

## Article

# Andean Blueberry of the Genus *Disterigma*: A High-Resolution Mass Spectrometric Approach for the Comprehensive Characterization of Phenolic Compounds

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**Abstract:** Wild neotropical blueberries, endemic of Central and South American areas, are promising yet still undisclosed sources of bioactive compounds. Most research studies have addressed wild and cultivated blueberries from Europe and North America, despite the extremely wide variety of wild neotropical species. In the present paper, for the first time, the phenolic composition of *Disterigma alaternoides* was investigated through ultra-high-performance liquid chromatography coupled to high-resolution mass-spectrometric analysis followed by accurate data analysis and compound validation with a dedicated structure-based workflow. *D. alaternoides*, which belongs to a closely related genus to that of the common blueberry, grows exclusively in the Andean regions over 2000 above sea level. Thanks to the dedicated analytical platform, 249 phenolic compounds were tentatively identified, including several anthocyanins, flavonoids, phenolic acids, and proanthocyanidins. The nature and heterogeneity of identified phenolic compounds demonstrate once more the need for a more profound knowledge of such still uncharted matrices.

**Keywords:** polyphenols; neotropical berries; *Disterigma alaternoides*; anthocyanins; Compound Discoverer



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## 1. Introduction

The vast majority of the research activity on the berries of the plant family Ericaceae has addressed temperate species of *Vaccinium* [1–3], which is only 1 of the 32 genera of the tribe Vaccinieae of the family Ericaceae. Among these species, the most known are blueberry (*Vaccinium corymbosum*) [4], bilberry (*Vaccinium myrtillus*) [5], cranberry (*Vaccinium macrocarpon*) [6], and lingonberry (*Vaccinium vitis-idaea*) [7]. Nevertheless, more than 600 species of berry-producing Ericaceae are native to the Neotropical realm, including South America, Central America, and the Caribbean islands [8]. Several neotropical blueberries in the Andean region of South America are widely consumed raw or in different preparations [9].

Berries of the genus *Vaccinium* have been raising interest for their extremely high content in flavonoids, anthocyanins, phenolic acids, and tannins, which have been demonstrated to exert a wide range of biological activities [10,11]. In a recent paper by Rutledge et al. [12], blueberry phenolics were associated with a cognitive enhancement in healthy elder adults. Likewise, Stull et al. [13] reported that consumption of the whole blueberry reduces the blood glucose level in vivo. For these reasons, blueberries are often referred to as “super-fruits”. At present, the composition of blueberries from North American and European regions has been widely investigated [4,14,15]. In a recent study by Ancillotti et al. [2], the polyphenol composition of cultivated *V. corymbosum* and wild *V. myrtillus* and *V. uliginosum* were evaluated by liquid chromatography coupled to high-resolution mass

spectrometry (HRMS). More than 200 compounds were tentatively identified in the hydroalcoholic extracts, comprising mainly anthocyanins, flavonols, and proanthocyanidins, with the wild berries presenting generally higher concentrations. As well as the common species of blueberries, other species native to South America have been the object of several studies. *V. floribundum*, a woody perennial shrub that is endemic in the Andean region and grows between 1600 and 4500 m above sea level (masl) [16], has been extensively studied for its potential beneficial effects and its vast consumption by the local population [17–20]. Despite the growing interest in the bioactive compounds in berries from South America, there is still a lack of knowledge in the phenol composition of berries belonging to other genera of the Ericaceae family. In the present paper, for the first time, the phenolic compound composition of *Disterigma alaternoides* was determined by ultra-high-performance liquid chromatography (UHPLC) coupled to HRMS. The genus *Disterigma* has more than 35 species of small shrubs, distributed from southern Mexico to Bolivia, generally above 2000 masl [21]. The paper aims to widen the knowledge on often neglected species that could possess peculiar characteristics since they grow in the unique Andean ecosystems.

## 2. Materials and Methods

### 2.1. Samples, Chemicals, and Reagents

*D. alaternoides* fruit samples were obtained by the National Agrarian University La Molina (Lima, Perú). Their taxonomy was certified by the Herbario San Marcos (National University of San Marcos, Lima, Perú). Berries were mashed, freeze-dried by a Heto PowerDry LL1500 (Thermo Fisher), finely ground in a mortar, and stored at  $-20\text{ }^{\circ}\text{C}$  until use. Optima<sup>®</sup> LC-MS grade water, methanol (MeOH), and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Acetone, acetic acid, formic acid, and sodium hypochlorite were purchased from Merck (Kenilworth, NJ, USA).

### 2.2. Phenolic Compound Extraction

Freeze-dried berries were extracted as previously reported with slight modifications [22]. Briefly, 0.2 g of freeze-dried berry samples were extracted with 10 mL  $\text{CH}_3\text{COCH}_3/\text{H}_2\text{O}/\text{CH}_3\text{COOH}$  (70:29.5:0.5, *v/v/v*). The extract was sonicated for 15 min in an ice bath and then centrifuged for 10 min at  $2000\times g$ . The supernatant was collected, and the procedure was repeated once. The supernatants were mixed and concentrated to 4.5 mL using a Speed-Vac SC 250 Express (Thermo 164 Avant, Holbrook, NY, USA). Then, 500  $\mu\text{L}$  of MeOH was added to the sample, and the final extract solution ( $\text{H}_2\text{O}/\text{MeOH}$ , 90:10 *v/v*) was filtered through a 13-mm Acrodisc Syringe filter with a 0.2  $\mu\text{m}$  GH Polypropylene membrane (Pall, Ann Arbor, MI, USA). Finally, the extract was aliquoted and stored at  $-20\text{ }^{\circ}\text{C}$  for further analysis.

### 2.3. UHPLC-HRMS Analysis

Phenolic compound chromatographic separation was carried out by a Vanquish binary pump H (Thermo Fisher Scientific, Bremen, Germany), equipped with a thermostated autosampler and column compartment, on a Kinetex core-shell C18 column (100 mm  $\times$  2.1 mm i.d.) with a particle size of 2.6  $\mu\text{m}$  (Phenomenex, Torrance, CA, USA) at  $40\text{ }^{\circ}\text{C}$  and with a flow-rate of 600  $\mu\text{L min}^{-1}$ . The injection volume was 10  $\mu\text{L}$ . The mobile phases consisted of  $\text{H}_2\text{O}/\text{HCOOH}$  (99.9:0.1, *v/v*; phase A) and  $\text{ACN}/\text{HCOOH}$  (99.9:0.1, *v/v*; phase B). The elution gradient was optimized in a previous study [22]. The chromatographic system was coupled to a Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) with a heated ESI source. The ESI source parameters were set as reported in our previous work [23]. The detection was conducted in TOP 5 data-dependent acquisition (DDA) mode for low- and high-molecular-weight phenolic compounds. An exclusion list containing the most intense ions detected in the blank sample, consisting of  $\text{H}_2\text{O}/\text{MeOH}$  (90:10, *v/v*), was added to the mass-spectrometric method. For low-molecular-weight phenolic compound analysis (flavonoids, anthocyanins, and phenolic acids) and high-molecular-weight polyphenol analysis (tannins), MS data were acquired in the range

150–1000  $m/z$  and 300–2000  $m/z$ , respectively, with a resolution (full width at half maximum, FWHM, at  $m/z$  200) of 70,000. In full scan mode, the automatic gain control (AGC) target value was 200,000 and the maximum ion injection time was 100 ms. The isolation window width was 2  $m/z$ . MS/MS fragmentation was performed with a resolution (FWHM, at  $m/z$  200) of 35,000 with AGC target value set at 100,000 and dynamic exclusion set to 3 s. Fragmentation was achieved in the higher-collision dissociation (HCD) cell at three values of normalized collision energy (NCE), namely, 20–50–80 NCE in the positive ion mode and 20–40–60 NCE in the negative ion mode, based on the results of a previous study [24]. All samples were run in triplicate.

#### 2.4. Phenolic Compound Identification

Raw data obtained from three consecutive injections and the blank sample were processed by Compound Discoverer 3.1 (Thermo Fisher Scientific) using a customized method specifically dedicated to phenolic compound analysis [24,25]. Customized databases were generated by combining free phenolic compounds (aglycones) with a series of sugars, aliphatic, and aromatic acids, and complete IDs, accurate masses, and molecular formulas were implemented in the *mass list* feature for the automatic matching of extracted  $m/z$  ratios (45,567 combinations). Moreover, detailed HCD fragmentation spectra for flavonoids and phenolic acids were implemented in the *compound class scoring* section for automatic MS/MS spectra matching. The parameters for the *predict composition* tool were adapted to phenolic compounds. Extracted  $m/z$  from the raw chromatograms were grouped, aligned, and filtered to remove background compounds found in the blank sample,  $m/z$  values not associated with compounds present in the databases, and the features lacking MS/MS spectra. Filtered compounds were manually validated by matching fragmentation spectra to those of available standards or spectra reported in the literature. When data were lacking, phenolic compounds were tentatively identified according to the characteristic fragmentation spectra. The identification data for the tentatively identified compounds are discussed in the following sections and summarized in Tables 1–4 and Tables S1–S4 with the related confidence level according to Schymanski et al. [26].

**Table 1.** Retention times (Rt, min), proposed formulas, experimental  $m/z$ , accuracy ( $\Delta$ , ppm), main diagnostic experimental product ions, and confidence level of the identification (c. l.) of the 18 tentatively identified anthocyanins in *Disterigma alaternoides* extract in ESI(+).

Id	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ mass (ppm)	Diagnostic Product Ions ( $m/z$ )	c. l.
1	Cyanidin O-hexoside (I)	1.63	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup>	449.1088	1.5	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
2	Delphinidin O-hexoside (I)	2.79	C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> <sup>+</sup>	465.1038	2.1	303.0502; 247.0605; 229.0499; 153.0185; 149.0237	2
3	Delphinidin O-hexoside (II)	3.77	C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> <sup>+</sup>	465.1034	1.4	303.0502; 247.0605; 229.0499; 153.0185; 149.0237	2
4	Delphinidin O-pentoside	4.98	C <sub>20</sub> H <sub>19</sub> O <sub>11</sub> <sup>+</sup>	435.0920	−0.5	303.0502; 247.0605; 229.0499; 153.0185; 149.0237	2
5	Cyanidin O-hexoside (II)	5.07	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup>	449.1088	2.1	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
6	Delphinidin O-hexoside (III)	5.95	C <sub>21</sub> H <sub>21</sub> O <sub>12</sub> <sup>+</sup>	465.1038	2.1	303.0502; 247.0605; 229.0499; 153.0185; 149.0237	2
7	Pelargonidin O-hexoside	6.25	C <sub>21</sub> H <sub>21</sub> O <sub>10</sub> <sup>+</sup>	433.1127	−0.4	271.0608; 215.0707; 197.0601; 149.0237; 121.0285	2
8	Cyanidin O-deoxyhexoside	6.54	C <sub>21</sub> H <sub>21</sub> O <sub>10</sub> <sup>+</sup>	433.1126	1.9	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
9	Cyanidin O-pentoside (I)	6.71	C <sub>20</sub> H <sub>19</sub> O <sub>10</sub> <sup>+</sup>	419.0976	0.8	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
10	Pelargonidin O-pentoside	7.66	C <sub>20</sub> H <sub>19</sub> O <sub>9</sub> <sup>+</sup>	403.1030	1.6	271.0608; 215.0707; 197.0601; 149.0237; 121.0285	2
11	Cyanidin O-hexoside (III)	7.98	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup>	449.1088	2.2	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
12	Peonidin 3-O-glucoside	8.34	C <sub>22</sub> H <sub>23</sub> O <sub>11</sub> <sup>+</sup>	463.1247	2.7	301.0707; 286.0472; 149.0237; 121.0285	1

Table 1. Cont.

Id	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ mass (ppm)	Diagnostic Product Ions ( $m/z$ )	c. l.
13	Peonidin O-pentoside	8.75	C <sub>21</sub> H <sub>21</sub> O <sub>10</sub> <sup>+</sup>	433.1136	1.5	301.0707; 286.0472; 149.0237; 121.0285	2
14	Cyanidin O-hexoside (IV)	8.88	C <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup>	449.1088	2.1	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
15	Delphinidin O-dihexoside	9.40	C <sub>27</sub> H <sub>31</sub> O <sub>17</sub> <sup>+</sup>	627.1570	2.2	303.0502; 247.0605; 229.0499; 153.0185; 149.0237	2
16	Cyanidin O-pentoside (II)	9.51	C <sub>20</sub> H <sub>19</sub> O <sub>10</sub> <sup>+</sup>	419.0976	0.8	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	2
17	Cyanidin	10.23	C <sub>15</sub> H <sub>11</sub> O <sub>6</sub> <sup>+</sup>	287.0541	−3.2	287.0553; 231.0656; 213.0550; 149.0237; 137.0235	1
18	Cyanidin isomer	12.06	C <sub>15</sub> H <sub>11</sub> O <sub>6</sub> <sup>+</sup>	287.0541	−3.2	287.0553; 231.0656; 213.0550; 149.0237; 147.0445; 137.0235	2

Table 2. Retention times (Rt, min), proposed formulas, experimental  $m/z$ , accuracy ( $\Delta$ , ppm), main diagnostic experimental product ions, and confidence level of the identification (c. l.) of the 87 tentatively identified flavonoids in *Disterigma alaternoides* extract in ESI(−).

Id	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ (ppm)	Diagnostic Product Ions ( $m/z$ )	c. l.
19	(Epi)catechin O-hexoside	3.47	C <sub>21</sub> H <sub>24</sub> O <sub>11</sub>	451.1253	1.6	289.0719; 245.0817; 137.0245; 125.0244;	2
20	Epicatechin	6.30	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	289.0724	1.8	289.0719; 245.0817; 137.0245; 125.0244;	1
21	Taxifolin isomer	7.05	C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>	303.0515	1.7	259.0613; 193.0142; 167.0350; 165.0193; 137.0245	2
22	Quercetin O-dihexoside	7.05	C <sub>27</sub> H <sub>30</sub> O <sub>17</sub>	625.1430	3.1	301.0354; 178.9985; 151.0036; 121.0295	2
23	Aromadendrin O-hexoside	7.25	C <sub>21</sub> H <sub>22</sub> O <sub>11</sub>	449.1100	2.4	287.0561; 151.0036; 125.0244; 107.0139	2
24	Taxifolin O-hexoside	7.65	C <sub>21</sub> H <sub>22</sub> O <sub>12</sub>	465.1057	4.0	303.0512; 177.0194; 151.0036; 125.0244	2
25	Eriodictyol O-pentoside	8.21	C <sub>20</sub> H <sub>20</sub> O <sub>10</sub>	419.0995	2.7	287.0561; 177.0194; 151.0036; 135.0452	2
26	Quercetin O-hexoside O-deoxyhexoside	8.70	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	609.1475	2.3	463.0859; 447.0949; 301.0354; 178.9985; 151.0036; 121.0295	2
27	Taxifolin	9.26	C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>	303.0520	3.3	303.0512; 241.0407; 177.0194; 151.0036; 125.0244	1
28	Quercetin O-dihexoside	9.38	C <sub>27</sub> H <sub>30</sub> O <sub>17</sub>	625.1430	3.2	463.0880; 301.0354; 178.9985; 151.0036; 121.0295	2
29	Myricetin 3-O-hexoside	9.55	C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>	479.0854	2.7	317.0303; 316.0242; 178.9985; 151.0036; 137.0244	2
30	Myricetin O-pentoside	9.58	C <sub>20</sub> H <sub>18</sub> O <sub>12</sub>	449.0738	2.7	317.0303; 178.9985; 151.0036; 137.0244	2
31	Myricetin 3-O-hexoside	9.86	C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>	479.0844	2.6	317.0303; 316.0242; 178.9985; 151.0036; 137.0244	2
32	Isorhamnetin O-glucuronide	9.96	C <sub>22</sub> H <sub>20</sub> O <sub>13</sub>	491.0844	2.7	315.0512; 300.0275; 255.0296; 151.0036	2
33	Naringenin O-hexoside	10.03	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	433.1150	2.2	271.0613; 227.0713; 177.0194; 151.0036; 119.0503	2
34	Myricetin O-hexoside O-pentoside	10.04	C <sub>26</sub> H <sub>28</sub> O <sub>17</sub>	611.1266	2.0	479.0833; 449.0729; 317.0303; 316.0242; 178.9985; 151.0036; 137.0244	2
35	Myricetin O-pentoside	10.07	C <sub>20</sub> H <sub>18</sub> O <sub>12</sub>	449.0735	2.0	317.0303; 178.9985; 151.0036; 137.0244	2
36	Quercetin O-dihexoside	10.08	C <sub>27</sub> H <sub>30</sub> O <sub>17</sub>	625.1426	2.6	463.0880; 301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
37	Myricetin O-pentoside	10.16	C <sub>20</sub> H <sub>18</sub> O <sub>12</sub>	449.0736	2.2	317.0303; 178.9985; 151.0036; 137.0244	2
38	Quercetin O-hexoside O-pentoside	10.33	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1314	1.5	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
39	Taxifolin isomer	10.36	C <sub>15</sub> H <sub>12</sub> O <sub>7</sub>	303.0520	3.2	285.0407; 241.0407; 177.0194; 151.0036; 125.0244	2
40	Quercetin O-hexosylpentoside	10.62	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1313	1.4	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2

Table 2. Cont.

Id	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ (ppm)	Diagnostic Product Ions ( $m/z$ )	c. l.
41	Myricetin isomer	10.63	C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>	317.0308	1.7	317.0303; 178.9985; 151.0036; 137.0244	2
42	Naringenin O-hexoside	10.81	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	433.1144	0.9	271.0613; 227.0713; 177.0194; 151.0036; 119.0503	2
43	Eriodictyol O-hexoside	10.84	C <sub>21</sub> H <sub>22</sub> O <sub>11</sub>	449.1101	2.7	287.0561; 177.0194; 151.0036; 135.0452	2
44	Shiikimoyl Kaempferol	11.10	C <sub>22</sub> H <sub>18</sub> O <sub>10</sub>	441.0838	2.5	285.0407; 243.0296; 241.0502; 151.0036; 133.0295	2
45	Myricetin 3-O-pentoside	11.11	C <sub>20</sub> H <sub>18</sub> O <sub>12</sub>	449.0735	2.0	317.0303; 316.0242; 178.9985; 151.0036; 137.0244	2
46	Quercetin O-hexoside O-pentoside	11.18	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1310	1.0	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
47	Myricetin O-pentoside	11.21	C <sub>20</sub> H <sub>18</sub> O <sub>12</sub>	449.0724	-0.4	317.0303; 178.9985; 151.0036; 137.0244	2
48	Myricetin 3-O-deoxyhexoside	11.25	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0895	2.8	317.0303; 316.0242; 178.9985; 151.0036; 137.0244	2
49	Quercetin O-hexoside O-pentoside	11.27	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1315	1.8	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
50	Quercetin O-hexoside O-pentoside	11.55	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1321	2.7	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
51	Quercetin 3-O-galactoside	11.58	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0883	0.2	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
52	Quercetin O-hexoside O-pentoside	11.69	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1319	2.3	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
53	Quercetin O-hexoside O-pentoside	11.84	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1321	2.7	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
54	Rutin	11.90	C <sub>27</sub> H <sub>30</sub> O <sub>16</sub>	609.1482	3.5	463.0859; 301.0354; 178.9985; 151.0036; 121.0295	1
55	Quercetin 3-O-glucoside	12.09	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0884	0.4	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	1
56	Aromadendrin	12.15	C <sub>15</sub> H <sub>12</sub> O <sub>6</sub>	287.0570	3.1	287.0561; 269.0456; 177.0194; 151.0036; 125.0244	2
57	Quercetin O-dipentoside	12.26	C <sub>25</sub> H <sub>26</sub> O <sub>15</sub>	565.1212	2.3	433.0776; 301.0354; 178.9985; 151.0036; 121.0295	2
58	Quercetin O-hexoside O-pentoside	12.30	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1318	2.3	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
59	Quercetin O-hexoside O-pentoside	12.37	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1317	2.0	463.0901; 433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
60	Kaempferol O-hexoside	12.45	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0950	3.9	285.0403; 257.0457; 229.0504; 151.0036	2
61	Quercetin 3-O-pentoside	12.62	C <sub>20</sub> H <sub>18</sub> O <sub>11</sub>	433.0776	-0.1	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
62	Quercetin O-hexosylpentoside	12.87	C <sub>26</sub> H <sub>28</sub> O <sub>16</sub>	595.1320	2.5	433.0777; 301.0354; 178.9985; 151.0036; 121.0295	2
63	Eriodictyol O-hexoside	12.91	C <sub>21</sub> H <sub>22</sub> O <sub>11</sub>	449.1100	2.4	287.0561; 177.0194; 151.0036; 135.0452	2
64	Quercetin O-pentoside	12.99	C <sub>20</sub> H <sub>18</sub> O <sub>11</sub>	433.0775	-0.2	301.0354; 178.9985; 151.0036; 121.0295	2
65	Kaempferol 3-O-hexoside	13.14	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0944	2.5	285.0403; 255.0300; 227.0351; 151.0036	2
66	Naringenin O-hexoside	13.28	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	433.1146	1.3	271.0613; 227.0713; 177.0194; 151.0036; 119.0503	2
67	Quercetin O-dipentoside	13.36	C <sub>25</sub> H <sub>26</sub> O <sub>15</sub>	565.1214	2.6	433.0776; 301.0354; 178.9985; 151.0036; 121.0295	2
68	Quercetin O-pentoside	13.51	C <sub>20</sub> H <sub>18</sub> O <sub>11</sub>	433.0775	-0.3	301.0354; 178.9985; 151.0036; 121.0295	2
69	Quercetin O-pentoside	13.84	C <sub>20</sub> H <sub>18</sub> O <sub>11</sub>	433.0776	-0.2	301.0354; 178.9985; 151.0036; 121.0295	2
70	Myricetin	13.89	C <sub>15</sub> H <sub>10</sub> O <sub>8</sub>	317.0310	2.2	317.0303; 178.9985; 151.0036; 137.0244	2
71	Quercetin 3-O-deoxyhexoside	14.04	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0933	0.0	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
72	Quercetin O-hexoside	14.18	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0899	3.7	301.0354; 178.9985; 151.0036; 121.0295	2
73	Isorhamnetin 3-O-hexoside	14.27	C <sub>22</sub> H <sub>22</sub> O <sub>12</sub>	477.1052	2.8	315.0512; 314.0436; 300.0275; 271.0244; 151.0036	2
74	Kaempferol O-pentoside	14.40	C <sub>20</sub> H <sub>18</sub> O <sub>10</sub>	417.0833	1.4	285.0403; 151.0036; 107.0139	2

Table 2. Cont.

Id	Name	Rt (min)	Proposed Formula	Experimental <i>m/z</i>	$\Delta$ (ppm)	Diagnostic Product Ions ( <i>m/z</i> )	c. l.
75	Isorhamnetin 3-O-hexoside	14.71	C <sub>22</sub> H <sub>22</sub> O <sub>12</sub>	477.1049	2.3	315.0512; 314.0433; 300.0275; 271.0244; 151.0036	2
76	Kaempferol O-pentoside	14.74	C <sub>20</sub> H <sub>18</sub> O <sub>10</sub>	417.0834	1.6	285.0403; 151.0036; 107.0139	2
77	Phloretin O-hexoside	14.95	C <sub>21</sub> H <sub>24</sub> O <sub>10</sub>	435.1310	3.0	273.0771; 167.0350; 123.0452	2
78	Myricetin isomer O-hexoside	15.17	C <sub>21</sub> H <sub>20</sub> O <sub>13</sub>	479.0846	3.1	317.0303; 271.0259; 178.9985; 151.0036; 137.0244	2
79	Quercetin O-acetylhexoside	15.23	C <sub>23</sub> H <sub>22</sub> O <sub>13</sub>	507.1140	1.3	ESI(+): 303.0502; 165.0186; 153.0185; 137.0237	2
80	Diosmetin O-hexoside	15.30	C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>	463.1248	2.8	ESI(+): 301.0715; 286.0479; 258.0530; 153.0185	2
81	Kaempferol 3-O-pentoside	15.46	C <sub>20</sub> H <sub>18</sub> O <sub>10</sub>	417.0832	1.2	285.0403; 284.0327; 151.0036; 107.0139	2
82	Quercetin O-hexoside	15.56	C <sub>21</sub> H <sub>20</sub> O <sub>12</sub>	463.0894	2.6	301.0354; 178.9985; 151.0036; 121.0295	2
83	Luteolin O-glucuronide	15.67	C <sub>21</sub> H <sub>18</sub> O <sub>12</sub>	461.0741	3.3	285.0407; 243.0296; 241.0502; 151.0036; 133.0295	2
84	Eriodictyol	15.86	C <sub>15</sub> H <sub>12</sub> O <sub>6</sub>	287.0568	2.5	287.0561; 177.0194; 151.0036; 135.0452; 107.0139	1
85	Isorhamnetin 3-O-pentoside	15.86	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0937	0.9	315.0512; 314.0433; 300.0275; 271.0244; 151.0036	2
86	Naringenin O-hexoside	15.88	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	433.1149	2.0	271.0613; 227.0713; 177.0194; 151.0036; 119.0503	2
87	Kaempferol O-pentoside	15.90	C <sub>20</sub> H <sub>18</sub> O <sub>10</sub>	417.0835	1.8	285.0403; 151.0036; 107.0139	2
88	Kaempferol O-deoxyhexoside	16.11	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	433.1136	1.5	ESI(+): 287.0553; 165.0187; 153.0185; 121.0286	2
89	Isorhamnetin 3-O-pentoside	16.41	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	447.0937	0.9	315.0512; 314.0437; 300.0275; 271.0244; 151.0036	2
90	Quercetin 3-O-malonyldeoxyhexoside	16.64	C <sub>24</sub> H <sub>22</sub> O <sub>14</sub>	533.0948	2.0	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
91	Quercetin	17.34	C <sub>15</sub> H <sub>10</sub> O <sub>7</sub>	301.0353	−0.1	273.0411; 245.0454; 178.9985; 151.0036; 121.0295	1
92	Quercetin 3-O-dihydroxybenzoylpentoside	17.68	C <sub>27</sub> H <sub>22</sub> O <sub>14</sub>	569.0950	2.3	301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
93	Luteolin	17.69	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	285.0412	2.7	243.0296; 241.0502; 151.0036; 133.0295	1
94	Quercetin 3-O-coumaroylhexoside	18.05	C <sub>30</sub> H <sub>26</sub> O <sub>14</sub>	609.1266	2.7	463.0883; 301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
95	Quercetin 3-O-coumaroylhexoside	18.28	C <sub>30</sub> H <sub>26</sub> O <sub>14</sub>	609.1262	2.0	463.0883; 301.0354; 300.0278; 178.9985; 151.0036; 121.0295	2
96	Naringenin isomer	18.41	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	271.0614	0.7	227.0713; 177.0194; 151.0036; 119.0503; 107.0139	2
97	Naringenin	18.76	C <sub>15</sub> H <sub>12</sub> O <sub>5</sub>	271.0614	0.7	227.0713; 177.0194; 151.0036; 119.0503; 107.0139	1
98	Quercetin 3-O-dihydroxybenzoylpentoside	19.06	C <sub>27</sub> H <sub>22</sub> O <sub>14</sub>	569.0937	0.0	301.0354; 300.0278; 273.0411; 151.0036; 121.0295	2
99	Quercetin O-dihydroxybenzoylpentoside	19.25	C <sub>27</sub> H <sub>22</sub> O <sub>14</sub>	569.0943	1.2	301.0354; 178.9985; 151.0036; 121.0295	2
100	Hesperetin	19.56	C <sub>16</sub> H <sub>14</sub> O <sub>6</sub>	301.0726	2.7	286.0480; 242.0581; 177.0195; 164.0115; 151.0036	1
101	Apigenin	20.05	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	269.0460	1.6	225.0557; 151.0036; 117.0346; 107.0139	1
102	Kaempferol	20.23	C <sub>15</sub> H <sub>10</sub> O <sub>6</sub>	285.0409	1.4	257.0457; 229.0504; 151.0036; 107.0139	1
103	Diosmetin	20.96	C <sub>16</sub> H <sub>12</sub> O <sub>6</sub>	299.0567	2.1	284.0327; 257.0411; 255.0303; 151.0036; 107.0139	1
104	Isorhamnetin	21.13	C <sub>16</sub> H <sub>12</sub> O <sub>7</sub>	315.0517	2.1	300.0275; 271.0244; 255.0296; 151.0036; 107.0139	1
105	Chrysin	25.50	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	253.0508	0.8	209.0608; 151.0036; 107.0139	2

**Table 3.** Retention times (Rt, min), proposed formulas, experimental  $m/z$ , accuracy ( $\Delta$ , ppm), main diagnostic experimental product ions, and confidence level of the identification (c. l.) of the 108 tentatively identified phenolic acid in *Disterigma alaternoides* extract in ESI(−).

ID	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ (ppm)	Main Product Ions ( $m/z$ )	c. l.
106	Quinic acid	0.50	C <sub>7</sub> H <sub>12</sub> O <sub>6</sub>	191.0555	−3.3	127.0401; 111.0088; 87.0084; 85.0292	2
107	Gallic acid	0.64	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	169.0145	1.4	169.0142; 125.0243	2
108	Methylgallic acid	0.79	C <sub>8</sub> H <sub>8</sub> O <sub>5</sub>	183.0300	0.4	183.0300; 139.0302	2
109	Hydroxybenzoyl hexose (I)	0.82	C <sub>13</sub> H <sub>16</sub> O <sub>8</sub>	299.0776	1.2	137.0244; 93.0343	2
110	Methyldihydroxybenzoic acid	1.04	C <sub>8</sub> H <sub>8</sub> O <sub>4</sub>	167.0352	1.3	167.0350; 123.0453	2
111	Dihydroxybenzoyl hexose (I)	1.08	C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>	315.0727	1.6	315.0727; 153.0194; 109.0296	2
112	Dihydroxybenzoic acid	1.19	C <sub>7</sub> H <sub>6</sub> O <sub>4</sub>	153.0196	1.8	153.0194; 109.0296	2
113	Phloroglucinol carboxylic acid	1.22	C <sub>7</sub> H <sub>6</sub> O <sub>5</sub>	169.0145	1.4	169.0142; 151.0036; 125.0244	2
114	Hexosyl caffeoyl hexose (I)	1.24	C <sub>21</sub> H <sub>28</sub> O <sub>14</sub>	503.1415	1.6	341.0881; 179.0350; 161.0245; 135.0453	2
115	Hydroxybenzoyl hexose (II)	1.29	C <sub>13</sub> H <sub>16</sub> O <sub>8</sub>	299.0777	1.4	299.0777; 137.0244; 93.0343	2
116	Methylhydroxybenzoyl hexose	1.38	C <sub>14</sub> H <sub>18</sub> O <sub>8</sub>	313.0936	2.2	151.0401; 107.0502	2
117	Dihydroxybenzoyl hexose (II)	1.43	C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>	315.0724	0.8	315.0727; 153.0194; 109.0296	2
118	Hydroxybenzoyl hexose (III)	1.62	C <sub>13</sub> H <sub>16</sub> O <sub>8</sub>	299.0777	1.6	299.0777; 137.0244; 93.0343	2
119	Neochlorogenic acid	1.68	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	353.0884	1.6	191.0562; 179.0350; 145.0452; 135.0453	2
120	Dihydroxybenzoyl pentose	1.72	C <sub>12</sub> H <sub>14</sub> O <sub>8</sub>	285.0621	1.7	285.0618; 153.0194; 109.0296	2
121	Chlorogenoyl hexose	1.91	C <sub>22</sub> H <sub>28</sub> O <sub>14</sub>	515.1409	0.6	353.0883; 191.0561; 179.0349; 135.0453	2
122	Hexosyl caffeoyl hexose (I)	2.07	C <sub>21</sub> H <sub>28</sub> O <sub>14</sub>	503.1418	2.3	341.0881; 179.0350; 161.0245; 135.0453	2
123	Caffeoyl hexose (I)	2.10	C <sub>15</sub> H <sub>18</sub> O <sub>9</sub>	341.0883	1.3	341.0881; 179.0350; 135.0453	2
124	Coumaroyl hexose (I)	2.10	C <sub>15</sub> H <sub>18</sub> O <sub>8</sub>	325.0933	1.3	325.0933; 163.0402; 119.0503	2
125	Dihydroxybenzoyl hexose (III)	2.14	C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>	315.0725	1.1	315.0727; 153.0194; 109.0296	2
126	Dihydroxybenzoyl hexose (IV)	2.37	C <sub>13</sub> H <sub>16</sub> O <sub>9</sub>	315.0725	1.1	315.0727; 153.0194; 109.0296	2
127	Hydroxyferuloyl hexose (I)	2.74	C <sub>16</sub> H <sub>20</sub> O <sub>10</sub>	371.0988	1.2	209.0456; 191.0352; 147.0452; 119.0503	2
128	Quinoyl coumaric acid	2.76	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	337.0935	1.7	191.0561; 173.0459; 163.0400; 119.0502	2
129	Caffeoyl hexose (II)	3.21	C <sub>15</sub> H <sub>18</sub> O <sub>9</sub>	341.0880	0.7	179.0350; 135.0453	2
130	Caffeoyl hexose (III)	3.49	C <sub>15</sub> H <sub>18</sub> O <sub>9</sub>	341.0879	0.2	179.0350; 161.0244; 135.0453	2
131	Caffeic acid	3.58	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	179.0352	1.2	179.0350; 135.0453	2
132	Feruloyl hexose (I)	3.60	C <sub>16</sub> H <sub>20</sub> O <sub>9</sub>	355.1040	1.5	193.0507; 178.0272; 149.0608; 134.0374	2
133	Coumaroyl hexose (II)	3.61	C <sub>15</sub> H <sub>18</sub> O <sub>8</sub>	325.0934	1.5	325.0933; 163.0402; 119.0503	2
134	Chlorogenic acid	3.76	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	353.0880	0.4	353.0879; 191.0562; 179.0350; 145.0452	2
135	Hydroxyferuloyl hexose (II)	4.04	C <sub>16</sub> H <sub>20</sub> O <sub>10</sub>	371.0988	1.2	209.0456; 191.0352; 147.0452; 119.0503	2
136	Hydroxyferulic acid	4.82	C <sub>10</sub> H <sub>10</sub> O <sub>5</sub>	209.0458	1.1	191.0352; 147.0452; 119.0503	2
137	Sinapoyl hexose (I)	5.23	C <sub>17</sub> H <sub>22</sub> O <sub>10</sub>	385.1145	1.2	223.0611; 208.0377; 193.0141; 179.0913; 164.0478; 149.0243; 121.0295	2
138	Feruloyl hexose (II)	5.68	C <sub>16</sub> H <sub>20</sub> O <sub>9</sub>	355.1037	0.7	193.0507; 178.0272; 149.0608; 134.0374	2
139	Chlorogenic acid isomer	5.93	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	353.0886	2.3	353.0879; 191.0562; 179.0350; 145.0452	2
140	Coumaric acid (I)	6.06	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	163.0404	2.0	163.0402; 119.0503	2
141	Coumaroyl quinic acid (I)	6.19	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	337.0936	2.0	191.0560; 173.0455; 163.0401; 119.0501	2
142	Caffeoyl shiikimoyl hexose (I)	6.46	C <sub>22</sub> H <sub>26</sub> O <sub>13</sub>	497.1307	1.3	335.0773; 179.0349; 135.0451	2
143	Hydroxyferulic acid isomer	6.61	C <sub>10</sub> H <sub>10</sub> O <sub>5</sub>	209.0458	1.1	209.0456; 165.0557; 123.0452; 81.0342	2
144	Coumaric acid (II)	6.69	C <sub>9</sub> H <sub>8</sub> O <sub>3</sub>	163.0404	2.1	163.0402; 119.0503	2
145	Acetyl dihydroxybenzoic acid	6.71	C <sub>9</sub> H <sub>8</sub> O <sub>5</sub>	195.0301	1.2	195.0299; 153.0194; 109.0296	2
146	Sinapoyl hexose (II)	7.06	C <sub>17</sub> H <sub>22</sub> O <sub>10</sub>	385.1146	1.4	223.0611; 208.0377; 193.0141; 179.0913; 164.0478; 149.0243; 121.0295	2
147	Caffeoyl shiikimic acid (I)	7.15	C <sub>16</sub> H <sub>16</sub> O <sub>8</sub>	335.0777	1.3	179.0349; 173.0452; 161.0242; 135.0451	2
148	Coumaroyl hexose (III)	7.35	C <sub>15</sub> H <sub>18</sub> O <sub>8</sub>	325.0936	2.1	325.0933; 163.0402; 119.0503	2
149	Feruloyl quinic acid	7.88	C <sub>17</sub> H <sub>20</sub> O <sub>9</sub>	367.1041	1.8	193.0507; 191.0560; 178.0272; 173.0455; 149.0608; 134.0374	2

Table 3. Cont.

ID	Name	Rt (min)	Proposed Formula	Experimental <i>m/z</i>	$\Delta$ (ppm)	Main Product Ions ( <i>m/z</i> )	c. l.
150	Coumaroyl quinic acid (II)	8.11	C <sub>16</sub> H <sub>18</sub> O <sub>8</sub>	337.0936	2.0	191.0560; 173.0455; 163.0401; 119.0501	2
151	Ferulic acid	8.16	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0509	1.5	178.0272; 149.0608; 134.0374	1
152	Feruloyl shiikimoyl hexose	8.25	C <sub>23</sub> H <sub>28</sub> O <sub>13</sub>	511.1468	2.1	193.0507; 178.0272; 149.0608; 134.0374	2
153	Caffeoyl hexosyl arbutin (I)	8.93	C <sub>27</sub> H <sub>32</sub> O <sub>15</sub>	595.1683	2.4	433.1143; 323.0779; 179.0348; 161.0245; 135.0454	2
154	Caffeoyl shiikimic acid (II)	9.01	C <sub>16</sub> H <sub>16</sub> O <sub>8</sub>	335.0780	2.3	179.0349; 173.0452; 161.0242; 135.0451	2
155	Caffeoyl shiikimoyl hexose (II)	9.29	C <sub>22</sub> H <sub>26</sub> O <sub>13</sub>	497.1310	1.8	335.0773; 179.0349; 161.0245; 135.0451	2
156	Sinapic acid	9.33	C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	223.0617	2.4	208.0377; 193.0141; 179.0913; 164.0478; 149.0243; 121.0295	2
157	Coumaroyl shiikimic acid	9.65	C <sub>16</sub> H <sub>16</sub> O <sub>7</sub>	319.0829	1.8	173.0454; 163.0402; 155.0350; 119.0503	2
158	Acetyl caffeoyl deoxyhexoside	9.91	C <sub>17</sub> H <sub>20</sub> O <sub>9</sub>	367.1037	0.6	179.0350; 161.0244; 135.0452	2
159	Caffeoyl hexosyl arbutin (II)	9.95	C <sub>27</sub> H <sub>32</sub> O <sub>15</sub>	595.1683	2.4	433.1143; 323.0779; 179.0348; 135.0454	2
160	Caffeoyl hexosyl arbutin (III)	10.05	C <sub>27</sub> H <sub>32</sub> O <sub>15</sub>	595.1686	2.9	433.1143; 323.0779; 179.0348; 135.0454	2
161	Hydroxybenzoyl arbutin (I)	10.12	C <sub>19</sub> H <sub>20</sub> O <sub>9</sub>	391.1044	2.4	281.0669; 137.0245; 109.0296; 93.0344	2
162	Caffeoyl hexosyl trihydroxymethoxyphenyl propanoic acid	10.16	C <sub>25</sub> H <sub>28</sub> O <sub>14</sub>	551.1416	1.8	389.0873; 345.0975; 327.0873; 179.0349; 165.0557; 161.0243; 135.0451; 121.0296	2
163	Caffeoyl arbutin (I)	11.05	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	433.1139	−0.3	323.0778; 179.0351; 161.0244; 135.0453; 133.0295; 109.0295	2
164	Galloyl valeryl hexoside	11.26	C <sub>18</sub> H <sub>24</sub> O <sub>11</sub>	415.1255	2.2	169.0142; 125.0244	2
165	Caffeoyl arbutin (II)	11.35	C <sub>21</sub> H <sub>22</sub> O <sub>10</sub>	433.1139	−0.3	323.0778; 179.0351; 161.0244; 135.0453; 133.0295; 109.0295	2
166	Ferulic acid isomer	11.85	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	193.0509	1.5	175.0402; 149.0608	2
167	Coumaroyl iridoid (I)	11.98	C <sub>25</sub> H <sub>28</sub> O <sub>13</sub>	535.1460	0.6	191.0350; 163.0400; 147.0452; 119.0502	2
168	Coumaroyl iridoid (II)	12.04	C <sub>25</sub> H <sub>28</sub> O <sub>13</sub>	535.1460	0.6	191.0350; 163.0400; 147.0452; 119.0502	2
169	Caffeoyl methoxyarbutin	12.31	C <sub>22</sub> H <sub>24</sub> O <sub>11</sub>	463.1249	0.7	323.0771; 179.0349; 161.0244; 139.0401; 135.0453; 124.0166	2
170	Coumaroyl coumaric acid	12.55	C <sub>18</sub> H <sub>14</sub> O <sub>5</sub>	309.0776	2.4	163.0401; 119.0503	2
171	Caffeoyl dihydroxybenzoyl hexose	13.63	C <sub>22</sub> H <sub>22</sub> O <sub>12</sub>	477.1046	1.6	315.0724; 179.0347; 153.0193; 135.0451; 109.0296	2
172	Dicaffeoyl hexoside	14.36	C <sub>24</sub> H <sub>24</sub> O <sub>12</sub>	503.1213	3.6	341.0875; 323.0778; 179.0351; 135.0453	2
173	Dihydroxybenzoyl valeryl hexose (I)	14.44	C <sub>18</sub> H <sub>24</sub> O <sub>10</sub>	399.1303	1.5	153.0193; 109.0294	2
174	Coumaroyl dihydroxybenzoyl hexose (II)	14.81	C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>	461.1101	2.6	315.0741; 153.0195; 109.0296	2
175	Dihydroxybenzoyl valeryl hexose (II)	14.95	C <sub>18</sub> H <sub>24</sub> O <sub>10</sub>	399.1303	1.5	399.1290; 153.0193; 109.0294	2
176	Coumaroyl coumaric acid	15.14	C <sub>18</sub> H <sub>14</sub> O <sub>5</sub>	309.0777	2.7	309.0776; 163.0401; 119.0503	2
177	Dihydroxybenzoyl benzoyl hexose (I)	15.15	C <sub>20</sub> H <sub>20</sub> O <sub>10</sub>	419.0991	1.8	315.0727; 153.0194; 109.0296	2
178	Coumaroyl hydroxybenzoyl hexose	15.24	C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>	445.1146	1.2	307.0822; 163.0403; 145.0294; 137.0243; 119.0501; 93.0343	2
179	Sinapoyl coumaroyl hexose	15.31	C <sub>26</sub> H <sub>28</sub> O <sub>12</sub>	531.1520	2.3	531.1481; 307.0823; 223.0612; 208.0378; 193.0141; 163.0400; 149.0243; 119.0503	2
180	Hydroxybenzoyl arbutin (II)	15.52	C <sub>19</sub> H <sub>20</sub> O <sub>9</sub>	391.1044	2.5	391.1047; 281.0669; 137.0244; 93.0343	2
181	Dihydroxybenzoyl benzoyl hexose (II)	15.58	C <sub>20</sub> H <sub>20</sub> O <sub>10</sub>	419.0994	2.5	315.0727; 153.0194; 109.0296	2
182	Caffeoyl acetyl arbutin (I)	15.71	C <sub>23</sub> H <sub>24</sub> O <sub>11</sub>	475.1246	0.0	179.0348; 161.0244; 135.0452; 133.0295	2
183	Caffeoyl acetyl arbutin (II)	15.84	C <sub>23</sub> H <sub>24</sub> O <sub>11</sub>	475.1246	0.0	179.0348; 161.0244; 135.0452; 133.0295	2
184	Chlorogenic acid derivative (I)	15.85	C <sub>26</sub> H <sub>28</sub> O <sub>12</sub>	531.1516	1.5	353.0879; 191.0562; 179.0350; 145.0452	3



Table 3. Cont.

ID	Name	Rt (min)	Proposed Formula	Experimental <i>m/z</i>	$\Delta$ (ppm)	Main Product Ions ( <i>m/z</i> )	c. l.
185	Hydroxybenzoyl benzoyl hexose (I)	15.86	C <sub>20</sub> H <sub>20</sub> O <sub>9</sub>	403.1080	2.0	137.0244; 93.0344	2
186	Caffeoyl coumaroyl hexose	16.06	C <sub>24</sub> H <sub>24</sub> O <sub>11</sub>	487.1257	2.2	323.0772; 179.0349; 163.0399; 119.0501	2
187	Dihydroxybenzoyl valeryl hexose (III)	16.32	C <sub>18</sub> H <sub>24</sub> O <sub>10</sub>	399.1303	1.5	153.0193; 109.0294	2
188	Caffeoyl feruloyl hexose (I)	16.43	C <sub>25</sub> H <sub>26</sub> O <sub>12</sub>	517.1358	1.5	337.0929; 179.0349; 175.0399; 135.0452	2
189	Coumaroyl dihydroxybenzoyl hexose (I)	16.46	C <sub>22</sub> H <sub>22</sub> O <sub>11</sub>	461.1101	2.5	315.0741; 153.0195; 109.0296	2
190	Dihydroxybenzoyl dihydroxybenzoic acid	16.60	C <sub>14</sub> H <sub>10</sub> O <sub>7</sub>	289.0362	2.8	153.0194; 109.0296	2
191	Caffeoyl feruloyl hexose (II)	16.64	C <sub>25</sub> H <sub>26</sub> O <sub>12</sub>	517.1358	1.5	179.0349; 175.0399; 135.0452	2
192	Dicoumaroyl hexose (I)	16.87	C <sub>24</sub> H <sub>24</sub> O <sub>10</sub>	471.1305	1.8	307.0826; 163.0400; 145.0294; 119.0502	2
193	Feruloyl dihydroxybenzoyl hexose	17.19	C <sub>23</sub> H <sub>24</sub> O <sub>12</sub>	491.1204	1.8	315.0724; 193.0505; 175.0401; 160.0165; 153.0192; 134.0371; 109.0294	2
194	Coumaroyl Feruloyl hexose (I)	17.28	C <sub>25</sub> H <sub>26</sub> O <sub>11</sub>	501.1413	2.0	337.0936; 307.0824; 193.0507; 178.0272; 163.0400; 149.0608; 134.0374; 119.0502	2
195	Caffeoyl valeryl hexose (I)	17.32	C <sub>20</sub> H <sub>26</sub> O <sub>10</sub>	425.1459	1.3	179.0350; 135.0451	3
196	Caffeoyl valeryl hexose (II)	17.39	C <sub>20</sub> H <sub>26</sub> O <sub>10</sub>	425.1457	0.9	179.0350; 135.0451	3
197	Caffeoyl benzoyl hexose (I)	17.40	C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>	445.1146	1.3	179.0345; 135.0452; 121.0296	2
198	Diferuloyl hexose	17.61	C <sub>26</sub> H <sub>28</sub> O <sub>12</sub>	531.1521	2.4	337.0936; 193.0507; 178.0272; 134.0374	2
199	Caffeoyl benzoyl hexose (II)	17.72	C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>	445.1149	1.9	179.0345; 135.0452	2
200	Hydroxybenzoyl benzoyl hexose (II)	17.84	C <sub>20</sub> H <sub>20</sub> O <sub>9</sub>	403.1080	2.0	137.0244; 93.0344	2
201	Dicafeoyl shiikimic acid (II)	17.94	C <sub>25</sub> H <sub>22</sub> O <sub>11</sub>	497.1080	−1.9	179.0350; 161.0243; 135.0451	2
202	Coumaroyl methylhydroxybenzoyl hexose (I)	17.95	C <sub>23</sub> H <sub>24</sub> O <sub>10</sub>	459.1302	1.2	307.0818; 163.0402; 145.0295; 119.0502	2
203	Coumaroyl valeryl hexose (I)	17.96	C <sub>20</sub> H <sub>26</sub> O <sub>9</sub>	409.1513	2.2	163.0399; 119.0501	3
204	Dicoumaroyl hexose (II)	18.04	C <sub>24</sub> H <sub>24</sub> O <sub>10</sub>	471.1306	2.0	307.0820; 163.0399; 145.0293; 119.0501	2
205	Dicafeoyl shiikimic acid (I)	18.06	C <sub>25</sub> H <sub>22</sub> O <sub>11</sub>	497.1068	−4.2	179.0350; 161.0243; 135.0451	2
206	Dihydroxybenzoyl benzoyl hexose (III)	18.36	C <sub>20</sub> H <sub>20</sub> O <sub>10</sub>	419.0992	1.9	153.0194; 109.0295	2
207	Feruloyl valeryl hexose	18.38	C <sub>21</sub> H <sub>28</sub> O <sub>10</sub>	439.1618	1.9	193.0507; 178.0272; 149.0608; 134.0374	2
208	Coumaroyl methylhydroxybenzoyl hexose (II)	18.42	C <sub>23</sub> H <sub>24</sub> O <sub>10</sub>	459.1302	1.2	307.0818; 163.0402; 145.0295; 119.0502	2
209	Coumaroyl Feruloyl hexose (II)	18.58	C <sub>25</sub> H <sub>26</sub> O <sub>11</sub>	501.1413	2.0	193.0507; 175.0401; 163.0400; 160.0165; 149.0608; 145.0295; 134.0374; 119.0502	2
210	Chlorogenic acid derivative (II)	19.14	C <sub>26</sub> H <sub>28</sub> O <sub>12</sub>	531.1512	0.7	353.0879; 191.0562; 179.0350; 145.0452	2
211	Caffeoyl dihydroxybenzoyl mevalonic acid	19.47	C <sub>22</sub> H <sub>22</sub> O <sub>10</sub>	445.1149	1.9	179.0345; 153.0194; 135.0452; 109.0296	2
212	Coumaroyl valeryl hexose (II)	19.77	C <sub>20</sub> H <sub>26</sub> O <sub>9</sub>	409.1512	1.8	409.1500; 163.0399; 119.0501	3
213	Caffeoyl cinnamoyl hexose	20.26	C <sub>24</sub> H <sub>24</sub> O <sub>10</sub>	471.1303	1.3	471.1230; 179.0350; 135.0452	2

**Table 4.** Retention times (Rt, min), proposed formulas, experimental  $m/z$ , accuracy ( $\Delta$ , ppm), main diagnostic experimental product ions, and confidence level of the identification (c. l.) of the 36 annotated proanthocyanidins in *Disterigma alaternoides* extract in ESI(-).

ID	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ (ppm)	Main Product Ions ( $m/z$ )	c. l.
214	A-Procyanidin tetramer	2.56	C <sub>60</sub> H <sub>48</sub> O <sub>24</sub>	575.1209	2.5	981.1918; 863.1859; 829.1519; 693.1247; 573.1050; 451.1032; 425.0874; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
215	B-Procyanidin dimer	5.73	C <sub>30</sub> H <sub>26</sub> O <sub>12</sub>	577.1365	2.3	451.1032; 425.0874; 407.0772; 289.0720; 287.0568; 245.0828; 137.0244; 125.0244	2
216	A-Procyanidin pentamer	5.78	C <sub>75</sub> H <sub>60</sub> O <sub>30</sub>	719.1533	3.0	981.1981; 863.1859; 711.1358; 693.1247; 575.1198; 573.1064; 451.1054; 449.0878; 423.0739; 411.0740; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
217	B-Procyanidin trimer	5.94	C <sub>45</sub> H <sub>38</sub> O <sub>18</sub>	865.2004	2.2	713.1520; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 137.0244; 125.0244	2
218	B-Procyanidin tetramer	7.08	C <sub>60</sub> H <sub>50</sub> O <sub>24</sub>	576.1287	2.4	577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2
219	A-Procyanidin trimer	7.33	C <sub>45</sub> H <sub>36</sub> O <sub>18</sub>	863.1846	2.0	711.1358; 693.1247; 573.1050; 451.1032; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
220	B-Procyanidin tetramer	7.59	C <sub>60</sub> H <sub>50</sub> O <sub>24</sub>	576.1291	3.0	577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2
221	A-Procyanidin tetramer	8.14	C <sub>60</sub> H <sub>48</sub> O <sub>24</sub>	575.1213	3.2	693.1247; 573.1050; 451.1032; 425.0874; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
222	A-Procyanidin trimer	8.38	C <sub>45</sub> H <sub>36</sub> O <sub>18</sub>	863.1840	1.3	711.1358; 693.1247; 573.1050; 451.1032; 425.0874; 411.0724; 289.0720; 285.0406; 137.0245; 125.0245	2
223	B-Procyanidin trimer	8.71	C <sub>45</sub> H <sub>38</sub> O <sub>18</sub>	865.2002	1.9	713.1520; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 137.0244; 125.0244	2
224	B-Procyanidin pentamer	8.92	C <sub>75</sub> H <sub>62</sub> O <sub>30</sub>	720.1611	2.9	863.1821; 693.1250; 577.1334; 575.1216; 451.1032; 425.0874; 289.0720; 287.0562; 137.0244; 125.0244	2
225	A-Procyanidin tetramer	9.60	C <sub>60</sub> H <sub>48</sub> O <sub>24</sub>	575.1209	2.6	863.1859; 711.1330; 693.1247; 573.1050; 451.1032; 425.0874; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
226	B-Procyanidin tetramer	9.65	C <sub>60</sub> H <sub>50</sub> O <sub>24</sub>	576.1288	2.5	577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2
227	A-Proanthocyanidin dimer (galcat-cat)	9.98	C <sub>30</sub> H <sub>24</sub> O <sub>13</sub>	591.1151	1.2	591.1118; 465.0753; 439.0693; 303.0506; 285.0406; 137.0245; 125.0245	2
228	B-Procyanidin pentamer	10.15	C <sub>75</sub> H <sub>62</sub> O <sub>30</sub>	720.1606	2.3	863.1821; 737.1509; 693.1250; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 137.0244; 125.0244	2
229	A-Procyanidin trimer	10.54	C <sub>45</sub> H <sub>36</sub> O <sub>18</sub>	863.1844	1.7	711.1358; 693.1247; 575.1198; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2

Table 4. Cont.

ID	Name	Rt (min)	Proposed Formula	Experimental <i>m/z</i>	$\Delta$ (ppm)	Main Product Ions ( <i>m/z</i> )	c. l.
230	B-Procyanidin pentamer	10.61	C <sub>75</sub> H <sub>62</sub> O <sub>30</sub>	720.1603	1.7	721.1294; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 137.0244; 125.0244	2
231	B-Procyanidin tetramer	10.75	C <sub>60</sub> H <sub>50</sub> O <sub>24</sub>	576.1290	2.9	577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2
232	A-Procyanidin pentamer	10.82	C <sub>75</sub> H <sub>60</sub> O <sub>30</sub>	719.1547	4.9	1115.2210; 861.1715; 719.1268; 577.1363; 451.1054; 449.0878; 425.0880; 411.0740; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
233	A-Procyanidin tetramer	10.94	C <sub>60</sub> H <sub>48</sub> O <sub>24</sub>	575.1213	3.1	575.1198; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
234	B-Procyanidin hexamer	11.07	C <sub>90</sub> H <sub>74</sub> O <sub>36</sub>	864.1883	-2.8	1151.2379; 865.1873; 863.1821; 695.1427; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2
235	B-Procyanidin hexamer	11.42	C <sub>90</sub> H <sub>74</sub> O <sub>36</sub>	864.1910	0.3	1151.2379; 865.1873; 863.1821; 695.1427; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2
236	A-Procyanidin pentamer	11.87	C <sub>75</sub> H <sub>60</sub> O <sub>30</sub>	719.1527	2.1	861.1613; 739.1692; 737.1482; 689.2198; 577.1314; 573.1050; 451.1032; 425.0874; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
237	A-Procyanidin dimer	12.02	C <sub>30</sub> H <sub>24</sub> O <sub>12</sub>	575.1204	1.5	449.0883; 423.0725; 407.0762; 289.0720; 285.0406; 137.0245; 125.0245	2
238	A-Procyanidin hexamer	12.02	C <sub>90</sub> H <sub>72</sub> O <sub>36</sub>	863.1840	1.9	575.1198; 539.0993; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
239	A-Procyanidin pentamer	12.30	C <sub>75</sub> H <sub>60</sub> O <sub>30</sub>	719.1530	2.5	693.1247; 573.1050; 451.1032; 425.0874; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
240	B-Procyanidin dimer	12.35	C <sub>30</sub> H <sub>26</sub> O <sub>12</sub>	577.1369	3.0	577.1334; 451.1032; 425.0874; 407.0772; 289.0720; 287.0568; 245.0828; 137.02443; 125.0244	2
241	A-Procyanidin tetramer	12.44	C <sub>60</sub> H <sub>48</sub> O <sub>24</sub>	575.1212	2.9	863.1859; 711.1358; 693.1247; 575.1198; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245	2
242	B-Procyanidin hexamer	12.49	C <sub>90</sub> H <sub>74</sub> O <sub>36</sub>	864.1878	-2.8	863.1859; 693.1280; 575.1198; 539.0993; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245	2
243	A-Proanthocyanidin trimer (cat-afz-galcat)	12.64	C <sub>45</sub> H <sub>36</sub> O <sub>18</sub>	863.1820	-1.0	711.1358; 693.1247; 575.1198; 449.0878; 433.0771; 407.0772; 301.0340; 289.0720; 137.0245; 125.0245	2
244	A-Procyanidin tetramer	13.15	C <sub>60</sub> H <sub>48</sub> O <sub>24</sub>	575.1210	2.7	573.1050; 451.1032; 425.0874; 411.0724; 407.0772; 289.0720; 285.0406; 137.0245; 125.0245	2
245	B-Procyanidin trimer	13.21	C <sub>45</sub> H <sub>38</sub> O <sub>18</sub>	865.2013	3.4	713.1520; 577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 137.0244; 125.0244	2
246	diA-Procyanidin trimer	13.44	C <sub>45</sub> H <sub>34</sub> O <sub>18</sub>	861.1694	-2.3	693.1247; 575.1198; 571.09454; 449.0878; 289.0720; 285.0406; 137.0245; 125.0245	2

Table 4. Cont.

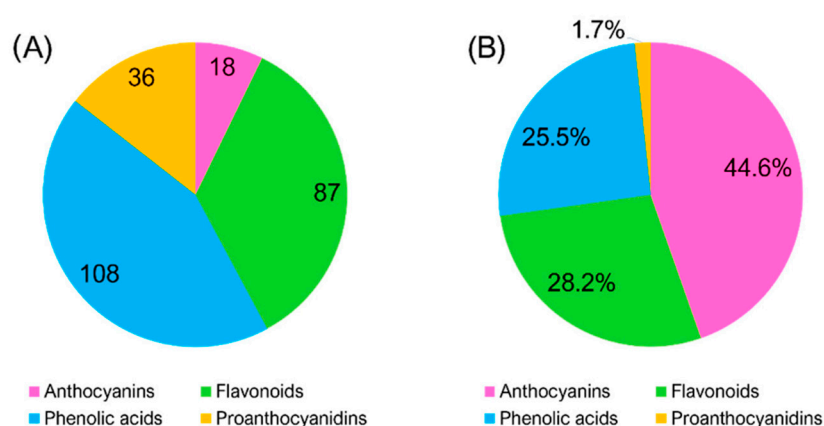
ID	Name	Rt (min)	Proposed Formula	Experimental $m/z$	$\Delta$ (ppm)	Main Product Ions ( $m/z$ )	c. l.
247	A-Procyanidin pentamer	14.11	C <sub>75</sub> H <sub>60</sub> O <sub>30</sub>	719.1526	2.0	863.1859; 711.1358; 693.1247; 575.1198; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245	2
248	A-Procyanidin hexamer	14.80	C <sub>90</sub> H <sub>72</sub> O <sub>36</sub>	863.1845	1.9	863.1859; 693.1280; 575.1198; 539.0993; 449.0878; 423.0739; 407.0772; 289.0720; 285.0406; 137.0245	2
249	B-Procyanidin tetramer	15.14	C <sub>60</sub> H <sub>50</sub> O <sub>24</sub>	576.1293	3.5	577.1334; 575.1216; 451.1032; 425.0874; 407.0772; 289.0720; 287.0562; 245.0828; 137.0244; 125.0244	2

### 3. Results and Discussion

The identification of phenolic compounds is a critical issue in the phytochemical analysis research field. Phenolic compounds are, in fact, a structurally diverse class of compounds, encompassing a wide range of molecular weights, acid-base properties, and structure complexity [27]. Flavonoids, anthocyanins, and phenolic acids are often present as glycoconjugates to sugars or sugar derivatives and/or acylated to aliphatic and aromatic acids. Historically, the analysis of phenolic compounds has been based on UV-Vis spectroscopy due to the extensively aromatic systems in their structures [28]. However, in recent years, untargeted HRMS has become the foremost technique for phenolic compound identification as it allows extending the characterization to a wide range of compounds, also without the need for analytical standards [29]. Because of their extreme complexity, HRMS raw data cannot be handled without using software programs that render accessible the large datasets by  $m/z$  extraction, adduct grouping, and feature alignment. Moreover, when highly composite phytocomplexes are analyzed by untargeted HRMS, there is a need for tools that simplify the datasets for a more accessible manual validation of the compounds.

For the characterization of *D. alaternoides*, untargeted HRMS followed by a suspect screening data processing was employed, based on a methodology that was previously implemented on Compound Discoverer 3.1 by our research group [24]. Because of the wide range of bond energies in phenolic compound structures (from the weak acetal to the strong aromatic bonds), the acquisition was performed with a three-stepped NCE of 20–50–80 and 20–40–60 for the positive and negative ion mode, respectively. To obtain a larger number of chromatographic points per peak, separate chromatographic runs for each polarity were preferred to polarity switching mode. Top 5 DDA mode is widely used for untargeted analysis with orbitrap-based instrumentation as it allows high-quality MS/MS spectra for the five most intense ions for each scan in full-scan mode [30]. Compound Discoverer is based on a system of blocks and nodes that can be customized by the user for the development of specific data-processing methods. For this purpose, an extensive database of 45,567 phenolic compound derivatives was generated by considering flavonoids, phenolic acids, and tannins in their free and conjugated to sugars and acids. The database, which was implemented in the *mass list* tool, was employed to filter the extracted and aligned features to remove calculated masses not included in the database. Manual validation was also aided by the *compound class scoring* tool, which matches the experimental MS/MS to theoretical fragmentation of the flavonoid or phenolic acid core. According to this approach, 16 and 233 phenolic compounds were identified and tentatively identified, respectively. The use of DDA mode for data acquisition allowed the annotation of several compounds even though some of them coeluted. In fact, as precursor ions are sequentially isolated and fragmented with an isolation window of 2  $m/z$ , whenever compounds differing from more than 2 Da coeluted, they could still be tentatively identified. In Figure 1, the classes of identified phenolics are reported in terms of the number of identifications and the total peak areas per

class. The largest class of compounds was phenolic acids with 108 compounds, followed by flavonoids (87 compounds); proanthocyanidins (36 compounds); and, finally, anthocyanins (18 compounds). Despite being the less numerous, anthocyanins were the most abundant class in terms of total peak area with almost 45% of the total. Flavonoids and phenolic acids were equally distributed (with 28.2% and 25.5%, respectively). Finally, proanthocyanidins comprised only the 1.7% of the total peak area. Identification for each class is discussed in the following sections.



**Figure 1.** Pie chart reporting the tentatively identified classes of compounds in terms of (A) number of identifications and (B) total peak area.

### 3.1. Anthocyanin and Flavonoid Composition

Anthocyanins are a peculiar class of phenolic compounds synthesized via the phenylpropanoid pathways but differently from the other flavonoids, characterized by a positive charge on the oxygen of the C-ring of the basic flavonoid structure [31]. Because of this positive charge, anthocyanins are commonly determined in positive ion mode in the form of molecular ions  $[M]^+$  since the corresponding adduct  $[M-2H]^-$  in negative mode is generated with a noticeably lower sensitivity. In Table 1, the 18 tentatively identified anthocyanins are listed, while in Table S1 further details on the identification are reported (adducts, molecular weights, confirming peaks, and peak areas).

Despite their generally high peak areas (with compound 5 comprising alone almost 40% of the total peak area), the tentatively identified anthocyanins are numerically inferior compared to European and North American blueberries, for which more than 50 anthocyanins were previously reported [2]. Regarding the aglycones, methylated compounds were significantly under-expressed compared to *V. myrtillus* and *V. corymbosum*, with only two minor peonidin derivatives identified (compounds 12–13). Malvidin and petunidin derivatives, which are major constituents of blueberries, were not found. It is worth mentioning that malvidin derivatives have not been identified in *V. floribundum*, an Andean blueberry that grows in the same regions as *D. alaternoides* [19,20]. In a previous paper by Ma et al. [9], phenolic markers for discriminate North American and Neotropical blueberries were studied, comprising another member of the genus *Disterigma* (*D. rimbachii*). Malvidin derivatives were effectively under-expressed in Neotropical blueberries compared to North American ones. The absence of malvidin derivatives was also apparent from the color of the extract, which is significantly more reddish (and less purplish) than those of blueberry and bilberry.

Besides anthocyanins, 87 other flavonoids were tentatively identified in *D. alaternoides*, mostly flavanol derivatives. Among the several aglycones belonging to this class, quercetin derivatives were the most abundant with more than 97% of the total flavanol peak area, followed by minor amounts of kaempferol, myricetin, and isorhamnetin. The flavanol composition of Andean blueberry was noticeably similar to that of other genus *Vaccinium* species, except for laricitrin and syringetin derivatives, which were not identified in the *D. alaternoides* extract [2,32]. Similar to malvidin, laricitrin and syringetin are *O*-methylated

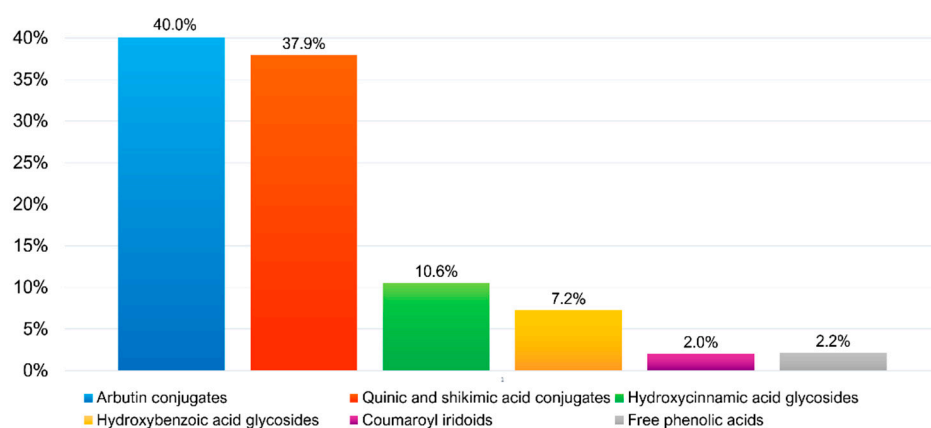
compounds. Their simultaneous absence could indicate a lower degree of methylation in the flavonoid constituents of Andean blueberries compared to European and North American blueberries.

Flavonoids were analyzed in both positive and negative ion modes, with the latter providing generally higher ionization efficiencies. In Table 2, the annotated flavonoids were reported alongside some details, i.e., retention time, proposed formula, experimental  $m/z$ , accuracy, main diagnostic product ions, and confidence level in ESI(−), except for compounds 79, 80, and 88, which were uniquely identified in ESI(+). In Table S2, further details were provided, including complete MS/MS spectra in both ion modes. The determination of the position of the glycoconjugation on the flavonol structure is a great analytical challenge when authentic standards are not available. The sugar-aglycone bond can undergo both heterolytic and homolytic cleavage in the negative ion mode, producing an aglycone ion  $[Y_0]^-$  and a radical aglycone ion  $[Y_0-H]^-$ , respectively. Differently from the hydroxyl position on the aromatic rings (e.g., position 7 on the A-ring or position 4' on the B-ring), when a sugar is bound to position 3 (on the non-aromatic C-ring of the flavanol structure), the homolytic cleavage is favored [33]. Based on these pieces of evidence, whenever the radical aglycone ion had a higher abundance than the aglycone ion, the compounds were ascribed to 3-O-monosaccharide derivatives. Whenever the aglycone ion was more abundant or in the case of more than one glycosylation, the position was not indicated, as positions 7 and 4' are not distinguishable by HRMS [34]. In agreement with previous findings on other species of the *Vaccinium* genus, the majority of flavonols were 3-O-glycosylated [2].

### 3.2. Phenolic Acid Composition

To date, phenolic acids in berries from the *Ericaceae* family have been largely neglected to date compared to flavonoids and flavonoid derivatives, despite their interesting biological activities and high abundance in species of the *Vaccinium* genus [1]. Anthocyanin-rich matrices are often only analyzed in the positive ion mode [19], which is unsuitable for analyzing strong acid compounds. Moreover, whereas flavonoid structures are somehow consistent in different matrices, phenolic acids encompass a more comprehensive range of compounds, resulting in the need for several analytical standards for targeted analyses. In the case of *V. floribundum*, which is the most similar berry to *D. alaternoides* in terms of anthocyanin and flavonoid composition, no more than 7 phenolic acids have been reported so far by previous liquid chromatography coupled to MS analyses [19,35,36]. The reported phenolic acids comprised mainly hydroxycinnamic acids conjugated to quinic and shikimic acid, with the most abundant being chlorogenic acid. In the present paper, a total of 108 phenolic acids and phenolic acid derivatives have been tentatively identified in the Andean blueberry extract by HRMS analysis in the negative ion mode, a number that was significantly higher than reported for blueberries of the genus *Vaccinium* [2,7,19,32,37]. In Table 3 and Table S3, details of the annotated phenolic acids were reported.

Unlike previous studies on blueberries, the annotated phenolic acids presented a more significant structural variability and could be grouped into six main categories, i.e., arbutin conjugates, quinic and shikimic acid conjugates, hydroxycinnamic acid glycosides, hydroxybenzoic acid glucosides, coumaroyl iridoids, and free phenolic acids. The large number of tentative identifications, with tremendous structural heterogeneity and a high total peak area (more than 25%), implied phenolic acids have a role more important than expected in the composition of *D. alaternoides*. Figure 2 shows the total peak area for each of the six classes of compounds. Arbutin derivatives were the most abundant compounds (40% of the total peak area), followed by quinic and shikimic acid conjugates (38%). Hydroxycinnamic and hydroxybenzoic glycosides contributed to the total peak area with 10.6% and 7.2%, while coumaroyl iridoids and free phenolic acids were present in minor amounts (ca. 2%).



**Figure 2.** Bar chart reporting the total peak area per class of phenolic acids tentatively identified in *Disterigma alaternoides* extract.

Arbutin conjugates presented extremely high concentrations despite being numerically a minor class (10 out of 108 compounds). As a matter of fact, caffeoyl arbutin (compound 165) was the single most abundant phenolic acid in terms of peak area. Arbutin is a glucoside of hydroquinone primarily found in blueberry leaves [38] and is known for its skin-whitening properties [39] as well as its efficacy in the treatment of various urinary tract infections [40]. Caffeoyl arbutin was identified by a prior loss of 110 Da (hydroquinone). This cleavage generates a dehydration on the sugar moiety due to the extremely strong C-O phenolic bond of hydroquinone. Therefore, the subsequent sugar cleavage led to a loss of 144 Da (glucose—2H<sub>2</sub>O) rather than the usual neutral loss of 162 Da (glucose—H<sub>2</sub>O). In a previous paper by Ieri et al. [41], several arbutin derivatives were identified in a bilberry leaf extract, including caffeoyl and coumaroyl arbutin, as well as their acetyl derivatives, in good agreement with our findings. Other than caffeoyl arbutin (compounds 163 and 165), other derivatives were identified, i.e., caffeoyl hexosyl arbutin (three isomers, compounds 153, 159, and 160), caffeoyl acetyl arbutin (two isomers, compounds 182 and 183), caffeoyl methoxyarbutin (compound 169), and hydroxybenzoyl arbutin (two isomers, compounds 161 and 180), which were identified with the same logic as described for caffeoyl arbutin. Considering how neglected phenolic acids generally are, arbutin derivatives are likely to be present in all blueberry fruits rather than be solely present in *D. alaternoides*.

The identified phenolic acid conjugates were mainly hydrophobic hydroxycinnamic derivatives (caffeoyl; coumaroyl; and, to a lesser extent, feruloyl, and sinapoyl conjugates) rather than hydrophilic hydroxybenzoic derivatives. Gallic acid and its polymeric derivatives (gallotannins and ellagitannins) were scarcely represented, while several minor glycoconjugates of benzoic, hydroxybenzoic, and dihydroxybenzoic acid have been tentatively identified. Despite a large number of identified compounds (60 compounds), phenolic acid glycoconjugates represented just 18% of the total peak area, likely due to a large number of minor positional isomers. Among the other minor constituents, two coumaroyl iridoids (compounds 166–167) were tentatively identified; these compounds, which are characteristic of cranberry (*V. macrocarpon*), are of great interest for their possible role in healing urinary tract infections [42].

### 3.3. Proanthocyanidin Composition

Proanthocyanidins are non-hydrolyzable oligomers of flavanols, mainly (epi)catechin and (epi)gallocatechin, and are distinguished into two subclasses according to their linkage. A-type proanthocyanidins present a double linkage from positions 7 and 8 on the ring A of the terminal unit to positions 2 and 4 on the ring C of the extension unit (2 $\beta$ →O→7; 4 $\beta$ →8), while B-type proanthocyanidins present a single interflavanoid bond (4 $\beta$ →8). For simplicity, species with one or more A-type interflavanoid bonds are commonly defined as A-type proanthocyanidins [43]. The standard nomenclature is perfectly suitable for dimers,



as there is either one A-type bond or one B-type bond. Nevertheless, it is worth specifying that, concerning oligomers with more than two units, there are more than two possibilities, e.g., in the case of trimers, two B-type bonds, two A-type bonds, and one for each kind, with the latter two both falling under the definition of A-type proanthocyanidins. For sake of clarity, whenever more than one A-type bond was present in the oligomer, a prefix was added to the name (compound 246). In Table 4 and Table S4, detailed data of the 36 tentatively identified proanthocyanidins were reported. Despite being efficiently ionized in both positive and negative ion mode, proanthocyanidins have been only analyzed in negative polarity for the generally higher ionization efficiency, the higher clarity of the MS/MS spectra, and the minor interference of contaminants and noise. Proanthocyanidin fragmentation pathways involve quinone methide (QM) fissions, retro-Diels-Alder (RDA) ring openings, and heterocyclic ring fissions (HRF). QM fissions generate two distinctive sections of the oligomer, named terminal and extension unit (or  $\beta$  unit). While terminal unit ions deriving from QM fissions are independent of the linkage, i.e.,  $[M_T-H]^-$ , product ions of the extension unit are distinctive, i.e.,  $[M_E-3H]^-$  and  $[M_E-5H]^-$  for B- and A-type oligomers, respectively. RDA C-ring opening generates neutral losses of the B-ring sections of the flavanol, producing diagnostic losses for the single flavanol (152.0423 and 168.0432 u for catechin and gallic catechin, respectively). Finally, the HRF pathway generates confirming peaks deriving from the loss of the A-ring sections of the flavanol (126.0317 u), independent from the flavanol's nature.

Compared to other proanthocyanidin-rich matrices, such as tea [44], strawberry [22], and even bilberry [2], the identified compounds were primarily A- and B-type procyanidins, oligomers of the sole catechin and epicatechin. A-type procyanidins were more abundant than B-type ones both in terms of the number of identifications (20 vs. 16) and the total peak area (74% vs. 26%), in good agreement with previously found for bilberry [2].

#### 4. Conclusions

Wild neotropical berries are still an undisclosed rich source of bioactive compounds. In the present paper, almost 250 phenolic compound derivatives were tentatively identified, including anthocyanins, flavonoids, phenolic acids, and proanthocyanidins, in *Disterigma alaternoides*, an Andean blueberry of the unfamiliar genus *Disterigma* of the family Ericaceae. These results indicated, once more, the need for more profound and capillary knowledge on these exotic berry species. Many of the identified compounds are indeed known to exert important biological activities. The high number of tentative identifications was achieved thanks to a dedicated analytical platform based on HRMS and data analysis that allowed a comprehensive yet accessible phenol characterization. The annotated compounds were in general agreement with the composition of other blueberry, with high anthocyanin and flavanol glycoconjugated. The anthocyanin and flavonoid pattern, however, was more similar to that of other Andean blueberries, such as *V. floribundum*, with the absence of highly methylated malvidin, petunidin, and syringetin derivatives. Phenolic acids, which are a generally less investigated class of phenolic compounds, were instead the most numerous and heterogenous class. Several phenolic acids that conjugated to arbutin, which is present in the leaves of blueberry plants, were reported for the first time in blueberry fruit extracts. The extremely rich composition of *D. alaternoides* represents a pivotal result in the field of neotropical berries, which are emerging as possible “super-fruits” for the biological activities of their compounds.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/separations8050058/s1>, Table S1: detailed identification data for the annotated anthocyanins in *Disterigma alaternoides*; Table S2: detailed identification data for the annotated flavonoids in *Disterigma alaternoides*; Table S3: detailed identification data for the annotated phenolic acids in *Disterigma alaternoides*; Table S4: detailed identification data for the annotated proanthocyanidins in *Disterigma alaternoides*.



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