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

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

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

Liquid chromatography-mass spectrometry (LC-MS). LC-MS analysis was performed on a Q-Tof Premier mass spectrometer (Waters Corporation, Micromass MS Technologies, Manchester, UK coupled to Alliance 2695 HPLC system (Waters Corporation, Milford, MA, USA). The Q-Tof Premier is equipped with a lockspray 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 dicafeoylquinic 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 dicafeoylquinic 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
derivatives mentioned above were detected in all the Lamiaceae spices examined by ESI-MS analyses (Table 1).

Flavonoids

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

For the remaining nine flavonoids listed in Table 1 for which there were no ‘in-house’ standards, their identifications were based solely on accurate mass measurements and the MS/MS data (Table 1). Acacetin found in rosemary, oregano and basil; cirsimaritin and methyl apigenin found in all 5 spices; and isorhamnetin found in rosemary, sage and thyme were the only four non-sugar based flavonoids. They had a characteristic feature in the MS/MS experiment where the loss of one or more methyl groups was observed. Acacetin ($m/z$ 283.1) eluting at 17.89 min, methyl apigenin ($m/z$ 283.1) eluting at 20.69 min and isorhamnetin ($m/z$ 315.0) eluting at 14.80 min lost one methyl group each producing $m/z$ 268.0, $m/z$ 268.0 and $m/z$ 300.0 respectively while cirsimaritin ($m/z$ 313.1) lost two consecutive methyl groups resulting fragment ions $m/z$ 298.0 and $m/z$ 283.1. 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 rutinos 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
ions 308 u (dehydrated rutinose moiety) lower than the \( m/z \) values of the precursor ions. The presence of rutin in rosemary and oregano extract has been reported by Papageorgiou, et al. (28) using reversed phase HPLC. However, only one glucuronide derivative of flavonoids could be detected in all the spices examined. This compound eluting at 12.15 min was identified as luteolin-3-\( O \)-glucoronide (Table 1). Subsequent CID of luteolin-3-\( O \)-glucoronide showed the loss of a glucoronic acid (\( m/z \) 176) and produced the predominant fragment at \( m/z \) 285.0 corresponding to deprotonated luteolin. Similar fragmentation of the compound was reported by Justesen (26) in analyzing thyme extracts.

**Phenolic terpenes and lignan**

There were 8 polyphenols detected in the spices examined that fall under the phenolic terpenes and lignan category (Table 1). Three of them, thymol, carnosol and carnosic acid, were identified as they showed identical LC-MS characteristics as that of the standards. Thymol detected only in thyme when subjected to CID produced fragments at \( m/z \) 131.0 and \( m/z \) 120.0 corresponding to the loss of water and an ethyl [-CH\(_2\)-CH\(_3\)] group (29 u) from the precursor ion \( (m/z \) 149.09). Carnosol detected in all the spices and carnosic acid found only in rosemary, oregano and sage showed major fragment ions following a loss of carbon dioxide as seen in all the phenolic acids. Decarboxylated carnosic acid further fragmented producing \( m/z \) 244.2 ions due to dissociation of a propyl group (CH\(_2\)CH\(_2\)CH\(_3\)). Methylated carnosic acid and methoxycarnosol were also identified in all the samples (Table 1). Methyl carnosate \( (m/z \) 345.20) eluting at 22.68 min produced two major fragments: \( m/z \) 301.2 due to loss of carbon dioxide molecule with
further loss of methyl group producing \textit{m/z} 286.2 ions. This fragmentation pattern was in agreement with that reported by Herrero et al., (2009) in analysing the phenolic antioxidant compounds of rosemary extracts. The methoxycarnosol (\textit{m/z} 359.17) eluting at 22.70 min also generated two major fragments in the MS/MS experiment: \textit{m/z} 329.2 and \textit{m/z} 285.2 corresponding to loss of a methoxy group and subsequent loss of carbon dioxide molecule. Epirosmannol which has the same nominal mass as that of methyl carnosate eluted 4.75 min earlier than the methyl carnosate in the LC separation (Figure 1 and Table 1). In addition to difference in elution time, the accurate mass measurement distinguished epirosmannol (calculated \textit{m/z} 345.1702, observed \textit{m/z} 345.1702) from methyl carnosate (calculated \textit{m/z} 345.2066, observed \textit{m/z} 345.2054). Furthermore the MS/MS data from epirosmannol, unlike methyl carnosate, showed the loss of water following decarboxylation. The last of the terpenes found in this study was rosmadial which had a unique fragmentation pathway compared to other terpenes described earlier. Rosmadial (\textit{m/z} 343.20) lost two and three methylene groups from the precursor ions resulting fragment ions \textit{m/z} 315.2 and \textit{m/z} 300.2 respectively. There was only one phenolic lignan, namely medioresinol, identified in the extracts of all Lamiaceae spices analysed.

Application of LC-ESI-MS/MS technique in the current study provided useful information to characterize 38 phenolic compounds in the extracts of five Lamiaceae spices. Fragments produced during CID analysis of the compounds mentioned above are the diagnostic features of these compounds which could be used to identify them in 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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LITERATURE CITED


(23) Gruz, J.; Novák, O.; Strnad, M. Rapid analysis of phenolic acids in beverages by UPLC-MS/MS. *Food Chem.* **2008**, *111*, 789-794.


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**Figure captions**

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

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

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.

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>Polyphenols</th>
<th>Empirical formula</th>
<th>Observed m/z</th>
<th>Calculated m/z</th>
<th>Major fragments (intensity)</th>
<th>RT (min)</th>
<th>Detected in</th>
</tr>
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<tr>
<td>1</td>
<td>Gallic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>169.0141</td>
<td>169.0137</td>
<td>125.0 (100 %)</td>
<td>1.25</td>
<td>R, O&lt;sup&gt;b&lt;/sup&gt;, S, B&lt;sup&gt;b&lt;/sup&gt;, T</td>
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<td>2</td>
<td>Caffeic acid hexoside</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;O&lt;sub&gt;9&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>341.0883</td>
<td>341.0873</td>
<td>179.0 (55 %), 161.0 (15 %)</td>
<td>1.53</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Caffeic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;4&lt;/sub&gt;O&lt;sup&gt;-&lt;/sup&gt;</td>
<td>179.0350</td>
<td>179.0344</td>
<td>161.0 (10 %), 135.0 (10 %)</td>
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<td>197.0453</td>
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<td>109.0 (100 %)</td>
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<td>315.2 (20 %), 300.2 (20 %)</td>
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<td>R</td>
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<td>C&lt;sub&gt;16&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;O&lt;sub&gt;9&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
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<td>353.0873</td>
<td>191.1 (42 %), 179.0 (62 %), 173.0 (100 %)</td>
<td>3.91</td>
<td>R, O, S, B, T</td>
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<tr>
<td>9</td>
<td>p-Coumaric acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;9&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sup&gt;-&lt;/sup&gt;</td>
<td>163.0402</td>
<td>163.0395</td>
<td>119.0 (100 %)</td>
<td>3.97</td>
<td>R, O, S, B, T</td>
</tr>
<tr>
<td>10</td>
<td>4-Hydroxybenzoic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;O&lt;sup&gt;-&lt;/sup&gt;</td>
<td>137.0247</td>
<td>137.0239</td>
<td>93.0 (40 %)</td>
<td>4.23</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>11</td>
<td>Quercetin-3-O-hexoside</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;19&lt;/sub&gt;O&lt;sub&gt;12&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>463.0880</td>
<td>463.0877</td>
<td>301.0 (50 %)</td>
<td>4.83</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>Medioresinol</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>387.1421</td>
<td>387.1444</td>
<td>207.1 (20 %)</td>
<td>4.84</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>Gallocatechin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;15&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>305.0665</td>
<td>305.0661</td>
<td>225.0 (88 %)</td>
<td>6.08</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>Luteolin-7-O-glucoside&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;11&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>447.0920</td>
<td>447.0927</td>
<td>285.0 (50 %)</td>
<td>8.87</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15</td>
<td>Ferulic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;O&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>193.0518</td>
<td>193.0501</td>
<td>178.0 (10 %), 149.0 (100 %)</td>
<td>8.98</td>
<td>R, O, S, B, T</td>
</tr>
<tr>
<td>16</td>
<td>Phloridzin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;3&lt;/sub&gt;O&lt;sub&gt;10&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>435.1302</td>
<td>435.1291</td>
<td>273.0 (65 %), 167 (40 %)</td>
<td>9.38</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>17</td>
<td>Isorhamnetin-3-O-hexoside</td>
<td>C&lt;sub&gt;22&lt;/sub&gt;H&lt;sub&gt;21&lt;/sub&gt;O&lt;sub&gt;12&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>477.1036</td>
<td>477.1033</td>
<td>462.0 (10 %), 315.0 (100 %), 300.0 (20 %)</td>
<td>9.85</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>18</td>
<td>Dicaffeoylquinic acid</td>
<td>C&lt;sub&gt;25&lt;/sub&gt;H&lt;sub&gt;23&lt;/sub&gt;O&lt;sub&gt;12&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>515.1163</td>
<td>515.1190</td>
<td>359.0 (15 %), 179.0 (54 %), 135.0 (25 %), 101.0 (6 %)</td>
<td>10.01</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>19</td>
<td>Apigenin-7-O-rutinoside</td>
<td>C&lt;sub&gt;27&lt;/sub&gt;H&lt;sub&gt;29&lt;/sub&gt;O&lt;sub&gt;14&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>577.1559</td>
<td>577.1557</td>
<td>269.0 (100 %)</td>
<td>10.54</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>Rutin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;27&lt;/sub&gt;H&lt;sub&gt;29&lt;/sub&gt;O&lt;sub&gt;16&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>609.1473</td>
<td>609.1456</td>
<td>301.0 (100 %)</td>
<td>10.59</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>21</td>
<td>Apigenin-7-O-glucoside&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;19&lt;/sub&gt;O&lt;sub&gt;10&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>431.0993</td>
<td>431.0978</td>
<td>269.1 (22 %)</td>
<td>10.62</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>22</td>
<td>Rosmarinic acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C&lt;sub&gt;18&lt;/sub&gt;H&lt;sub&gt;15&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>359.0763</td>
<td>359.0767</td>
<td>197.0 (50 %), 179.0 (20 %), 161.0 (100 %), 135.0 (10 %)</td>
<td>11.27</td>
<td>R, O, S, B, T</td>
</tr>
<tr>
<td>23</td>
<td>Luteolin-3-O-glucoronide</td>
<td>C&lt;sub&gt;21&lt;/sub&gt;H&lt;sub&gt;17&lt;/sub&gt;O&lt;sub&gt;12&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>461.0725</td>
<td>461.0720</td>
<td>285.0 (100 %)</td>
<td>12.15</td>
<td>R&lt;sup&gt;b&lt;/sup&gt;, O&lt;sup&gt;b&lt;/sup&gt;, S&lt;sup&gt;b&lt;/sup&gt;, B&lt;sup&gt;b&lt;/sup&gt;, T&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>No.</td>
<td>Name</td>
<td>Molecular Formula</td>
<td>Exact Mass</td>
<td>Tentative Mass</td>
<td>Purity (%)</td>
<td>MW</td>
<td>Retention Time</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------</td>
<td>-------------------</td>
<td>------------</td>
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<td>------------</td>
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<td>----------------</td>
</tr>
<tr>
<td>24</td>
<td>Luteolin-7-O-rutinoside</td>
<td>C(<em>{27})H(</em>{30})O(_{15})(^-)</td>
<td>593.1533</td>
<td>593.1506</td>
<td>285.0 (28 %)</td>
<td>14.09</td>
<td>R(^b), O(^b), S(^b), B(^b), T(^b)</td>
</tr>
<tr>
<td>25</td>
<td>Isorhamnetin</td>
<td>C(<em>{16})H(</em>{11})O(_{7}) (^-)</td>
<td>315.0489</td>
<td>315.0505</td>
<td>300.0 (100 %)</td>
<td>14.80</td>
<td>R(^b), S(^b), T(^b)</td>
</tr>
<tr>
<td>26</td>
<td>Quercetin(^a)</td>
<td>C(<em>{15})H(</em>{9})O(_{7}) (^-)</td>
<td>301.0334</td>
<td>301.0349</td>
<td>227.1 (10 %), 151.1 (10 %)</td>
<td>16.86</td>
<td>R(^b), O(^b), S(^b), B(^b), T</td>
</tr>
<tr>
<td>27</td>
<td>Apigenin(^a)</td>
<td>C(<em>{15})H(</em>{9})O(_{5}) (^-)</td>
<td>269.0441</td>
<td>269.0450</td>
<td>158.9 (15 %)</td>
<td>17.09</td>
<td>R, O(^b), S(^b), B, T</td>
</tr>
<tr>
<td>28</td>
<td>Thymol(^a)</td>
<td>C(<em>{10})H(</em>{13})O(_{5}) (^-)</td>
<td>149.0981</td>
<td>149.0966</td>
<td>131.0 (10 %), 120.0 (25 %)</td>
<td>17.30</td>
<td>T(^b)</td>
</tr>
<tr>
<td>29</td>
<td>Acacetin</td>
<td>C(<em>{16})H(</em>{11})O(_{5}) (^-)</td>
<td>283.0613</td>
<td>283.0606</td>
<td>268.0 (45 %)</td>
<td>17.89</td>
<td>R(^b), O(^b), B(^b)</td>
</tr>
<tr>
<td>30</td>
<td>Epirosmanol</td>
<td>C(<em>{20})H(</em>{25})O(_{5}) (^-)</td>
<td>345.1702</td>
<td>345.1702</td>
<td>301.2 (100 %), 283.2 (25 %)</td>
<td>17.93</td>
<td>R, O(^b), S(^b), B(^b), T(^b)</td>
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<tr>
<td>31</td>
<td>Cirsimaritin</td>
<td>C(<em>{17})H(</em>{15})O(_{5}) (^-)</td>
<td>313.0700</td>
<td>313.0712</td>
<td>298.0 (55 %), 283.0 (18 %)</td>
<td>19.01</td>
<td>R, O(^b), S(^b), B(^b), T</td>
</tr>
<tr>
<td>32</td>
<td>Methyl apigenin</td>
<td>C(<em>{16})H(</em>{11})O(_{5}) (^-)</td>
<td>283.0616</td>
<td>283.0606</td>
<td>268.0 (100 %)</td>
<td>20.69</td>
<td>R(^b), O(^b), S(^b), B(^b), T(^b)</td>
</tr>
<tr>
<td>33</td>
<td>Hydroxybenzoic acid-O-hexoside</td>
<td>C(<em>{13})H(</em>{15})O(_{8}) (^-)</td>
<td>299.0752</td>
<td>299.0767</td>
<td>137.0 (100 %)</td>
<td>22.64</td>
<td>R(^b), O(^b), S(^b), B(^b), T(^b)</td>
</tr>
<tr>
<td>34</td>
<td>Methyl carnosate</td>
<td>C(<em>{21})H(</em>{20})O(_{4}) (^-)</td>
<td>345.2054</td>
<td>345.2066</td>
<td>301.2 (100 %), 286.2 (20 %)</td>
<td>22.68</td>
<td>R(^b), O(^b), S(^b), B(^b), T(^b)</td>
</tr>
<tr>
<td>35</td>
<td>Methoxy carnosol</td>
<td>C(<em>{21})H(</em>{27})O(_{5}) (^-)</td>
<td>359.1855</td>
<td>359.1858</td>
<td>329.2 (100 %), 285.2 (40 %)</td>
<td>22.70</td>
<td>R(^b), O(^b), S(^b), B(^b), T(^b)</td>
</tr>
<tr>
<td>36</td>
<td>Luteolin(^a)</td>
<td>C(<em>{15})H(</em>{9})O(_{6}) (^-)</td>
<td>285.0392</td>
<td>285.0399</td>
<td>267.0 (60 %)</td>
<td>22.95</td>
<td>R(^b), O(^b), S(^b), B(^b), T(^b)</td>
</tr>
<tr>
<td>37</td>
<td>Carnosol(^a)</td>
<td>C(<em>{20})H(</em>{25})O(_{4}) (^-)</td>
<td>329.1747</td>
<td>329.1753</td>
<td>285.1 (40 %)</td>
<td>23.09</td>
<td>R, O(^b), S, B(^b), T(^b)</td>
</tr>
<tr>
<td>38</td>
<td>Carnosic acid(^a)</td>
<td>C(<em>{20})H(</em>{27})O(_{4}) (^-)</td>
<td>331.1903</td>
<td>331.1909</td>
<td>287.2 (100 %), 244.2 (10 %)</td>
<td>24.97</td>
<td>R, O(^b), S</td>
</tr>
</tbody>
</table>

\(^a\) 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

[Graph with various time markers and peaks labeled with numbers.]
(Dissociation products of the phenol ring)
Figure 3

161.0 \([179.0-\text{H}_2\text{O}]\)

179.0

135.0

197.0

198.0

197.0

359.1

360.1

\([\text{M-H}]\) rosmarinic acid