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Characterization of Phenolics Composition in Lamiaceae Spices by LC-ESI-MS/MS

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1 **Characterization of phenolics composition in Lamiaceae spices by LC-ESI-MS/MS**

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24 **ABSTRACT**

25 A total of 38 phenolic compounds in the solid/liquid extracts of five Lamiaceae spices
26 such as rosemary, oregano, sage, basil and thyme were identified in the present study
27 using LC-ESI-MS/MS. These compounds were distributed in four major categories
28 namely hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, flavonoids
29 and phenolic terpenes. Among them, the category of flavonoids was the largest with 17
30 compounds. Identification of the phenolic compounds was carried out by comparing
31 retention times and mass spectra with those of authentic standards. In case of
32 unavailability of standards, phenolic compounds were identified based on accurate mass
33 of pseudomolecular $[M-H]^-$ ions and tandem mass spectrometry (MS/MS) data. The
34 results of accurate mass measurements fitted well with the elemental composition of the
35 compounds. The diagnostic fragmentation patterns of the compounds during collision
36 induced dissociation (CID) elucidated structural information of the compounds analysed.

37

38 **KEYWORDS:** Spice, accurate mass, phenolics, LC-ESI-MS/MS, fragments

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47 **INTRODUCTION**

48 It is well known that Lamiaceae spices have potent antioxidant properties, mostly due to
49 the polyphenolic compounds present in them (1, 2). Recently, interest has increased
50 considerably in naturally occurring antioxidant for use in foods as replacements for
51 synthetic antioxidants such as BHA and BHT, whose use is being restricted due to
52 concerns over safety (3, 4). Natural antioxidants can protect the human body from free
53 radicals and could retard the progress of many chronic diseases as well as lipid oxidative
54 rancidity in foods (5-7). Oxidation of lipids in food not only lowers the nutritional value
55 (8), but is also associated with cell membrane damage, aging, heart disease and cancer in
56 living organisms (9). Therefore the addition of natural antioxidants to food products has
57 become popular as a means of increasing shelf life and to reduce wastage and nutritional
58 losses by inhibiting and delaying oxidation (10). As previously stated spices in the
59 Lamiaceae family are a well known source of antioxidants particularly polyphenols.
60 Furthermore, spices have been used for many years to enhance the sensory attributes such
61 as taste and aroma of foods (11). Since these spices are commonly consumed in most
62 countries, there are no legal barriers to use them in foods. However, their use in foods as
63 either a control measure for lipid oxidation or increase inherent antioxidant capacity
64 requires detailed characterization of the compounds responsible for their antioxidant
65 properties. Liquid chromatography-electrospray ionization-tandem mass spectrometry
66 (LC-ESI-MS/MS) has been recognized as a powerful analytical tool with its high
67 sensitivity, short run time and less use of toxic organic solvents used as mobile phase
68 compared to reversed phase stand alone HPLC coupled with Diode-Array Detector (12-
69 15). A previous LC-ESI-MS study of polyphenols in Lamiaceae family by Møller et al.

70 (16) investigated the major fingerprint ions in methanolic extracts of three variants of
71 oregano and rosemary, however, only two polyphenols, rosmarinic acid and kaempferol,
72 were identified in these extracts despite the fact that many other polyphenolic compounds
73 have been identified in these species by other methods. However, Herrero et al. (17)
74 reported 14 compounds in the pressurized liquid extract of rosemary by LC-ESI-MS
75 method. Other studies (18-22) also identified similar number of compounds in different
76 members of the family. In the present study we examined 38 polyphenols in five
77 Lamiaceae spices using liquid chromatographic separation and collision induced
78 dissociation analysis. Furthermore, accurate mass measurement technique was
79 successfully applied for the first time in this spice family to elucidate the elemental
80 composition of the polyphenols studied.

81

82 **MATERIALS AND METHODS**

83 **Samples and reagents.** Dried and ground rosemary, oregano, sage, basil and thyme were
84 provided by AllinAll Ingredients Ltd., Dublin 12, Ireland. According to product
85 specifications, the country of origin of the spices used was Turkey. The spices were air
86 dried after heat treatment (steam sterilization at 120 °C for 30 sec). The dried spices were
87 ground (particle size range: 500 to 600 µm) and stored at -20 °C in darkness. Seventeen
88 standards namely caffeic acid, chlorogenic acid, carnosic acid, carnosol, ferulic acid,
89 gallic acid, gallic acid, gallic acid, gallic acid, gallic acid, gallic acid, gallic acid, gallic acid,
90 coumaric acid, quercetin, rosmarinic acid, rutin, syringic acid, thymol and vanillic acid
91 were purchased from Sigma-Aldrich. Four flavonoid standards, such as apigenin,
92 apigenin-7-*O*-glucoside, luteolin and luteolin-7-*O*-glucoside were purchased from

93 Extrasynthese, France. HPLC grade methanol and water were purchased from VWR
94 International Limited, Leicestershire, UK and Lennox Laboratory Supplies Limited,
95 Dublin, Ireland respectively. The purity of standards and solvents were in the range of 95
96 % to 99.8 %. Only luteolin-7-*O*-glucoside and carnosic acid had 90 % and 91 % purity
97 respectively.

98

99 **Preparation of solid/liquid extracts.** Dried and ground spice samples (1 g) were
100 homogenised for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogenizer
101 (Janke & Kunkel, IKA[®]-Labortechnik, Saufen, Germany) in 25 mL of 80% methanol in
102 the dark at room temperature (~23 °C). Aqueous methanol (80 %) was chosen for its high
103 efficiency in extracting polyphenols from plant samples (2). The homogenised sample
104 suspension was shaken overnight with a V400 Multitude Vortexer (Alpha laboratories,
105 North York, Canada) at 1,500 rpm and room temperature. The mixture was then
106 centrifuged for 15 min at 2,000 g (MSE Mistral 3000i, Sanyo Gallenkamp,
107 Leicestershire, UK) and filtered through 0.22 µm polytetrafluoroethylene (PTFE) filters
108 (Sigma-Aldrich, Steinheim, Germany). The extracts were analyzed immediately after
109 extraction.

110

111 **Liquid chromatography-mass spectrometry (LC-MS).** LC-MS analysis was
112 performed on a Q-ToF Premier mass spectrometer (Waters Corporation, Micromass MS
113 Technologies, Manchester, UK coupled to Alliance 2695 HPLC system (Waters
114 Corporation, Milford, MA, USA). The Q-ToF Premier is equipped with a lockspray
115 source where an internal reference compound (Leucine-Enkephalin) was introduced

116 simultaneously with the analyte for accurate mass measurements. Compounds were
117 separated on an Atlantis T3 C18 column (Waters Corporation, Milford, USA, 100 mm x
118 2.1 mm; 3 μ m particle size) using 0.5% aqueous formic acid (solvent A) and 0.5% formic
119 acid in 50/50 v/v acetonitrile:methanol (solvent B). Column temperature was maintained
120 at 40 °C. A stepwise gradient from 10% to 90% solvent B was applied at a flow rate of
121 0.2 mL/min for 26 min. Electrospray mass spectra data were recorded on a negative
122 ionisation mode for a mass range m/z 100 to m/z 1000. Capillary voltage and cone
123 voltage were set at 3 kV and 30 V respectively. Collision induced fragmentation (CID) of
124 the analytes was achieved using 12 eV to 20 eV energy with argon as the collision gas.

125

126 **RESULTS AND DISCUSSION**

127 A total of 38 polyphenols distributed in four major categories; hydroxycinnamic acid
128 derivatives, hydroxybenzoic acid derivatives, flavonoids and phenolic terpenes have been
129 analyzed in the present study. Figure 1 shows the total ion current (TIC) chromatogram
130 of rosemary extract and the major peaks observed has been assigned in Table 1. Since
131 polyphenols contain one or more hydroxyl and/or carboxylic acid groups, MS data were
132 acquired in negative ionization mode. Identification of the phenolic compounds was
133 carried out by comparing retention times and their masses with those of the 21 authentic
134 standards. For the remaining 17 compounds for which no standards were available
135 identification was based on accurate mass measurements of the pseudomolecular [M-H]⁻
136 ions and CID fragment ions. Results of accurate mass measurements matched the
137 elemental composition of all the compounds analyzed (Table 1). Data obtained from the
138 ESI-MS analyses of the extracts of five Lamiaceae spices are summarized in Table 1. The

139 following sections outline conditions used to identify each of the compounds (arranged
140 into their constituent groups), fragmentation patterns and occurrence in each of the spice
141 extracts.

142

143 **Hydroxycinnamic acid derivatives**

144 Seven different polyphenols in the category of hydroxycinnamic acid derivatives were
145 found to occur in all the spices examined. Five of them namely caffeic acid, chlorogenic
146 acid, p-coumaric acid, rosmarinic acid and ferulic acid were identified by comparing their
147 retention times and characteristic MS spectral data with those of authentic standards
148 (Table 1). Accurate mass measurements and fragmentation pattern during CID further
149 confirmed their structural composition. The pseudomolecular ions of p-coumaric acid
150 (m/z 163.04) and ferulic acid (m/z 193.05) produced the major fragment ions at m/z 119.0
151 and m/z 149.0 respectively during CID corresponding to the loss of carbon dioxide from
152 the precursor ion. Gruz et al. (23) reported the same fragmentation pattern of these
153 compounds in white wine. The other fragment generated during CID of ferulic acid was
154 at m/z 178.0 due to initial loss of a methyl group from the precursor ion. The remaining
155 two hydroxycinnamic acid derivatives; caffeic acid hexoside and dicaffeoylquinic acid
156 were identified by their accurate mass measurements and MS/MS spectral data.

157 The tentative mass spectrum for caffeic acid showed the deprotonated molecule $[M-H]^-$
158 ion at m/z 179.03 at 1.57 min. The major fragment ions produced by CID analysis were
159 m/z 161.0 and m/z 135.0 corresponding to loss of water and carbon dioxide molecules
160 respectively from the precursor ion. Generally, deprotonated phenolic acids $[M-H]^-$
161 produce a typical fragmentation pattern after collision induced dissociation, characterised

162 by the loss of a CO₂ (44 u) from the carboxylic acid group, providing an anion of [M-H-
163 COO]⁻ (24). Other fragment ions *m/z* 113.0, 101.0 and 71.0 unique to caffeic acid were
164 also observed. These ions were produced as a result of the cleavage of the phenolic ring
165 of the precursor ion at *m/z* 179.0 at different sites as illustrated in Figure 2. Similar
166 fragment ions were seen when the precursor [M-H]⁻ ions of *m/z* 341.10 eluting at 1.53
167 min, *m/z* 353.09 at 3.91 min and *m/z* 515.10 eluting at 10.01 min were subjected to CID.
168 This confirmed that these precursor molecular ions were associated with caffeic acid. For
169 instance *m/z* 341.10 ions were identified as deprotonated caffeic acid hexoside. The loss
170 of a hexose moiety (162 u) resulted in a dominant fragment ion at *m/z* 179.0
171 corresponding to deprotonated caffeic acid. It must also be noted that a dicaffeic acid
172 would also generate similar precursor and fragment ions as that of caffeic acid hexoside.
173 In this context, application of accurate mass measurement discriminated caffeic acid
174 hexoside (calculated from [M-H]⁻ = 341.0873) from dicaffeic acid (calculated from [M-
175 H]⁻ = 341.0660). The MS/MS on the precursor *m/z* 353.09 ions identified as chlorogenic
176 acid gave dominant product ions *m/z* 191.1, *m/z* 179.0 and *m/z* 173.0. The product ions
177 *m/z* 191.1 for quinic acid and 179.0 for caffeic acid revealed the constituent of
178 chlorogenic acid prior to condensation. Loss of a caffeoyl moiety yielded the other
179 dominant fragment ion *m/z* 173.0. The MS/MS on precursor [M-H]⁻ ion at *m/z* 515.10
180 showed product ions of *m/z* 353.0, *m/z* 191.0 and *m/z* 179.0 corresponding to the
181 pseudomolecular ions of caffeoylquinic acid, quinic acid and caffeic acid respectively in
182 addition to the finger-print fragment ions of caffeic acid. Thus this compound was
183 identified as dicaffeoylquinic acid. A similar fragmentation of the compound was
184 reported by Parejo et al. (24) in fennel extract. The CID experiment on [M-H]⁻ ion at *m/z*

185 359.08 identified as rosmarinic acid gave the two main constituents of rosmarinic acid
186 namely caffeic acid at m/z 179.0 and the 2-hydroxy derivative of hydrocaffeic acid at m/z
187 197.0 as illustrated in Figure 3. Similar pattern of fragmentation of rosmarinic acid
188 during CID analysis has been reported by several authors (17, 25, 26) in analyzing
189 extracts of Lamiaceae spices.

190

191 **Hydroxybenzoic acid derivatives**

192 The ESI-MS signals at m/z 169.01, m/z 197.04, m/z 167.04, m/z 153.02 and m/z 137.02
193 were identified as gallic acid, syringic acid, vanillic acid, protocatechuic acid and 4-
194 hydroxybenzoic acid respectively by comparing their retention time and MS spectral data
195 with those of an authentic standard. Accurate mass measurements further confirmed their
196 elemental composition (Table 1). Upon fragmentation by CID gallic acid, vanillic acid,
197 protocatechuic acid and 4-hydroxybenzoic acid produced the ions at m/z 125.0, m/z
198 123.0, m/z 109.0 and m/z 93.0 respectively due to loss of CO₂ from their respective
199 precursor ions. This pattern of fragmentation was characteristic feature of
200 hydroxybenzoic acid derivatives like other phenolic acids. Syringic acid on the other
201 hand first lost a water molecule generating a major fragment ion at m/z 179.0 followed by
202 a loss of carbon dioxide producing the other fragment at m/z 135.0. A sugar conjugate of
203 hydroxybenzoic acid eluting at 22.64 min showed [M-H]⁻ ions of m/z 299.10. Accurate
204 mass measurement suggested the molecular composition as that of hydroxybenzoic acid-
205 *O*-hexoside. Subsequent MS/MS experiment revealed the loss of hexose moiety
206 producing deprotonated 4-hydroxybenzoic acid at m/z 137.0. All the hydroxybenzoic acid

207 derivatives mentioned above were detected in all the Lamiaceae spices examined by ESI-
208 MS analyses (Table 1).

209

210 **Flavonoids**

211 Flavonoids constituted the largest number of polyphenols in the spices investigated in this
212 study (Table 1). With the aid of reference standards and complemented by the accurate
213 mass measurement data, eight flavonoids were identified in all the spices studied by LC-
214 MS. The eight flavonoids were apigenin, luteolin, apigenin-7-*O*-glucoside, luteolin-7-*O*-
215 glucoside, galocatechin, phloridzin, quercetin and rutin. Furthermore, the fragmentation
216 pattern of these flavonoids was similar to those described previously where the most
217 common fragment lost was a water molecule and a glucose moiety in the two glucosides
218 (26, 27).

219 For the remaining nine flavonoids listed in Table 1 for which there were no ‘in-house’
220 standards, their identifications were based solely on accurate mass measurements and the
221 MS/MS data (Table 1). Acacetin found in rosemary, oregano and basil; cirsimaritin and
222 methyl apigenin found in all 5 spices; and isorhamnetin found in rosemary, sage and
223 thyme were the only four non-sugar based flavonoids. They had a characteristic feature in
224 the MS/MS experiment where the loss of one or more methyl groups was observed.
225 Acacetin (m/z 283.1) eluting at 17.89 min, methyl apigenin (m/z 283.1) eluting at 20.69
226 min and isorhamnetin (m/z 315.0) eluting at 14.80 min lost one methyl group each
227 producing m/z 268.0, m/z 268.0 and m/z 300.0 respectively while cirsimaritin (m/z 313.1)
228 lost two consecutive methyl groups resulting fragment ions m/z 298.0 and m/z 283.1.
229 Despite the fact that acacetin and methyl-apigenin are isomers differing only in the

230 position of methyl group, they separated well in the reversed phase LC. Since acacetin is
231 slightly polar than methyl-apigenin, it eluted earlier in the LC-separation. Justesen (26)
232 described similar fragmentation of acacetin in analyzing extracts from different herbs.
233 Similar to our findings, Herrero et al. (17) have previously reported on cirsimaritin in
234 rosemary extracts using LC-ESI-MS/MS. Parejo et al. (24), unlike our data, have noted
235 three fragment ions from isorhamnetin, i.e. m/z 300, m/z 271 and m/z 255, in fennel
236 extracts by ESI-MS/MS analysis. The difference could probably be due to different set of
237 collision energy being used in the two different instruments.

238 Glycosylated flavonoids constituted the bulk of the polyphenols in the spices. Hexose and
239 rutinose conjugates of flavonoids were most commonly observed. The MS/MS
240 experiments revealed that the $[M-H]^-$ ions at m/z 477.10 eluting at 9.85 min and m/z
241 463.09 eluting at 4.83 min were isorhamnetin-3-*O*-hexoside and quercetin-3-*O*-hexoside
242 respectively. Similar to the MS/MS data from apigenin-7-*O*-glucoside and luteolin-7-*O*-
243 glucoside, these hexosides also showed the loss of a hexose moiety (162 u). In addition to
244 the fragment ion at m/z 315.0 corresponding to deprotonated molecular ion of
245 isorhamnetin, the isorhamnetin-3-*O*-hexoside produced a fragment ion at m/z 300.0
246 further confirming that the hexose derivative was that of isorhamnetin. As expected
247 isorhamnetin-3-*O*-hexoside was only detected in the extracts of rosemary, sage and
248 thyme of the five spices examined (Table 1). Similar approach and conclusions were
249 made for quercetin-3-*O*-hexoside. The present study also identified two phenolic
250 rutosides, namely apigenin-7-*O*-rutinoside and luteolin-7-*O*-rutinoside apart from
251 quercetin-7-*O*-rutinoside (commonly known as rutin) in all the spices examined (Table
252 1). The product ion scan experiments of these compounds produced the intense fragment

253 ions 308 u (dehydrated rutinose moiety) lower than the m/z values of the precursor ions.
254 The presence of rutin in rosemary and oregano extract has been reported by
255 Papageorgiou, et al. (28) using reversed phase HPLC. However, only one glucuronide
256 derivative of flavonoids could be detected in all the spices examined. This compound
257 eluting at 12.15 min was identified as luteolin-3-*O*-glucuronide (Table 1). Subsequent
258 CID of luteolin-3-*O*-glucuronide showed the loss of a glucuronic acid (m/z 176) and
259 produced the predominant fragment at m/z 285.0 corresponding to deprotonated luteolin.
260 Similar fragmentation of the compound was reported by Justesen (26) in analyzing thyme
261 extracts.

262

263 **Phenolic terpenes and lignan**

264 There were 8 polyphenols detected in the spices examined that fall under the phenolic
265 terpenes and lignan category (Table 1). Three of them, thymol, carnosol and carnosic
266 acid, were identified as they showed identical LC-MS characteristics as that of the
267 standards. Thymol detected only in thyme when subjected to CID produced fragments at
268 m/z 131.0 and m/z 120.0 corresponding to the loss of water and an ethyl [-CH₂-CH₃]
269 group (29 u) from the precursor ion (m/z 149.09). Carnosol detected in all the spices and
270 carnosic acid found only in rosemary, oregano and sage showed major fragment ions
271 following a loss of carbon dioxide as seen in all the phenolic acids. Decarboxylated
272 carnosic acid further fragmented producing m/z 244.2 ions due to dissociation of a propyl
273 group (CH₂CH₂CH₃). Methylated carnosic acid and methoxycarnosol were also identified
274 in all the samples (Table 1). Methyl carnosate (m/z 345.20) eluting at 22.68 min
275 produced two major fragments: m/z 301.2 due to loss of carbondioxide molecule with

276 further loss of methyl group producing m/z 286.2 ions. This fragmentation pattern was in
277 agreement with that reported by Herrero et al., (2009) in analysing the phenolic
278 antioxidant compounds of rosemary extracts. The methoxycarnosol (m/z 359.17) eluting
279 at 22.70 min also generated two major fragments in the MS/MS experiment: m/z 329.2
280 and m/z 285.2 corresponding to loss of a methoxy group and subsequent loss of
281 carbondioxide molecule. Epirosmannol which has the same nominal mass as that of
282 methyl carnosate eluted 4.75 min earlier than the methyl carnosate in the LC separation
283 (Figure 1 and Table 1). In addition to difference in elution time, the accurate mass
284 measurement distinguished epirosmannol (calculated m/z 345.1702, observed m/z
285 345.1702) from methyl carnosate (calculated m/z 345.2066, observed m/z 345.2054).
286 Furthermore the MS/MS data from epirosmannol, unlike methyl carnosate, showed the
287 loss of water following decarboxylation. The last of the terpenes found in this study was
288 rosmadial which had a unique fragmentation pathway compared to other terpenes
289 described earlier. Rosmadial (m/z 343.20) lost two and three methylene groups from the
290 precursor ions resulting fragment ions m/z 315.2 and m/z 300.2 respectively. There was
291 only one phenolic lignan, namely medioresinol, identified in the extracts of all Lamiaceae
292 spices analysed.

293

294 Application of LC-ESI-MS/MS technique in the current study provided useful
295 information to characterize 38 phenolic compounds in the extracts of five Lamiaceae
296 spices. Fragments produced during CID analysis of the compounds mentioned above are
297 the diagnostic features of these compounds which could be used to identify them in
298 different extracts. Results of accurate mass measurements are another diagnostic feature

299 of these compounds and proved useful to differentiate compounds with same nominal
300 mass but dissimilar exact masses (Table 1). Equally mass spectrometry showed
301 advantageous in identification of polyphenols for those that did not separate as different
302 entities in the reversed phase column. Nonetheless, when isomeric polyphenols such as
303 acacetin and methyl apigenin which posed challenge for MS, the LC was able to resolve
304 the isomers. One inherent weakness of the low collision energy MS/MS studies was that
305 it could not localise the position in the native phenolic ring that underwent modification.
306 In such scenario, the application of nuclear magnetic resonance (NMR) spectroscopy
307 would be helpful. The NMR would also have the capability to reveal the identity of the
308 compound responsible for the modification. As far as the authors are aware, there is no
309 literature providing a comprehensive analysis of polyphenols in the extracts of Lamiaceae
310 spices. Furthermore, of the 38 polyphenols identified, 20 compounds in rosmmary, 26
311 compounds in oregano, 23 compounds in sage, 24 compounds in basil and 20 compounds
312 in thyme have been reported in the present study for the first time (Table 1). In
313 conclusion, the combination of accurate mass measurement to determine the elemental
314 composition and the LC's ability to separate isomeric compounds provided a powerful
315 tool in identification of polyphenolic diversity in five species of Lamiaceae family even
316 in the absence of standards.

317

318 **Unidentified compounds.** Pseudomolecular ions at m/z 597.10 (observed exact mass
319 597.1288), m/z 503.10 (observed exact mass 503.0831), m/z 394.07 (observed exact mass
320 394.0667) and m/z 301.17 (observed exact mass 301.1758) eluting at 8.32 min, 13.96
321 min, 23.5 min and 24.45 min respectively could not be identified.

322

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325

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420

421 **Figure captions**

422 Figure 1. Total ion current (TIC) chromatogram of aqueous methanol extract of
423 rosemary.

424

425 Figure 2. Schematic diagram of the production of fragments from caffeic acid hexoside
426 (m/z 341.09) during CID analysis.

427

428 Figure 3. ESI-MS/MS spectrum of product ion scan of rosmarinic acid (m/z 359.10).

Table 1. Peak assignments of aqueous methanol extract of rosemary.

Peak No.	Polyphenols	Empirical formula	Observed m/z	Calculated m/z	Major fragments (intensity) m/z	RT (min)	Detected in
1	Gallic acid ^a	C ₇ H ₅ O ₅ ⁻	169.0141	169.0137	125.0 (100 %)	1.25	R, O ^b , S, B ^b , T
2	Caffeic acid hexoside	C ₁₅ H ₁₇ O ₉ ⁻	341.0883	341.0873	179.0 (55 %), 161.0 (15 %)	1.53	R ^b , O ^b , S ^b , B ^b , T ^b
3	Caffeic acid ^a	C ₉ H ₇ O ₄ ⁻	179.0350	179.0344	161.0 (10 %), 135.0 (10 %)	1.57	R, O, S, B, T
4	Syringic acid ^a	C ₉ H ₉ O ₅ ⁻	197.0453	197.0450	179.0 (60 %), 135.0 (100 %)	2.62	R, O, S, B, T
5	Vanillic acid ^a	C ₈ H ₇ O ₄ ⁻	167.0366	167.0344	123.0 (70 %)	2.70	R, O, S, B, T
6	Protocatechuic acid ^a	C ₇ H ₅ O ₄ ⁻	153.0190	153.0188	109.0 (100 %)	3.16	R ^b , O ^b , S ^b , B ^b , T ^b
7	Rosmadial	C ₂₀ H ₂₃ O ₅ ⁻	343.1526	343.1545	315.2 (20 %), 300.2 (20 %)	3.19	R
8	Chlorogenic acid ^a	C ₁₆ H ₁₇ O ₉ ⁻	353.0951	353.0873	191.1 (42 %), 179.0 (62 %), 173.0 (100 %)	3.91	R, O, S, B, T
9	p-Coumaric acid ^a	C ₉ H ₇ O ₃ ⁻	163.0402	163.0395	119.0 (100 %)	3.97	R, O, S, B, T
10	4-Hydroxybenzoic acid ^a	C ₇ H ₅ O ₃ ⁻	137.0247	137.0239	93.0 (40 %)	4.23	R ^b , O ^b , S ^b , B ^b , T ^b
11	Quercetin-3- <i>O</i> -hexoside	C ₂₁ H ₁₉ O ₁₂ ⁻	463.0880	463.0877	301.0 (50 %)	4.83	R ^b , O ^b , S ^b , B ^b , T ^b
12	Medioresinol	C ₂₁ H ₂₃ O ₇ ⁻	387.1421	387.1444	207.1 (20 %)	4.84	R ^b , O ^b , S ^b , B ^b , T ^b
13	Gallocatechin ^a	C ₁₅ H ₁₃ O ₇ ⁻	305.0665	305.0661	225.0 (88 %)	6.08	R, O ^b , S ^b , B, T ^b
14	Luteolin-7- <i>O</i> -glucoside ^a	C ₂₁ H ₁₉ O ₁₁ ⁻	447.0920	447.0927	285.0 (50 %)	8.87	R ^b , O ^b , S ^b , B ^b , T
15	Ferulic acid ^a	C ₁₀ H ₉ O ₄ ⁻	193.0518	193.0501	178.0 (10 %), 149.0 (100 %)	8.98	R, O, S, B, T
16	Phloridzin ^a	C ₂₁ H ₂₃ O ₁₀ ⁻	435.1302	435.1291	273.0 (65 %), 167 (40 %)	9.38	R ^b , O ^b , S ^b , B ^b , T ^b
17	Isorhamnetin-3- <i>O</i> -hexoside	C ₂₂ H ₂₁ O ₁₂ ⁻	477.1036	477.1033	462.0 (10 %), 315.0 (100 %), 300.0 (20 %)	9.85	R ^b , S ^b , T ^b
18	Dicaffeoylquinic acid	C ₂₅ H ₂₃ O ₁₂ ⁻	515.1163	515.1190	359.0 (15 %), 179.0 (54 %), 135.0 (25 %), 101.0 (6 %)	10.01	R ^b , O ^b , S ^b , B ^b , T ^b
19	Apigenin-7- <i>O</i> -rutinoside	C ₂₇ H ₂₉ O ₁₄ ⁻	577.1559	577.1557	269.0 (100 %)	10.54	R ^b , O ^b , S ^b , B ^b , T ^b
20	Rutin ^a	C ₂₇ H ₂₉ O ₁₆ ⁻	609.1473	609.1456	301.0 (100 %)	10.59	R ^b , O ^b , B ^b , T
21	Apigenin-7- <i>O</i> -glucoside ^a	C ₂₁ H ₁₉ O ₁₀ ⁻	431.0993	431.0978	269.1 (22 %)	10.62	R ^b , O ^b , S ^b , B ^b , T
22	Rosmarinic acid ^a	C ₁₈ H ₁₅ O ₈ ⁻	359.0763	359.0767	197.0 (50 %), 179.0 (20 %), 161.0 (100 %), 135.0 (10 %)	11.27	R, O, S, B, T
23	Luteolin-3- <i>O</i> -glucuronide	C ₂₁ H ₁₇ O ₁₂ ⁻	461.0725	461.0720	285.0 (100 %)	12.15	R ^b , O ^b , S ^b , B ^b , T

24	Luteolin-7- <i>O</i> -rutinoside	C ₂₇ H ₂₉ O ₁₅ ⁻	593.1533	593.1506	285.0 (28 %)	14.09	R ^b , O ^b , S ^b , B ^b , T ^b
25	Isorhamnetin	C ₁₆ H ₁₁ O ₇ ⁻	315.0489	315.0505	300.0 (100 %)	14.80	R ^b , S ^b , T ^b
26	Quercetin ^a	C ₁₅ H ₉ O ₇ ⁻	301.0334	301.0349	227.1 (10 %), 151.1 (10 %)	16.86	R ^b , O, S ^b , B ^b , T
27	Apigenin ^a	C ₁₅ H ₉ O ₅ ⁻	269.0441	269.0450	158.9 (15 %)	17.09	R, O ^b , S ^b , B, T
28	Thymol ^a	C ₁₀ H ₁₃ O ⁻	149.0981	149.0966	131.0 (10 %), 120.0 (25 %)	17.30	T ^b
29	Acacetin	C ₁₆ H ₁₁ O ₅ ⁻	283.0613	283.0606	268.0 (45 %)	17.89	R, O ^b , B ^b
30	Epirosmanol	C ₂₀ H ₂₅ O ₅ ⁻	345.1702	345.1702	301.2 (100 %), 283.2 (25 %)	17.93	R, O ^b , S, B ^b , T ^b
31	Cirsimaritin	C ₁₇ H ₁₃ O ₆ ⁻	313.0700	313.0712	298.0 (55 %), 283.0 (18 %)	19.01	R, O ^b , S ^b , B ^b , T
32	Methyl apigenin	C ₁₆ H ₁₁ O ₅ ⁻	283.0616	283.0606	268.0 (100 %)	20.69	R ^b , O ^b , S ^b , B ^b , T ^b
33	Hydroxybenzoic acid- <i>O</i> -hexoside	C ₁₃ H ₁₅ O ₈ ⁻	299.0752	299.0767	137.0 (100 %)	22.64	R ^b , O ^b , S ^b , B ^b , T ^b
34	Methyl carnosate	C ₂₁ H ₂₉ O ₄ ⁻	345.2054	345.2066	301.2 (100 %), 286.2 (20 %)	22.68	R, O ^b , S ^b , B ^b , T ^b
35	Methoxy carnosol	C ₂₁ H ₂₇ O ₅ ⁻	359.1855	359.1858	329.2 (100 %), 285.2 (40 %)	22.70	R ^b , O ^b , S ^b , B ^b , T ^b
36	Luteolin ^a	C ₁₅ H ₉ O ₆ ⁻	285.0392	285.0399	267.0 (60 %)	22.95	R ^b , O ^b , S ^b , B ^b , T ^b
37	Carnosol ^a	C ₂₀ H ₂₅ O ₄ ⁻	329.1747	329.1753	285.1 (40 %)	23.09	R, O ^b , S, B ^b , T ^b
38	Carnosic acid ^a	C ₂₀ H ₂₇ O ₄ ⁻	331.1903	331.1909	287.2 (100 %), 244.2 (10 %)	24.97	R, O ^b , S

^a Identification confirmed using commercial standards

^b Compounds characterized for the first time by LC-ESI-MS/MS

R = Rosemary, O = Oregano, S = Sage, B = Basil, T = Thyme

Figure 1

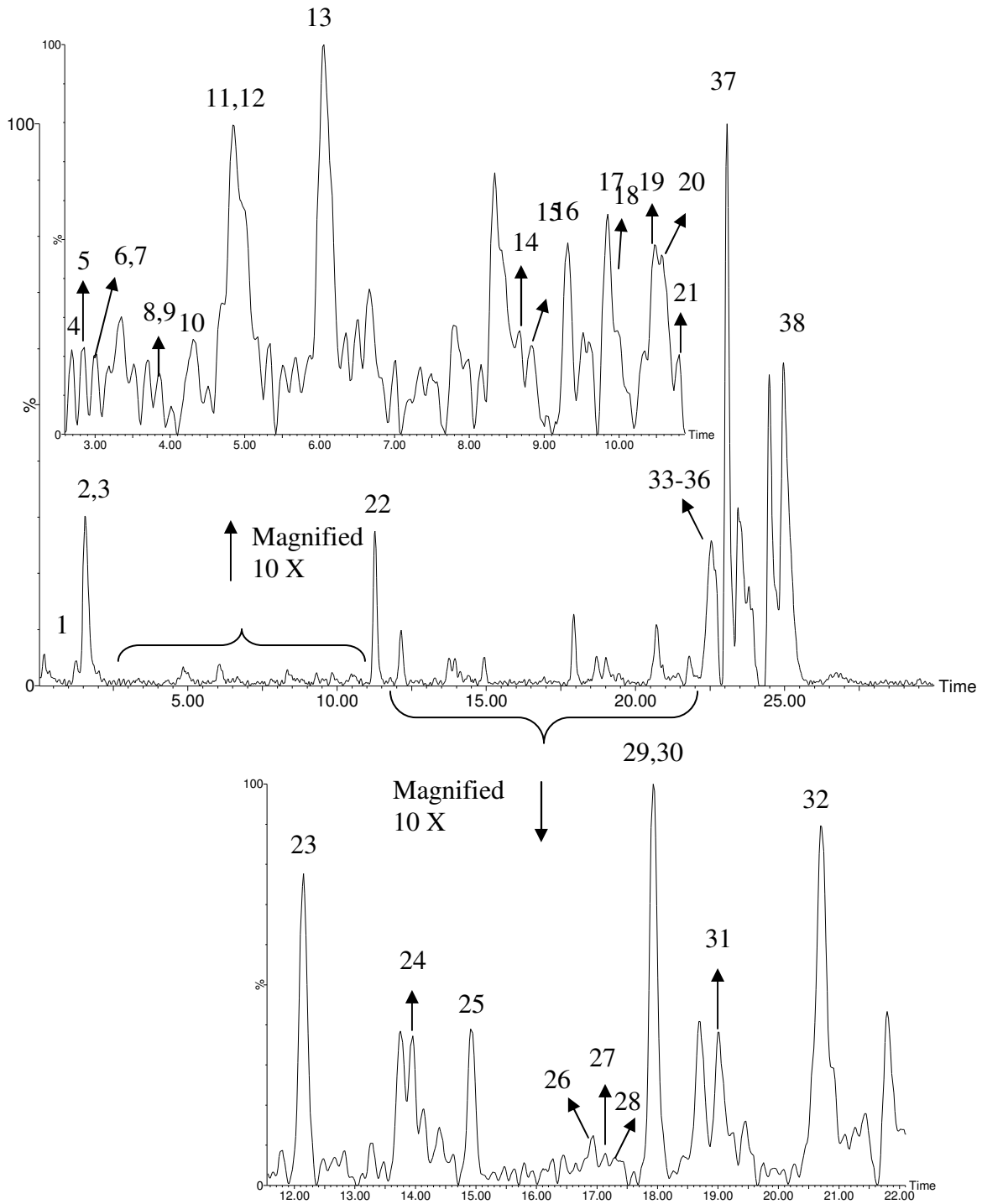


Figure 2

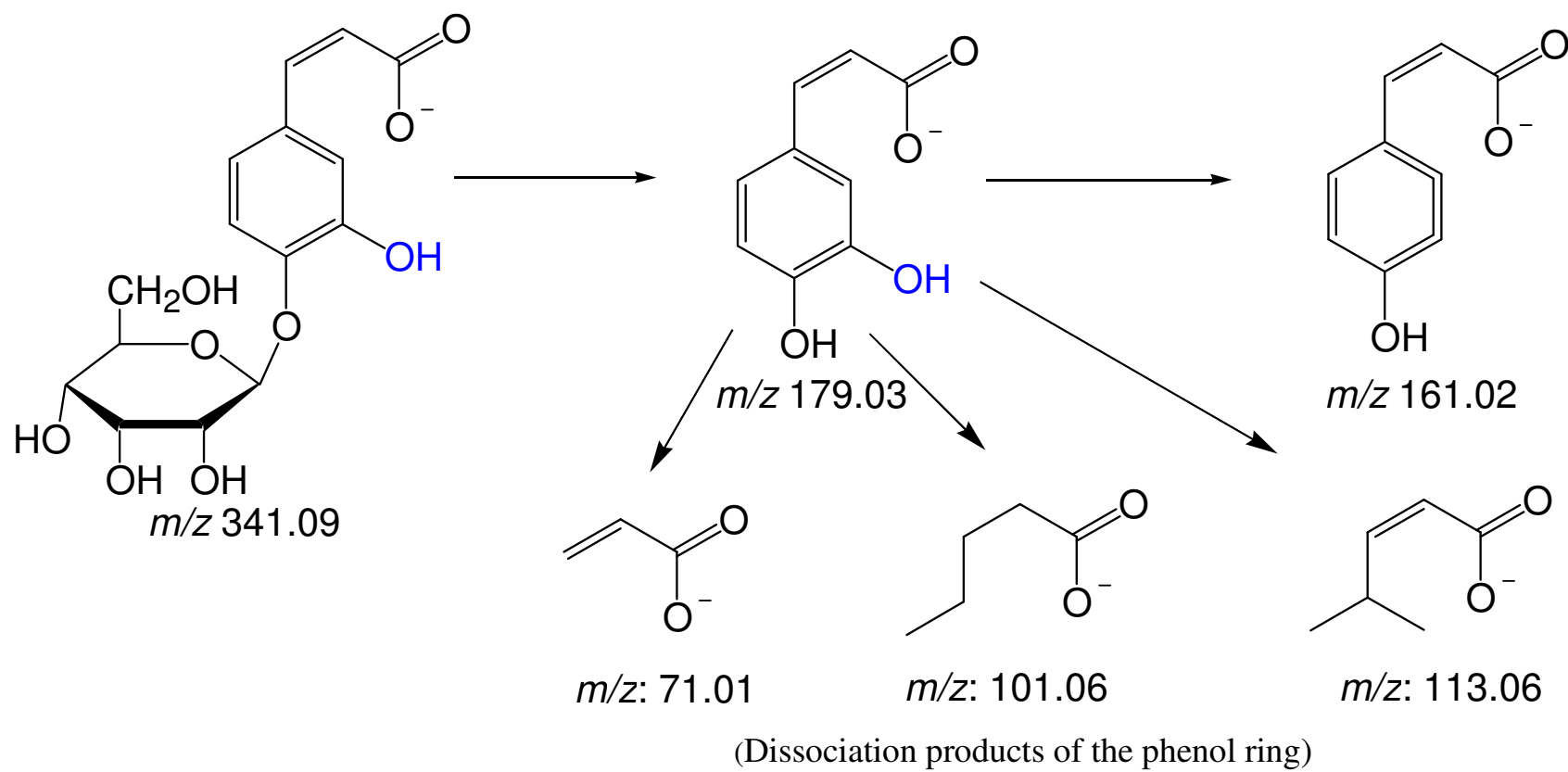


Figure 3

