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Edible *Myrciaria vexator* fruits: Bioactive phenolics for potential COPD therapy

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Abstract

The edible fruits of *Myrciaria vexator* McVaugh (Myrtaceae), from northern South America, are eaten in certain locales, either fresh or processed into jellies and drinks. Activity-guided fractionation of *M. vexator* resulted in identification of ellagic acid (**1**), cyanidin-3-O-glucoside (**2**), delphinidin-3-O-glucoside (**3**), 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid (**4**), and jaboticabin (**5**), and latter two compounds are being reported for the first time in this species. Ellagic acid was further examined, and found to inhibit cigarette smoke extract induced MMP-1 expression in vitro, and may be of significance in the treatment of chronic obstructive pulmonary (COPD). Other compounds identified for the first time from *M. vexator* include cyanidin-3-O-galactoside (**6**), cyanidin-3-O-arabinoside (**7**), cyanidin-3-O-rutinoside (**8**), petunidin (**9**), peonidin-3-O-galactoside (**10**) malvidin (**11**), hyperoside (**12**), quercetin-3-O-glucoside (**13**), and guajaverin (**14**), methyl protocatechuate (**15**), and protocatechuic acid (**16**).

Keywords

Myrciaria vexator; COPD; Ellagic acid; Depsides; Anthocyanins; MMP-1 inhibition

1. Introduction

Chronic obstructive pulmonary disease (COPD) is expected to be the fifth most common cause of disease worldwide by 2020 and currently in the United States is the fourth leading cause of death.¹ The disease is characterized by irreversible small airway obstruction, and is associated with an increase in oxidative stress, inflammatory cell infiltration into the lung,

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apoptosis, mucus hypersecretion, endothelial dysfunction, and an imbalance of proteinases and antiproteinases.² The inflammation and proteolysis observed in COPD is secondary to the normal inflammatory response to cigarette smoke, which is the main etiological factor associated with the disease.^{2,3} A direct role for matrix metalloproteinases (MMPs), specifically MMP-1 and MMP-9, in emphysema causation has been demonstrated.^{3,4} Therefore, inhibiting pulmonary inflammation, reduction of MMP-1 expression, and decreasing oxidative stress in the lungs of patients with COPD could be effective in the treatment of the disease.^{5,6} Currently there is no drug approved to cure COPD in the United States. Although, some therapeutic strategies, such as formoterol, salmeterol, and corticosteroids offer symptomatic relief, their effects are either limited and, or they are associated with major side effects.⁷⁻⁹

Diet may play a role in the prevention of COPD.¹⁰ Based on these findings, some authors have hypothesized that the antioxidants present in edible fruits can protect lung tissue from oxidative damage caused by cigarette smoke and air pollution.^{10,11} Thus research on plant phenolic antioxidants such as resveratrol and quercetin-3'-sulfate have generated considerable interest in the field of COPD therapy.¹²

The fruit of *Myrciaria vexator*, commonly known as the blue grape or false jaboticaba, is edible and native to Mesoamerica and northern South America.¹³ The fruit, known locally as 'pésjua' or 'guyabo morado' is eaten fresh and also processed into jellies and drinks like many other *Myrciaria* fruits.^{14,15} The plant which grows as small tree is a cultivated in Venezuela, and a single tree produces several thousand fruits.¹⁴

Fruits of several Myrtaceae species, including *M. vexator*, had been studied previously by us for their compositional fingerprint analysis and DPPH• scavenging activities.¹⁶ *M. vexator* fruit demonstrated strong DPPH• scavenging activity and six polyphenolic constituents were identified.¹⁶ However, in the current study a more detailed phytochemical and bioactivity assessment of the fruit extract, partitions and fractions is carried out. In order to identify chemical constituents with potential therapeutic relevance to COPD, activity-guided fractionation of *M. vexator* was employed and the identified compound from the most active fraction, ellagic acid (**1**), was tested for its modulatory effect on cigarette smoke extract induced MMP-1 expression in an in vitro model system of COPD.

2. Results and discussion

Activity-guided fractionation of *M. vexator* led to the identification of ellagic acid (**1**), cyanidin-3-O-glucoside (**2**), delphinidin-3-O-glucoside (**3**), 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid (**4**), and jaboticabin (**5**) (Fig. 1).

2.1. ABTS^{•+} scavenging activity of fractions

The ABTS^{•+} scavenging activity of the fruit extract, EtOAc and *n*-BuOH increased throughout the assay period, but the activity of the positive control cyanidin-3-O-glucoside remained higher than the extracts and the partitions (Fig. 2A). However, the ABTS^{•+} scavenging activity of the fruit extract was much higher than the activity reported for so

called ‘superfruits’ such as such as açai and high-bush blueberry.^{17,18} The scavenging activities of the extract and the liquid partitions were not significantly different ($P > 0.05$).

The phenolic enriched *n*-BuOH partition was chosen for further fractionation and antioxidant screening. All the fractions with the exception of fractions 1, 2, and 19 showed ABTS^{•+} scavenging activities. Fraction 5 remained the most potent fraction throughout the assay period. Its scavenging activity initially was significantly higher than that of the positive control ($P < 0.05$), however at the end of the assay the activities of fraction 5 and cyanidin-3-O-glucoside were not significantly different ($P > 0.05$) (Fig. 2B). The ABTS^{•+} scavenging data of most of the fractions were much higher than the partitions and the crude extract, likely due to the higher concentration of antioxidant phenolic constituents in the fractions (Fig. 2B).

2.2. Inhibition of MMP-1 production by fractions

All the fractions at a concentration of 0.1 mg/mL were screened for their ability to inhibit CSE-induced MMP-1 expression in SAE cells. Fractions 12, 14, 17, and 18 significantly reduced ($P < 0.05$) MMP-1 expression in the non-induced SAE cells (Fig. 3). However, in the CSE-induced cells fractions 2–5, 7–14 significantly inhibited MMP-1 expression ($P < 0.05$) (Fig. 3). Fractions 12 and 14 were capable of significantly decreasing MMP-1 expression in both CSE-induced and non-induced cells (Fig. 3). The strongest MMP-1 inhibitory activity was demonstrated by fraction 12. Due to the efficacy of this fraction is important to identify of lead compound possessing the bioactivity.

2.3. HPLC and LC–TOF compositional analysis of fractions

The most potent fractions inhibiting MMP-1 expression, fractions 12 and 14, contained ellagic acid (**1**) (Table 1), and the most potent antioxidant fraction, fraction 5, contained cyanidin-3-O-glucoside (**2**), delphinidin-3-O-glucoside (**3**), 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid (**4**), and jaboticabin (**5**) (Table 1). These compounds were identified based on the comparison of their retention times, mass spectra, and PDA profile with those of authentic standards (Table 1). The presence of these compounds in the fraction was also confirmed by spiking experiments. The two depsides, compounds **4** and **5**, are being reported for the first time in *M. vexator* fruit. In addition eleven phenolic compounds in *M. vexator* fractions were detected for the first time in this species based on their LC retention times and PDA profile (Table 1). The identity of each of the compounds in the fraction was confirmed by spiking with authentic standards (Table 1). The phenolic constituents can be grouped into three classes, which are (1) anthocyanins, (2) flavonols, and (3) benzoic acids. The anthocyanins consisted of cyanidin-3-O-galactoside (**6**), cyanidin-3-O-arabinoside (**7**), cyanidin-3-O-rutinoside (**8**), petunidin (**9**), peonidin-3-O-galactoside (**10**) and malvidin (**11**) (Fig. 1). The flavonoids identified were hyperoside (**12**), quercetin-3-O-glucoside (**13**), and guajaverin (**14**) (Fig. 1). The benzoic acid derivatives detected were methyl protocatechuate (**15**) and protocatechuic acid (**16**) (Fig. 1). In addition to the chemical constituents mentioned above, some compounds which have been previously reported in *M. vexator* were also detected in the fruit including 1, 2, 3, quercitrin (**17**), rutin (**18**), and myricetin (**19**) (Table 1).¹⁶

2.4. Inhibition of MMP-1 expression of ellagic acid (1)

The MMP-1 inhibitory-guided screening lead to the identification of ellagic acid (**1**) (Table 1), which unlike the compounds **2–5** from antioxidant-guided fractionation has not been screened in an in vitro model of COPD. Therefore, compound **1** was assessed for its ability to inhibit CSE-induced MMP-1 expression in SAE cells. MMP-1 expression increased six fold as a result of CSE exposure (Fig. 4). Compound **1** significantly inhibited MMP-1 expression levels in CSE-induced SAE cells in a dose dependent manner ($P < 0.05$) (Fig. 4). There was also reduction in MMP-1 expression in non-induced cells. The compound did not reduce cell viability at all concentrations (data not shown). In comparison to reported MMP-1 inhibitory activities of antioxidant phenolics such as vitexin and isoorientin in the same COPD model, ellagic reduced MMP-1 expression at a far lower concentration.¹⁹ The two flavone C-glyco-sides have already been identified as potential drug candidates for COPD.¹⁹

Ellagic acid (**1**), a dilactone of hexahydroxyphenolic acid, is a well known antioxidant compound with strong free radical scavenging and metal chelating properties.²⁰ The compound is known to decrease the expression of MMP-2 and MMP-9 both in vitro and in vivo.^{21,22} Compound **1** also demonstrated anti-inflammatory effect in murine model of asthma, by inhibiting IL-4, IL-5, and IL-13 production.²³ At physiological pH the compound chelates divalent cations such as Zn^{2+} , Ca^{2+} , Cd^{2+} , and Cu^{2+} , which enhances its ability to inactivate metalloproteinases involved in the pathogenesis of the disease.²⁴

A major problem associated with compound **1** has been its poor bioavailability,^{25,26} but there has been formulation research done which addresses this shortcoming.²⁷ Based on the bioactivity demonstrated in the current study and its reported biological and pharmacological properties, ellagic acid (**1**) may be a potential candidate for further in vivo studies of COPD.

3. Conclusion

In the current study antioxidant- and MMP-1 inhibitory-guided fractionation of *M. vexator* resulted in the identification of five bioactive phenolic constituents, **1–5**. Compounds **2–5**, have already been identified as potential drug candidates for COPD therapy. Therefore ellagic acid (**1**) was investigated further and was found to have the ability to inhibit CSE-induced MMP-1 in SAE cells. This bioactivity along with other reported biological and pharmacological activities demonstrates that compound **1** can be a potential candidate for further studies in COPD treatment. Nineteen phenolic constituents were identified in *M. vexator*, out of which thirteen are being reported for the first time in the fruit of this species.

4. Experimental section

4.1. Reagents

Potassium peroxosulfate, Trolox, and ellagic acid were purchased from Sigma-Aldrich (St. Louis, MO). 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS) was from TCI-Ace (Tokyo, Japan). Sephadex LH-20 from Pharmacia Fine Chemicals (Piscataway, NJ) and RP-18 F₂₅₄ plates from EMD Chemicals Inc. (Gibbstown, NJ) were

used for open column and thin-layer chromatographic separations respectively. HPLC analysis was carried out on a 250 × 4.6 mm, 4 μm Synergi Hydro-RP 80A column which was purchased from Phenomenex (Torrance, CA). Cyanidin-3-O-arabinoside, cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, cyanidin-3-rutinoside, and malvidin were from Extrasynthase (Genay, France). Hyperoside, quercetin-3-O-glucoside, and protocatechuic acid were from Chromadex (Irvine, CA). HPLC-grade acetonitrile, MeOH and formic acid were purchased from Baker (Philipsburg, NJ) and GR grade MeOH, and EtOH, ethyl acetate, and n-butanol from VWR Inc. (Bridgeport, PA). Ultrapure water was prepared using a Millipore Milli-RO 12 plus system, Millipore Corp. (Bedford, MA).

4.2. Fruit material

Fruits of *M. vexator* McVaugh were collected from the Fruit and Spice Park in Homestead, FL. The fruits were immediately frozen and transferred overnight by courier over dry ice, and then freeze-dried (21 g).

4.3. Extraction, partitioning, and fractionation

The freeze-dried fruits were homogenized using a blender, and 70% (v/v) MeOH as the extracting solvent. The ratio of material to the extracting solvent was 1:20 (w/v). Extract was filtered and the marc was extracted two more times. Extracts were then combined and concentrated in vacuo (40 °C), freeze-dried and stored at 4 °C.

The crude extract was suspended in water and sequentially partitioned three times with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH). The combined EtOAc and *n*-BuOH partitions were dried under vacuum (40 °C) and stored at 4 °C.

A portion of the *n*-BuOH partition (1.9 g) was then subjected to fractionation over Sephadex LH-20 (98 g) using 1% solution of formic acid in MeOH as the eluent. In total 36 fractions were collected from the column. These fractions were then recombined into 19 fractions using RP-18 TLC (60:40 H₂O, 5% formic acid/acetonitrile) analysis. The fractions were then subjected to ABTS^{•+} scavenging, and MMP-1 inhibitory activities. The most potent fractions were subjected to HPLC–PDA and LC–TOF analyses.

4.4. ABTS free radical ABTS^{•+} scavenging

The determination of ABTS^{•+} scavenging was based on the method of Re et al.²⁸ The ABTS^{•+} was generated by reacting an ABTS (7 mM) aqueous solution with K₂S₂O₈ (2.45 mM) in the dark for 12–16 h, at ambient temperature, and adjusting the absorbance to 0.700 (±0.020) at 734 nm with ethanol. To 2 μL aliquot of the sample 198 μL ABTS^{•+} was added and the absorbance at 734 nm was recorded after initial mixing and subsequently at 5 min intervals (for 40 min in total) using a Molecular Devices *Versa*_{max} microplate reader (Sunnyvale, CA). The results were expressed as the TEAC (mmol Trolox/g dry sample) values at different time intervals.²⁹

$$\text{Percentage Inhibition} = \left[\frac{(\text{Absorbance control}) - (\text{Absorbance sample})}{(\text{Absorbance control})} \right] \times 100 \quad (1)$$

4.5. Human primary cells

Human small airway epithelial (SAE) cells were cultured according to the supplier's instructions (Lonza, Walkersville, MD) and maintained in a controlled atmosphere of air 5% CO₂ at 37 °C. Cell populations that were 80% confluent and between passages 2–5, were used for all experiments.

4.6. Cigarette smoke extract (CSE) preparation and cell treatment

CSE was prepared using a modified protocol as previously described.³⁰ Briefly, a Barnet vacuum pump operating at constant flow was used to draw the smoke of one 3R4F research grade cigarette (University of Kentucky, Lexington, KY) through 25 mL of Dulbecco's phosphate-buffered saline. This solution (100% CSE) was adjusted to pH 7.4, filtered, diluted with small airway growth medium to a final concentration of 5%, and added to the cells immediately. Cells were incubated with 5% CSE, or pretreated with pure compounds 1 h prior to 5% CSE exposure. Cell viability was assessed following CSE exposure using the AlamarBlue kit (Invitrogen, Carlsbad, CA) according to manufacturer's specifications.

4.7. MMP-1 mRNA expression

The cells were cultured as described above. After 24 h of treatment, total RNA from the human SAE cells was isolated (RNeasy kit, Qiagen, Valencia, CA) and converted by reverse transcription into cDNA (high capacity cDNA kit, Applied Biosystems, Carlsbad, CA). Relative expression of MMP-1 was measured using real-time quantitative PCR (qPCR) and Taqman probes with GAPDH as an endogenous control (Applied Biosystems, Carlsbad, CA).

4.8. HPLC–PDA analysis

HPLC–PDA analyses of the extracts were performed using Waters (Milford, MA) Alliance 2695 system equipped with 2695 separation module unit and 2996 PDA detector using a 250 × 4.6 mm, 4μ Phenomenex Synergi Hydro-RP 80A column (Torrance, CA). Two different methods (A and B) were employed for chromatographic separation of different phytochemical constituents.

4.8.1. Method A—The mobile phase consisted of solvents (A) 1% aqueous formic acid solution and (B) acetonitrile. Step-wise non linear gradient elution was performed using 13% B for 7 min and 13–15% B in 3 min, 15–30% B in 18 min, 30–60% B in 7 min, 60–100% B in 10 min. The composition was then changed to the initial condition in 5 min, and maintained for 10 min. The flow rate and the injection volume were 1 mL/min and 10 μL, respectively.

4.8.2. Method B—The mobile phase consisted of solvents (A) 10% aqueous formic acid solution and (B) acetonitrile. Step-wise non linear gradient elution was performed starting with 5% B, changing to 11% B in 4 min, maintaining the condition for 12 min, increasing to 17% B in 16 min, followed by a final rise to 100% B in 13 min. The composition was maintained for 10 min and then changed to the initial condition in 5 min, and maintained for

10 min. The flow rate and the injection volume were 1 mL/min and 10 μ L, respectively. The results from methods A and B were monitored using a wavelength range of 210–800 nm.

4.9. LC–TOF analysis

High-resolution electrospray ionization mass spectrometry (HR-ESI-MS) was performed using a LCT premier XE TOF mass spectrometer (Waters, Manifold, MA) equipped with an ESI interface and controlled by MassLynx V4.1 software. Mass spectra were acquired in both positive and negative modes over the range m/z 100–1000. The capillary voltages were set at 3000 V (positive mode) and 2800 V (negative mode), respectively, and the cone voltage was 20 V. Nitrogen gas was used for both the nebulizer and in desolvation. The desolvation and cone gas flow rates were 300 and 20 L/h, respectively. The desolvation temperature was 400 °C, and the source temperature was 120 °C. For the dynamic range enhancement (DRE) lockmass, a solution of leucine enkephalin (Sigma–Aldrich, Steinheim, Germany) was infused by a secondary reference probe at 200 pg/mL in acetonitrile/water (1:1) containing 0.1% formic acid with a second LC pump (Waters 515 HPLC pump). The reference mass was scanned once every five scans for each positive and negative data collection. Both positive and negative ESI data were collected using a scan time of 0.2 s, with an interscan time of 0.01 s, and a polarity switch time of 0.3 s. The full chromatograms were recorded at two different aperture voltages. The most intense fragmental ions and molecular ions could be obtained, when the aperture voltage were set at 60 and 0 V, respectively. V-optics mode was used for increased intensity.

Method A from the previous Section 4.8 was used for chromatographic separation of the constituents in the fractions of *M. vexator*.

4.10. Statistical analysis

Data are presented as mean values $\pm$ 95% confidence interval. Analysis of variance was performed using ANOVA procedures. Significant differences between means were determined by Tukey's pairwise comparison test at a level of $P < 0.05$. The JMP version 8 software was used for statistical analyses.

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Figure 1.

Structural formulas of the constituents from *M. vexator*: **1**, ellagic acid; **2**, cyanidin-3-O-glucoside; **3**, delphinidin-3-O-glucoside; **4**, 2-O-(3,4-dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid; **5**, jaboticabin; **6**, cyanidin galactoside; **7**, cyanidin arabinoside; **8**, cyanidin-3-O-rutinoside; **9**, petunidin; **10**, peonidin-3-O-galactoside; **11**, malvidin; **12**, hyperoside; **13**, quercetin-3-O-glucoside; **14**, gujaverin; **15**, methyl protocatechuate; **16**, protocatechuic acid; **17**, quercitrin; **18**, rutin; **19**, myricetin.

Figure 2. ABTS•⁺ scavenging activity of (A) cyanidin-3-O-glucoside, *M. vexator* extract, and partitions (B) *M. vexator* fractions. Results are expressed as mean $\pm$ standard error mean (n = 8).

Figure 3. Inhibition of MMP-1 mRNA expression by *M. vexator* fractions in SAE cell non-induced (open bars) and CSE-induced (bold bars). Results are expressed as mean $\pm$ standard error mean (n = 3). * Indicates significant reduction (P < 0.05) as compared to the control cells.

Figure 4.

Inhibition of MMP-1 mRNA expression by compound 1 in SAE cell non-induced (open bars) and CSE-induced (bold bars). Results are expressed as mean $\pm$ standard error mean ($n = 3$). Bars with the same lower case letters (a–c) and upper case letters (A–D) are not significantly different ($P > 0.05$).

Table 1

LC-MS-TOF data of the identified constituents

Compound ^a	Retention time (min)	UV λ_{max}	Major ions		Other ions
			m/z [M+X] ⁺ /[M-X] ⁻	(L.F., ppm)	
1 Ellagic acid	21.87	253, 364	303.0163 [M+H] ⁺ 603.0037 [2M-H] ⁻	(C ₁₄ H ₇ O ₈ , 7.3) (C ₂₈ H ₁₁ O ₁₆ , -1.7)	301.0137 [M-H] ⁻
2 Cyanidin-3- <i>O</i> -glucoside	7.28	279, 516	449.0106 [M] ⁺ 447.0948 [M-2H] ⁻	(C ₂₁ H ₂₁ O ₁₁ , -5.1) (C ₂₁ H ₁₉ O ₁₁ , -4.7)	897.2083 [2M+H] ⁺ 465.1067 [M+H ₂ O] ⁺ 493.1016 [M-2H+HCOOH] ⁻ 285.0710 [M-Glu-2H] ⁻
3 Delphinidin-3- <i>O</i> -glucoside	5.54	263, 526	465.1046 [M] ⁺ 463.0875 [M-2H] ⁺	(C ₂₁ H ₂₁ O ₁₂ , 2.8) (C ₂₁ H ₂₁ O ₁₂ , -0.4)	913.1995 [2M+H] ⁺ 321.1370 [M-Glu+H ₂ O] ⁺ 911.1868 [2M-H] ⁻ 509.1201 [M-2H+HCOOH] ⁻
4 2- <i>O</i> -(3,4-Dihydroxybenzoyl)-2,4,6-trihydroxyphenylacetic acid	18.39	267, 297	343.0446 [M+Na] ⁺	(C ₁₅ H ₁₂ O ₈ Na, 4.7)	321.0217 [M+H] ⁺ 365.0624 [M-H+HCOOH] ⁻ 319.0553 [M-H] ⁻ 639.0927 [2M-H] ⁻
5 Jaboticabin	24.93	266, 297	357.0607 [M+Na] ⁺	(C ₁₆ H ₁₄ O ₈ Na, -0.8)	691.1258 [2M+Na] ⁺ 333.0756 [M-H] ⁻ 379.0802 [M-H+HCOOH] ⁻ 667.1255 [2M-H] ⁻
6 Cyanidin galactoside	7.19	279, 516	449.1065 [M] ⁺ 447.0992 [M-2H] ⁻	(C ₂₁ H ₂₁ O ₁₁ , -4.2) (C ₂₁ H ₁₉ O ₁₁ , 14.5)	287.0577 [M-Gal] ⁺ 305.0700 [M-Gal+H ₂ O] ⁺ 511.1097 [M-2H+HCOOH+H ₂ O] ⁻ 895.1922 [2M-H] ⁻ 913.1996 [2M+H ₂ O-H] ⁻
7 Cyanidin arabinoside	12.09	279, 516	419.0985 [M] ⁺ 417.0923 [M-2H] ⁻	(C ₂₀ H ₁₆ O ₁₀ , 1.7) (C ₂₀ H ₁₇ O ₁₀ , 24.2)	257.1029 [M-Ara] ⁺ 463.0917 [M-2H+HCOOH] ⁻ 481.1015 [M-2H+HCOOH+H ₂ O] ⁻
8 Cyanidin-3- <i>O</i> -rutoside	10.38	280, 518	593.1464 [M-2H] ⁻	(C ₂₇ H ₃₁ O ₁₅ , -7.1)	
9 Petunidin	34.99	280, 518	317.0685 [M] ⁺	(C ₁₆ H ₁₃ O ₇ , 7.6)	339.0655 [M+Na] ⁺ 315.0488 [M-H] ⁻ 631.1020 [2M-H] ⁻ 361.0642 [M-H+HCOOH] ⁻
10 Peonidin-3- <i>O</i> -galactoside	12.70	280, 513	463.1247 [M-2H] ⁺	(C ₂₂ H ₂₃ O ₁₁ , 1.5)	
11 Malvidin	37.22	270, 530	331.0814 [M] ⁺	(C ₁₇ H ₁₅ O ₇ , -1.2)	353.2317 [M+Na] ⁺ 661.2470 [2M+H] ⁺ 329.0563 [M-H] ⁻ 705.6577 [2M-H+HCOOH] ⁻

Compound ^a	Retention time (min)	UV λ_{max}	Major ions		Other ions
			m/z [M+X] ⁺ /[M-X] ⁻	(I.F., ppm)	
12 Hyperoside	21.89	254, 356	465.1060 [M+H] ⁺	(C ₂₁ H ₂₁ O ₁₂ , 5.8)	m/z [M+X] ⁺ /[M-X] ⁻ 695.0497 [2M+CL] ⁻ 303.0197 [M+H-Gal] ⁺ 301.0175 [M-H-Gal] ⁻ 463.1000 [M-H] ⁻
13 Quercetin-3- <i>O</i> -glucoside	22.37	254, 352	465.1040 [M+H] ⁺	(C ₂₁ H ₂₁ O ₁₂ , 1.5)	487.0900 [M+Na] ⁺ 303.0127 [M+H-Glu] ⁺ 463.0925 [M-H] ⁻ 509.0916 [M-H+HCOOH] ⁻ 301.0127 [M-H-Glu] ⁻
14 Gujaverin	23.42	254, 356	435.0943 [M+H] ⁺	(C ₂₀ H ₁₉ O ₁₁ , 3.7)	457.0757 [M+Na] ⁺ 433.0849 [M-H] ⁻ 479.0880 [M-H+HCOOH] ⁻
15 Methyl protocatechua	18.45	258, 295	167.0319 [M-H] ⁻	(C ₈ H ₉ O ₄ , -15.0)	381.2081 [2M-H+HCOOH] ⁻
16 Protocatechuic acid	6.15	260, 290	307.0428 [2M-H] ⁻	(C ₁₄ H ₁₁ O ₈ , -8.5)	
17 Quercitrin	25.06	255, 349	449.1100 [M+H] ⁺	(C ₂₁ H ₂₁ O ₁₁ , 3.6)	447.1010 [M-H] ⁺ 483.0289 [M+Cl] ⁻ 301.0144 [M-H-Rha] ⁻
18 Rutin	20.85	225, 350	611.1614 [M+H] ⁺	(C ₂₇ H ₃₁ O ₁₆ , 0.3)	633.1418 [M+Na] ⁺ 303.0542 [M+H-Rha-Glu] ⁺ 465.2947 [M+H-Rha] ⁺ 609.1406 [M-H] ⁺ 655.1432 [M-H+HCOOH] ⁻
19 Myricetin	27.70	249, 362	319.0481 [M+H] ⁺	(C ₁₅ H ₁₁ O ₈ , 8.5)	

^aThe identity of all the compounds were confirmed with spiking with authentic standards.