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

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ABSTRACT

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

Samples and reagents. Dried and ground rosemary, oregano, sage, basil and thyme were provided by AllinAll Ingredients Ltd., Dublin 12, Ireland. According to product specifications, the country of origin of the spices used was Turkey. The spices were air dried after heat treatment (steam sterilization at 120 °C for 30 sec). The dried spices were ground (particle size range: 500 to 600 µm) and stored at -20 °C in darkness. Seventeen standards namely caffeic acid, chlorogenic acid, carnosic acid, carnosol, ferulic acid, gallic acid, gallocatechin, 4-hydroxybenzoic acid, phloridzin, protocatechuic acid, p-coumaric acid, quercetin, rosmarinic acid, rutin, syringic acid, thymol and vanillic acid were purchased from Sigma-Aldrich. Four flavonoid standards, such as apigenin, 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 polytetrafluoethylene (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

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

RESULTS AND DISCUSSION

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

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

Hydroxycinnamic acid derivatives

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

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

by the loss of a CO_2 (44 u) from the carboxylic acid group, providing an anion of [M-H-COO]$^-$ (*24*). Other fragment ions *m/z* 113.0, 101.0 and 71.0 unique to caffeic acid were also observed. These ions were produced as a result of the cleavage of the phenolic ring of the precursor ion at *m/z* 179.0 at different sites as illustrated in Figure 2. Similar fragment ions were seen when the precursor [M-H]$^-$ions of *m/z* 341.10 eluting at 1.53 min, *m/z* 353.09 at 3.91 min and *m/z* 515.10 eluting at 10.01 min were subjected to CID. This confirmed that these precursor molecular ions were associated with caffeic acid. For instance *m/z* 341.10 ions were identified as deprotonated caffeic acid hexoside. The loss of a hexose moiety (162 u) resulted in a dominant fragment ion at *m/z* 179.0 corresponding to deprotonated caffeic acid. It must also be noted that a dicaffeic acid would also generate similar precursor and fragment ions as that of caffeic acid hexoside. In this context, application of accurate mass measurement discriminated caffeic acid hexoside (calculated from [M-H]$^-$= 341.0873) from dicaffeic acid (calculated from [M-H]$^-$= 341.0660). The MS/MS on the precursor *m/z* 353.09 ions identified as chlorogenic acid gave dominant product ions *m/z* 191.1, *m/z* 179.0 and *m/z* 173.0. The product ions *m/z* 191.1 for quinic acid and 179.0 for caffeic acid revealed the constituent of chlorogenic acid prior to condensation. Loss of a caffeoyl moiety yielded the other dominant fragment ion *m/z* 173.0. The MS/MS on precursor [M-H]$^-$ ion at *m/z* 515.10 showed product ions of *m/z* 353.0, *m/z* 191.0 and *m/z* 179.0 corresponding to the pseudomolecular ions of caffeoylquinic acid, quinic acid and caffeic acid respectively in addition to the finger-print fragment ions of caffeic acid. Thus this compound was identified as dicaffeoylquinic acid. A similar fragmentation of the compound was reported by Parejo et al. (*24*) in fennel extract. The CID experiment on [M-H]$^-$ ion at *m/z*

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

Hydroxybenzoic acid derivatives

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 were identified as gallic acid, syringic acid, vanillic acid, protocatechuic acid and 4-hydroxybenzoic acid respectively by comparing their retention time and MS spectral data with those of an authentic standard. Accurate mass measurements further confirmed their elemental composition (Table 1). Upon fragmentation by CID gallic acid, vanillic acid, protocatechuic acid and 4-hydroxybenzoic acid produced the ions at *m/z* 125.0, *m/z* 123.0, *m/z* 109.0 and *m/z* 93.0 respectively due to loss of CO_2 from their respective precursor ions. This pattern of fragmentation was characteristic feature of hydroxybenzoic acid derivatives like other phenolic acids. Syringic acid on the other hand first lost a water molecule generating a major fragment ion at *m/z* 179.0 followed by a loss of carbon dioxide producing the other fragment at *m/z* 135.0. A sugar conjugate of hydroxybenzoic acid eluting at 22.64 min showed $[M-H]^-$ ions of *m/z* 299.10. Accurate mass measurement suggested the molecular composition as that of hydroxybenzoic acid-*O*-hexoside. Subsequent MS/MS experiment revealed the loss of hexose moiety 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, gallocatechin, 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 isorhamentin (*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

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

Glycosylated flavonoids constituted the bulk of the polyphenols in the spices. Hexose and rutinose conjugates of flavonoids were most commonly observed. The MS/MS experiments revealed that the $[M-H]^-$ ions at *m/z* 477.10 eluting at 9.85 min and *m/z* 463.09 eluting at 4.83 min were isorhamnetin-3-*O*-hexoside and quercetin-3-*O*-hexoside respectively. Similar to the MS/MS data from apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside, these hexosides also showed the loss of a hexose moiety (162 u). In addition to the fragment ion at *m/z* 315.0 corresponding to deprotonated molecular ion of isorhamnetin, the isorhamnetin-3-*O*-hexoside produced a fragment ion at *m/z* 300.0 further confirming that the hexose derivative was that of isorhamnetin. As expected isorhamnetin-3-*O*-hexoside was only detected in the extracts of rosemary, sage and thyme of the five spices examined (Table 1). Similar approach and conclusions were made for quercetin-3-O-hexoside. The present study also identified two phenolic rutinosides, namely apigenin-7-*O*-rutinoside and luteolin-7-*O*-rutinoside apart from quercetin-7-*O*-rutinoside (commonly known as rutin) in all the spices examined (Table 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 glucoronide
256 derivrative of flavonoids could be detected in all the spices examined. This compound
257 eluting at 12.15 min was identified as luteolin-3-*O*-glucoronide (Table 1). Subsequent
258 CID of luteolin-3-*O*-glucoronide showed the loss of a glucoronic 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 uder 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 [$-CH_2-CH_3$]
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 ($CH_2CH_2CH_3$). 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

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

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

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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).

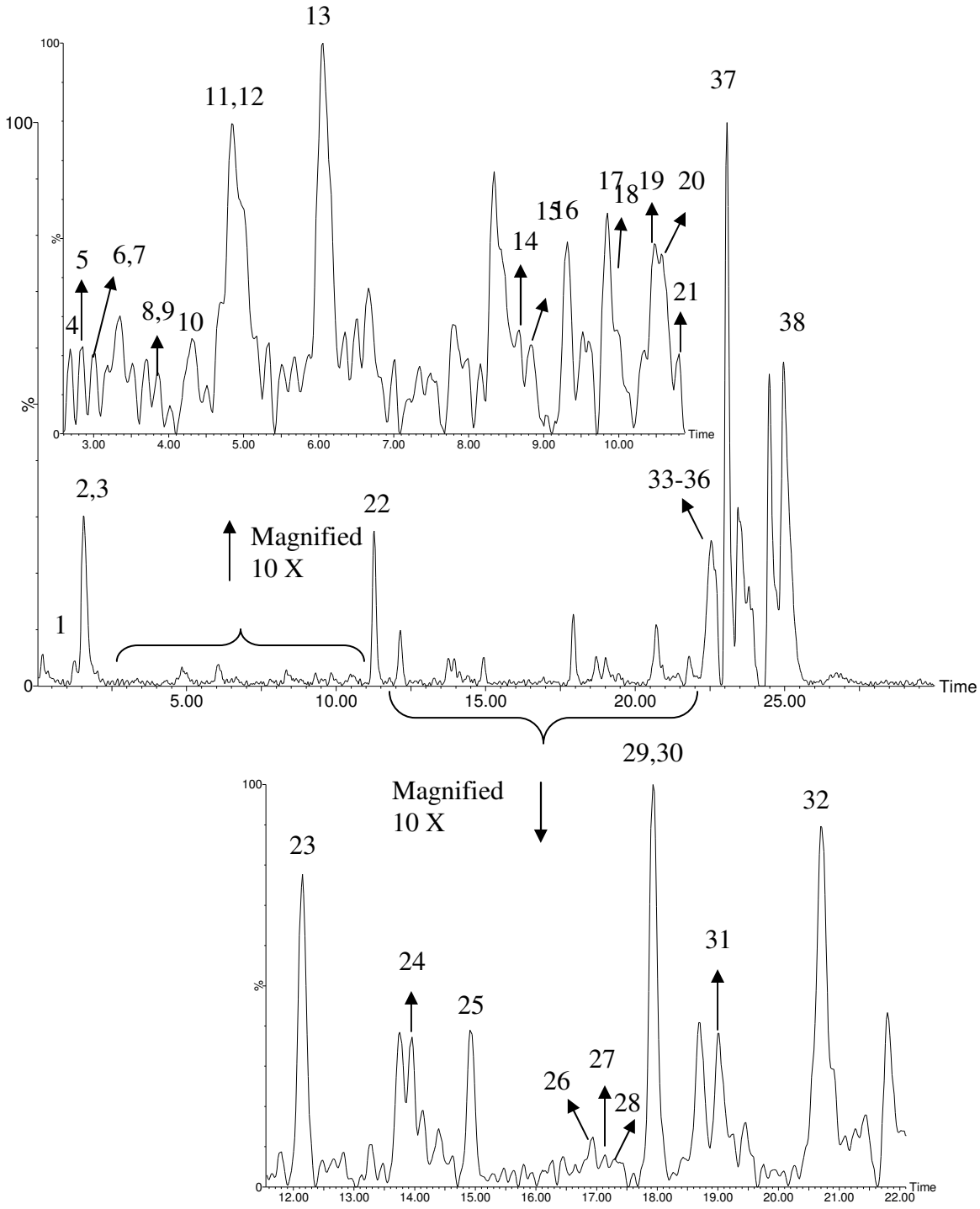
Table 1. Peak assignments of aqueous methanol extract of rosemary.

Peak No.	Polyphenols	Empirical formula	Observed *m/z*	Calculated *m/z*	Major fragments (intensity) *m/z*	RT (min)	Detected in
1	Gallic acid[a]	$C_7H_5O_5^-$	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]	$C_9H_7O_4^-$	179.0350	179.0344	161.0 (10 %), 135.0 (10 %)	1.57	R, O, S, B, T
4	Syringic acid[a]	$C_9H_9O_5^-$	197.0453	197.0450	179.0 (60 %), 135.0 (100 %)	2.62	R, O, S, B, T
5	Vanillic acid[a]	$C_8H_7O_4^-$	167.0366	167.0344	123.0 (70 %)	2.70	R, O, S, B, T
6	Protocatechuic acid[a]	$C_7H_5O_4^-$	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]	$C_9H_7O_3^-$	163.0402	163.0395	119.0 (100 %)	3.97	R, O, S, B, T
10	4-Hydroxybenzoic acid[a]	$C_7H_5O_3^-$	137.0247	137.0239	93.0 (40 %)	4.23	R[b], O[b], S[b], B[b], T[b]
11	Quercetin-3-*O*-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-*O*-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]	$C_{10}H_9O_4^-$	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-*O*-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-*O*-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-*O*-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-*O*-glucoronide	$C_{21}H_{17}O_{12}^-$	461.0725	461.0720	285.0 (100 %)	12.15	R[b], O[b], S[b], B[b], T

24	Luteolin-7-*O*-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]	$C_{15}H_9O_7^-$	301.0334	301.0349	227.1 (10 %), 151.1 (10 %)	16.86	R[b], O, S[b], B[b], T
27	Apigenin[a]	$C_{15}H_9O_5^-$	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-*O*-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]	$C_{15}H_9O_6^-$	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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11,12
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2,3
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33-36
Magnified
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Time
29,30
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10 X
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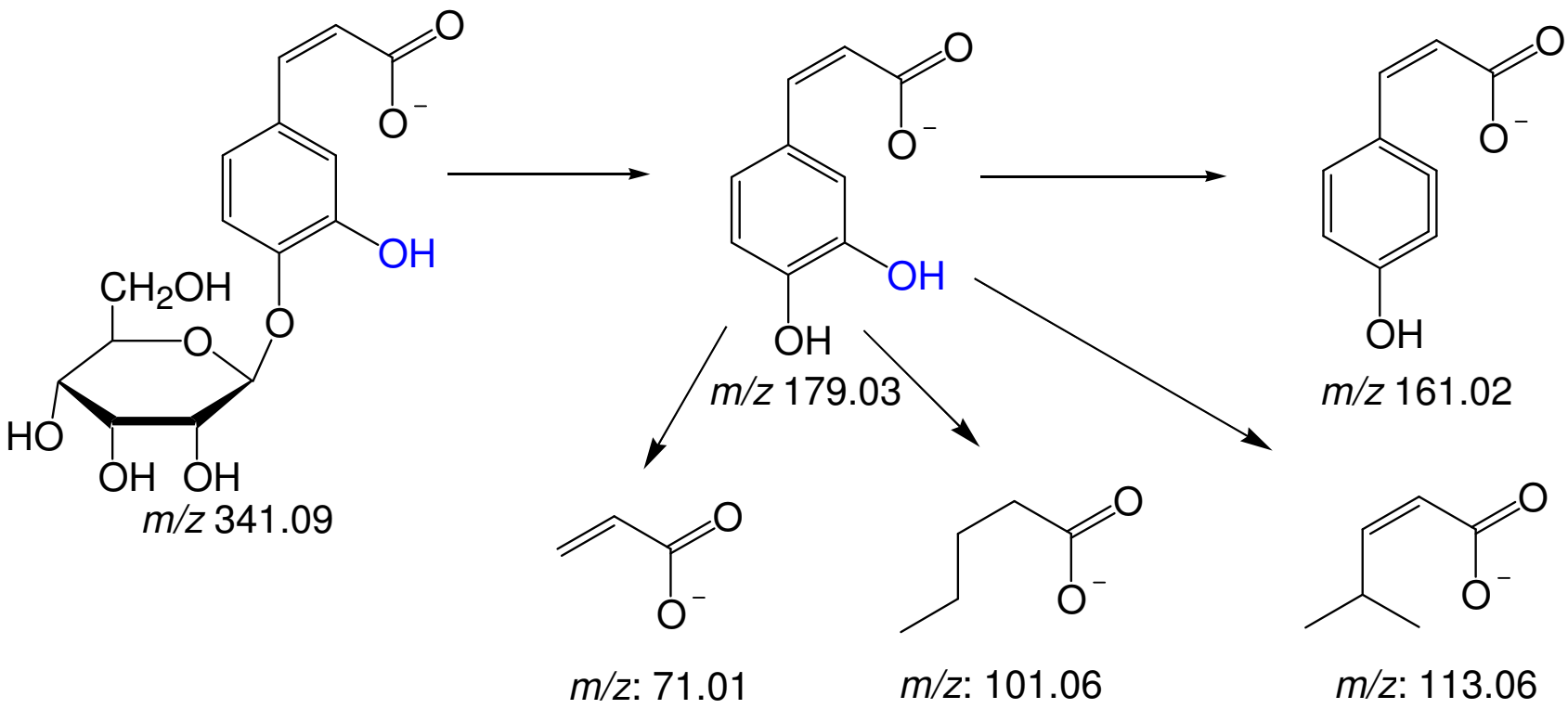
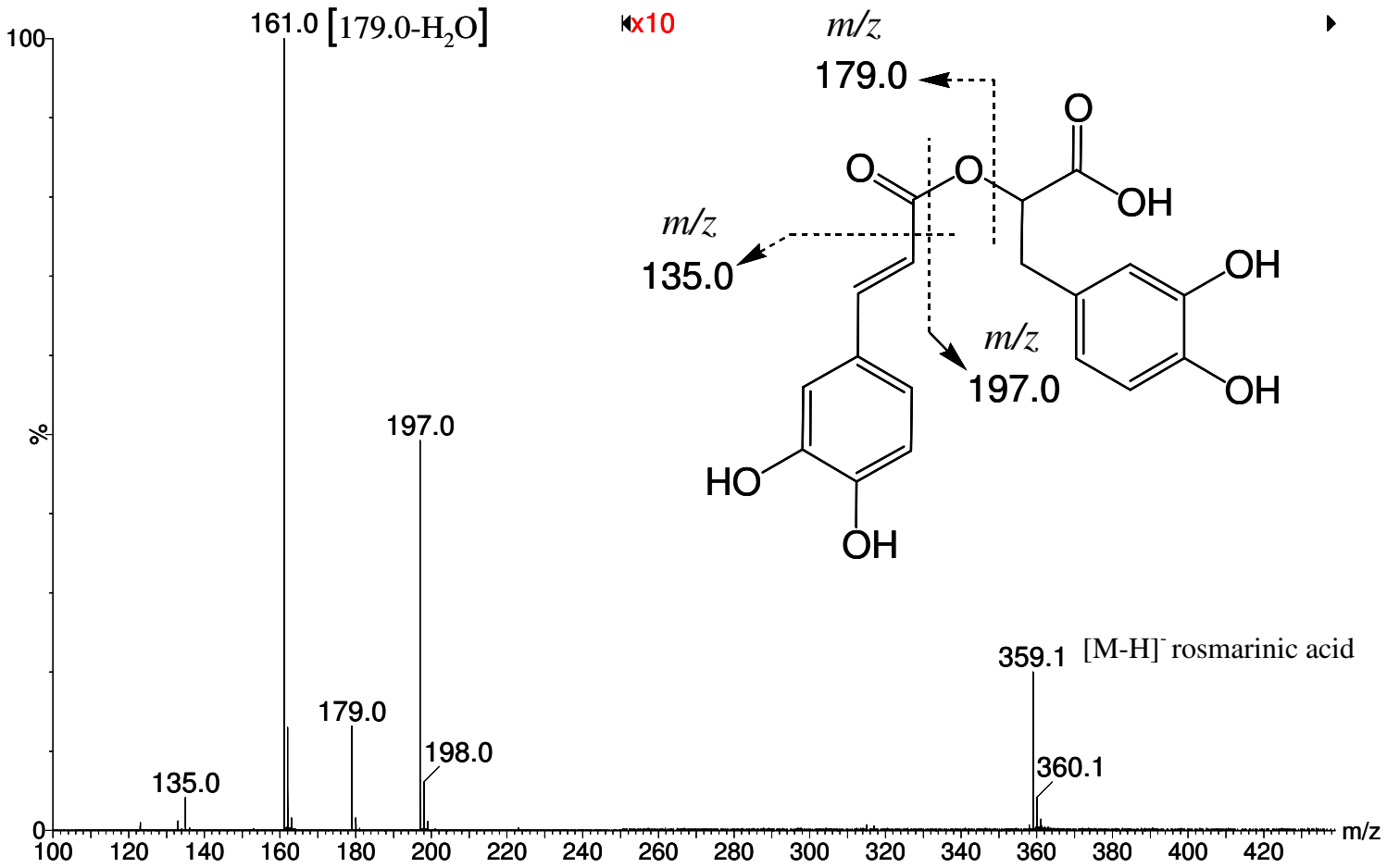
27
26
28
Time

Figure 2



(Dissociation products of the phenol ring)

Figure 3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x10
m/z
179.0
m/z
135.0
m/z
197.0
197.0
179.0
198.0
135.0
359.1
[M-H]- rosmarinic acid
360.1
100
%
0
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420
m/z
O
O
O
OH
OH
OH
HO
OH