20] It showed $[M - H]^-$ ion at m/z 935 and main fragment at m/z 633 (HHDP-galloylhexoside) along with other product ions at m/z 615 (dehydrated derivative), 481 (HHDP-hexoside), and 299 (ellagic acid). This finding marks the first report of an ellagitannin in *Myrica* species.

Additionally, some other nonphenolic compounds were detected in this analysis: organic acids, monoterpenes, phenylethanoids, and fatty acids.

Quinic acid showed $[M - H]^-$ at m/z 191 (compound **5**) and was plausibly characterized according to literature data.[19] Quinic acid dimer (compound **7**) with $[M - H]^-$ at m/z 383 showed a direct loss of 191 Da, with further fragmentation behavior identical to compound **5**.

Compound **3**, with $[M - H]^-$ ion at m/z 533, exhibited a fragment ion at m/z 191, which displayed the typical fragmentation pattern of quinic acid. Without further information, it was identified as a quinic acid derivative.

Benzyl alcohol hexose pentose (compound **15**) displayed $[M + HCOO]^-$ ion at m/z 447, and sequential losses of 46 (formate) and 132 (pentose) Da were observed. This fragmentation pattern was similar to that previously described in *Melicoccus bijugatus* Jacq. fruits.[29]

Compound **16** exhibited $[M + HCOO]^-$ ion at m/z 431 and suffered the loss of 46 Da (formate) to produce the ion at m/z 385, which was identified as a roseoside (vomifoliolglucoside). It produced a fragment ion at m/z 223 by loss of a sugar moiety (162 Da), and followed the exact behavior reported by Liet al. to what they called drovomifoliol-*O*-*B*-D-glucopyranoside (a terpenoid).[30] Roseoside has been previously identified in *Myrica*'s barks and leaves, but not in fruits.[14]

Another monoterpene was characterized (compound **45**), with ions at m/z 507 $[M + HCOO]^-$, 461 $[M - H]^-$, 443 $[M - H - H_2O]^-$, and 293 $[M - H - C_7H_6O_2]^-$. It followed the exact same pattern described for lactiflorin,[31] being documented here for the first time in this genus.

Compound **24** displayed an $[M - H]^-$ ion at m/z 597, and its sequential fragmentation led to typical glucaric acid ions (at m/z 209, 191, 147).[32] Thus, with no other information available, **24** was identified as a glucaric acid derivative, reported here for the first time in *Myrica*.

The MS^n spectrum of compound **34** showed an $[M - H]^-$ ion at m/z 539 which produced fragment ions at m/z 377 $[M - 162 - H]^-$ and 307 $[M - 162 - C_4H_6O - H]^-$. Sequential fragmentation was consistent with that reported for oleuropein.[33] This compound is one of the major phenolics (phenylethanoid) present in olive leaves and pulp and to our best knowledge have not been reported, so far, in *Myrica.*

Compound **35** exhibited $[M - H]^-$ at m/z 633 and during MS^2 fragmentation lost 163 Da, which could be attributed to a coumaric acid. Further fragmentation gave product ions at m/z 347 and 323, which corroborated the presence of benzoyl and coumaroyl groups (122 and 146 Da, respectively). This behavior was similar to that reported for benzoyl-*p*-coumaryl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid,[34,35] with **35** being identified as this compound. With an extra neutral loss of 146 Da, compound **54** displayed $[M - H]^-$ ion at m/z 779 and was characterized as benzoyl-*p*-tricoumaryl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid. The presence of another coumaryl group instead of a rhamnose unit attached to the molecule was consistent with the longer retention time (t_R = 31.9 min). To our best knowledge, octulosonic acid derivatives were reported here, for the first time, in *Myrica*.

Compound **57** showed $[M - H]^-$ ion at m/z 327. The neutral loss of 98 Da in MS^2 corresponded to the loss of an end-group HO—CH═CH$(CH_2)_3CH_3$ from an oxylipin molecule.[36] Compound **57** was thus identified as an oxo-dihydroxyoctadecenoic acid (oxo-DHODE). This compound, together with trihydroxyoctacedenoic acid (THODE), has been found by our group in the leaves of other species from Madeira endemic flora (unpublished results).

Other peaks (compounds **1**, **41**, **55**, **56**, and **58**) were detected, but their UV and MS^n data did not provide any valuable information about their chemical nature. Thus, their structures could not be elucidated.

Positive Mode Ionization. *Faya* berries are red or dark in color, attributed mainly to anthocyanins, which are more easily characterized with electrospray ionization operating in the positive mode (ESI^+) in combination to the characteristic UV-DAD absorptions.[20,21] The ESI^+ analysis was only relevant for the berries extracts.

Compound **2** gave an $[M + H]^+$ ion at m/z 449, and the main MS^2 fragment ion was observed at m/z 287, corresponding to the neutral loss of 162 Da. Further fragmentation of the ion at m/z 287 suggested that the aglycone was cyanidin based on literature data.[13] Thus, **2** was characterized as cyanidin-3-glucoside, which has been reported as the dominant anthocyanin (95% of total anthocyanins) present in *Myrica rubra* fruits.

Compound **9** exhibited $[M - H]^-$ ion at m/z 465, forming a fragment ion at m/z 303 (by the loss of 162 Da). MS^n fragment ions at m/z 257 and 229 were consistent with those reported for delphinidin.[37] Therefore, **9** was characterized for the first time in *Myrica* as delphinidin-*O*-hexoside.

Quantification of Phenolic Compounds. In the present study, 21 polyphenols were quantified by HPLC-DAD using the corresponding standards for calibration for each group, and the obtained results are shown in Table 3.

The phenolic composition of leaves and berries varied quantitatively. The results indicated that flavonols, flavanols, and phenolic acids were the most abundant compounds in the leaves. Myricetin-*O*-deoxyhexoside presented the highest concentration in leaves, which is in agreement with bibliographic data on *M. rubra*.[11] Leaves were also rich in myricetin-*O*-(*O*-galloyl)deoxyhexoside, gallo(epi)catechin-*O*-gallate dimer, and galloyl-bis-HHDP-*O*-hexoside (casuarin). TIPC of the leaves was comparable to those reported previously in *M. rubra* (1133−2255 mg GAE/100 g of dried leaves).

For berries, anthocyanins, flavonols, and flavones represented the dominant class of polyphenols. C3G was the major compound, followed by myricetin-*O*-hexoside and luteolin-*O*-hexoside derivative. Previous work[7,8,13] on juice and pomace from *M. rubra* also reported C3G as one of the main polyphenols in berries. Flavonoids (in particular flavonols)

Table 3. Contents of Total and Individual Phenolic Compounds (mg/100 g DW) in Leaves and Berries from *Myrica faya*[a]

phenolic compounds	leaves	berries
Phenolic Acids		
caffeic acid-*O*-hexoside		45.73 ± 0.97
protocatechuic acid-*O*-pentoside	7.53 ± 0.9	
total	7.53 ± 0.9	45.73 ± 0.97
Flavanols		
galloyl-di(epi)-gallocatechin	43.42 ± 0.98	
(epi)catechin	2.56 ± 0.41	nd
gallo(epi)catechin-*O*-gallate dimer	132.06 ± 8.27	
(epi)catechin-*O*-gallate		37.80 ± 1.22
total	178.04 ± 4.33	37.80 ± 1.22
Flavonols		
myricetin-*O*-hexoside	62.79 ± 2.15	80.98 ± 2.65
myricetin-O-(O-galloyl)hexoside	53.59 ± 1.59	nd
myricetin-*O*-deoxyhexoside	770.35 ± 11.31	44.20 ± 1.23
galloylquercetin-*O*-hexoside	33.88 ± 2.11	41.73 ± 2.06
kaempferol-*O*-rutinoside	13.25 ± 0.85	-
kaempferol-*O*-hexoside	16.65 ± 1.94	8.44 ± 0.46
kaempferol-*O*-(*O*-galloyl)hexoside	14.35 ± 0.79	
quercetin-*O*-deoxyhexoside	16.86 ± 0.86	33.95 ± 1.35
myricetin-*O*-(*O*-galloyl) deoxyhexoside	233.98 ± 6.55	59.51 ± 2.64
kaempferol-*O*-rhamnoside	26.27 ± 2.69	nd
total	1241.97 ± 10.79	268.81 ± 9.43
Flavones		
luteolin-*O*-hexoside derivative		77.68 ± 5.44
tricin-*O*-hexoside	26.38 ± 1.48	5.01 ± 0.94
total	26.38 ± 1.48	82.69 ± 4.79
Ellagitannins		
galloyl-bis-HHDP-*O*-hexoside (casuarin)	86.46 ± 6.75	
total	86.46 ± 6.75	
Anthocyanins		
cyanidin-*O*-hexoside		368.57 ± 5.42
delphinidin-*O*-hexoside		16.52 ± 0.36
total		385.09 ± 6.61
TIPC	1540.38 ± 87.76	820.11 ± 46.71

[a]nd = not detected.

were also present in higher amounts than phenolic acids. (epi)Catechin, myricetin-*O*-(*O*-galloyl)hexoside, and kaempferol-*O*-rhamnoside were not quantified in berries due to their low concentration.

The TIPC of leaves and berries was lower than those determined by the Folin−Ciocalteu method (Table 4). This difference is attributed to the fact that the Folin−Ciocalteu method tends to overestimate the contents of total phenolics, since it gives positive answer to other substances, and also because not all the identified compounds could be quantified.

Analysis of L-AA Content. The data regarding L-AA content, pH, and °Brix of *faya* berries are presented in Table 4.

The amounts of L-AA present in *M. faya* berries had not been determined before and were within the range of those reported for bayberries (Table 4). No data were found about vitamin C content in *M. esculenta*. Apart from *Myrica* species, the L-AA contents obtained here were higher than others reported previously in other berry fruits like blackberry, blueberry, chokeberry, raspberry, and redcurrant, but lower than in blackcurrant and strawberry.[17,38] The sugar content, evaluated through the °Brix, was higher than in *M. rubra* and within the range reported for most commercial berries (usually between 10 and 18), and the acidity was low.

TPC, TFC, and Antioxidant Capacity Tests. The results obtained for total phenolic and flavonoid contents of *Myrica faya* leaves and berries are presented in Table 3. L-AA is a powerful antioxidant, and its presence in plant extracts produces inaccurate estimations of TPC values because L-AA reduces FCR. One approach to improve the TPC values is the calculation of a corrected TPC value based on the L-AA reducing activity present in the extract.[39,40] The L-AA standard was tested for TPC using the same procedure previously described and it was found to present reducing activity of 0.683 mg GAE/g L-AA. So, for each sample, L-AA contribution was calculated by multiplying the L-AA content by 0.683. The corrected TPC of the samples, calculated by subtracting L-AA contribution, is also shown in Table 4.

Our tests revealed that leaves had a much higher content of total phenolics than berries, which is in agreement with previous literature reports for other subspecies of *Myrica*. TPC values of *Myrica faya* (leaves and berries) are comparable to those reported for other *Myrica* species (with *faya* showing a slightly higher content). Compared with other commonly consumed berries, *faya* presented higher TPC values than blackberry, blueberry, raspberry, and strawberry.[17,38] For TFC assay the same pattern was observed; however, no data regarding flavonoid content of leaves was found in the literature for comparison. Rawat et al. reported TFC values of *M. esculenta* (131−238 mg quercetin equivalent/100 g of dried weight), but those results were expressed in different units, which makes a comparison difficult.[15]

Table 4. Overview of L-AA, pH, TSS, TPC, TFC, and Antioxidant Capacity Assays (ABTS, DPPH) Determinations in *Myrica faya* (Leaves and Berries)[a]

	faya (*Myrica faya*)		bayberry (*Myrica rubra*)		box myrtle (*Myrica esculenta*)	
parameters	leaves	berries	leaves	berries	leaves	berries
pH		4.02				
TSS (°Brix)		14.87		11.6−13.4[41]		
L-AA content (mg/100 g FW)		48.42 ± 1.93		11−114[41]	4.16	
TPC (g GAE/100 g DE)	24.80 ± 0.28	5.26 ± 0.13	8.14−19.63[11]	0.07−4.74[6−8,12,13]	15[15]	0.18−2.86[15,16]
TPC (g GAE/100 g DE)[b]		5.23 ± 0.13				
TFC (g RUE/100 g DE)	12.72 ± 0.16	4.21 ± 0.12		0.01−0.12[6,12]		
ABTS (g TE/100 g DE)	24.10 ± 0.13	12.51 ± 0.15		0.33−10.06[6,8,12]	38.45[15]	
DPPH (g TE/100 g DE)	20.36 ± 0.12	9.24 ± 0.11		0.74−9.10[12,13]		

[a]All measurements were performed in triplicate (mean ± SD). [b]corrected TPC value (subtracted L-AA contribution).

In this study, both ABTS and DPPH were used to evaluate the antioxidant capacity of *Myrica faya*, and the results are shown in Table 4. *Myrica faya* presented a considerable free-radical scavenging capacity, with leaves showing a stronger reducing power than fruits, which corroborated the measured phenolic and flavonoid contents.

The values obtained for *M. faya* in the ABTS assay were slightly higher than the range of values reported for *M. rubra*, but lower than those from *M. esculenta*. *Faya* berries were much more active than, for instance, strawberries (1455.50 μmol TE) evaluated in the same experimental conditions (data to be published elsewhere). According to Sun et al.,[9] many structure−activity relationship studies have confirmed that the strong antioxidant capacities of *Myrica* species are attributed to the high content of galloyl esters that enhance such properties and confer high radical scavenging activities.[9]

In conclusion, over 50 compounds were characterized, for the first time, in different morphological parts of *Myrica faya* by means of an HPLC-DAD-ESI/MS^n method. *M. faya* shared some characteristics in phenolic profile with other *Myrica* species. Nevertheless, we reported for the first time the presence of some flavonoids, ellagitannins, lignans, phenylethanoids, and other organic compounds in this genus. The levels of L-AA and C3G observed in the berries were high, so they can constitute a good source of these nutrients when compared to other fruits. This study provides scientific evidence that *M. faya* is a rich source of bioactive compounds with great potential as natural antioxidants. *Faya* berries are underutilized, mainly due to the lack of scientific studies about their potential health benefits, and consumption and marketing deserve promotion, representing an opportunity for growers and collectors to reach niche markets to increase their revenues.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

FCR, Folin−Ciocalteu's phenol reagent; trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ABTS, 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; MPA, metaphosphoric acid; EDTA, ethylenediaminetetraacetic acid disodium salt; DAD, diode-array detector; AR, analytical reagent; L-AA, L-ascorbic acid; CH_3CN, acetonitrile; TSS, total soluble solids; Na_2CO_3, sodium carbonate; CH_3COOK, sodium acetate; $AlCl_3{\cdot}6H_2O$, aluminum chloride hexahydrated; PBS, phosphate buffered saline; DE, dried extract; ANOVA, analysis of variance; TE, trolox equivalent; RUE, rutin equivalent; GA, gallic acid equivalent; HPLC-DAD-ESI/MS^n, high performance liquid chromatography with online UV and electrospray ionization mass spectrometric detection; SD, standard deviation

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