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ARTICLE

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

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A total of 38 phenolic compounds in the solid/liquid extracts of five Lamiaceae spices, 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. If standards were unavailable, phenolic compounds were identified on the basis of accurate mass of pseudomolecular $[M - H]^-$ ions and tandem mass spectrometry (MS/MS) data. The results of accurate mass measurements fit well with the elemental composition of the compounds. The diagnostic fragmentation patterns of the compounds during collision-induced dissociation (CID) elucidated the structural information of the compounds analyzed.

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, the use of which 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 reducing 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 sensory attributes such as the taste and aroma of foods (*11*). Because these spices are commonly consumed in most countries, there are no legal barriers to their use in foods. However, their use in foods as either a control measure for lipid oxidation or to 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 standalone HPLC coupled with diode array detection (*12*−*15*). A previous LC-ESI-MS study of polyphenols in the 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 a LC-ESI-MS method. Other studies (*18*−*22*) also identified similar numbers of compounds in different members of the family. In the present study we examined 38 polyphenols in 5 Lamiaceae spices using liquid chromatographic separation and collision-induced dissociation analysis. Furthermore, an accurate mass measurement technique was successfully applied for the first time in this spice family to elucidate the elemental composition of the polyphenols studied.

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MATERIALS AND METHODS

Samples and Reagents. Dried and ground rosemary, oregano, sage, basil, and thyme were provided by AllinAll Ingredients Ltd., Dublin, 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 s). The dried spices were ground (particle size range = 500−600 μm) and stored at −20 °C in darkness.

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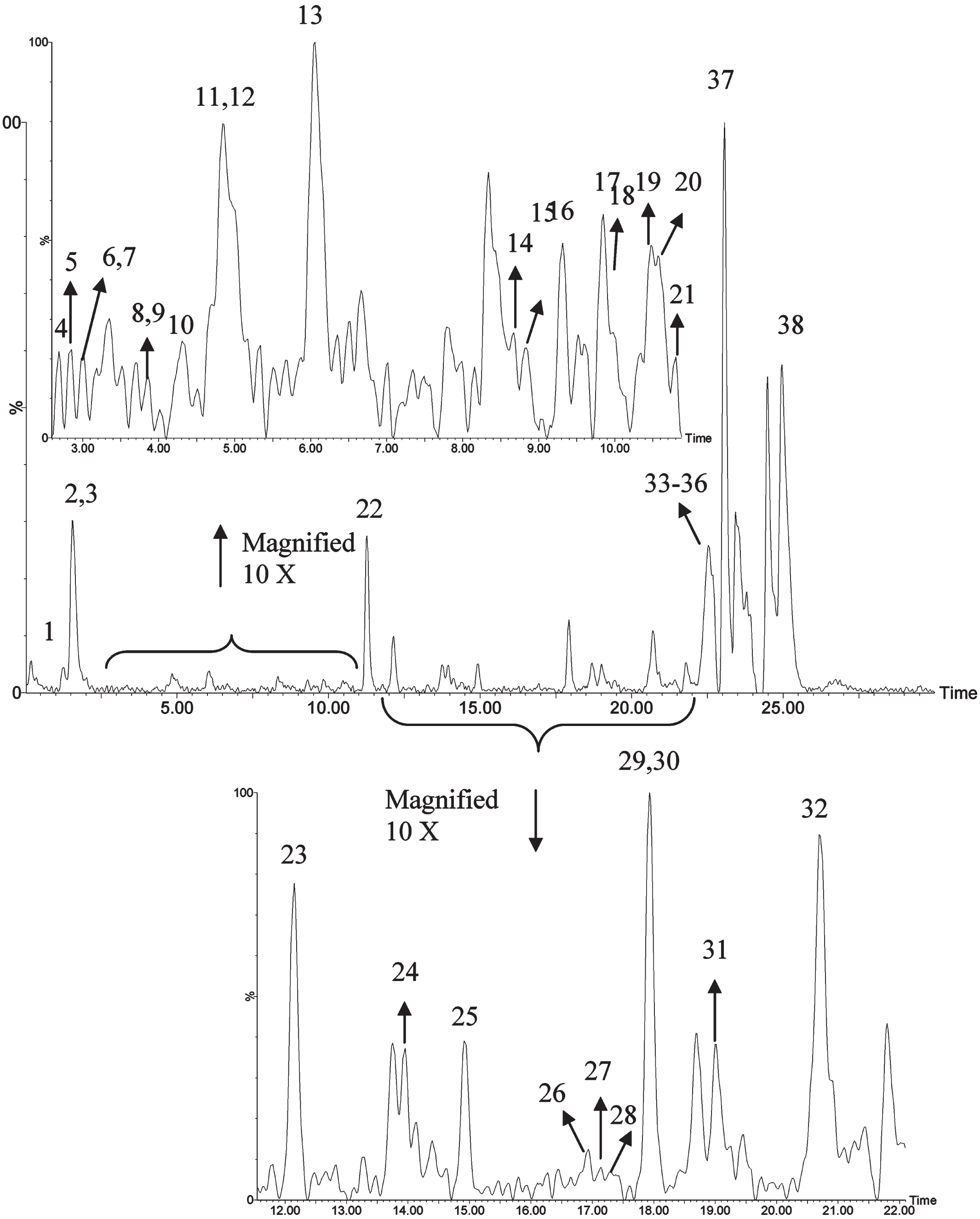

Figure 1. Total ion current (TIC) chromatogram of aqueous methanol extract of rosemary.

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, Steinheim, Germany. Four flavonoid standards, apigenin, apigenin-7-*O*-glucoside, luteolin, and luteolin-7-*O*-glucoside, were purchased from Extrasynthese, Genay, France. HPLC grade methanol and water were purchased from VWR International Limited, Leicestershire, U.K., and Lennox Laboratory Supplies Limited, Dublin, Ireland, respectively. The purities of standards and solvents were in the range of 95−99.8%. 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 homogenized for 1 min at 24000 rpm using an Ultra-Turrax T-25 tissue homogenizer (Janke & Kunkel, IKA-Labortechnik, Staufen, 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 homogenized sample suspension was shaken overnight with a V400 Multitude Vortexer (Alpha Laboratories, North York, Canada) at 1500 rpm and room temperature. The mixture was then centrifuged for 15 min at 2000*g* (MSE Mistral 3000i, Sanyo Gallenkamp, Leicestershire, U.K.) and filtered through 0.22 μm polytetrafluoroethylene (PTFE) filters (Sigma-Aldrich). 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 Corp., Micromass MS Technologies, Manchester, U.K.), coupled to an Alliance 2695 HPLC system (Waters Corp., Milford, MA). 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 Corp., Milford, MA; 100 mm × 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

Table 1. Peak Assignments of Aqueous Methanol Extract of Rosemary

peak	polyphenol	empirical formula	obsd *m/z*	calcd *m/z*	major fragments (intensity) *m/z*	RT (min)	detected in[b]
1	gallic acid[a]	$C_7H_5O_5^-$	169.0141	169.0137	125.0 (100%)	1.25	R, O,[c] S, B,[c] T
2	caffeic acid hexoside	$C_{15}H_{17}O_9^-$	341.0883	341.0873	179.0 (55%), 161.0 (15%)	1.53	R,[c] O,[c] S,[c] B,[c] T[c]
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,[c] O,[c] S,[c] B,[c] T[c]
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,[c] O,[c] S,[c] B,[c] T[c]
11	quercetin-3-*O*-hexoside	$C_{21}H_{19}O_{12}^-$	463.0880	463.0877	301.0 (50%)	4.83	R,[c] O,[c] S,[c] B,[c] T[c]
12	medioresinol	$C_{21}H_{23}O_7^-$	387.1421	387.1444	207.1 (20%)	4.84	R,[c] O,[c] S,[c] B,[c] T[c]
13	gallocatechin[a]	$C_{15}H_{13}O_7^-$	305.0665	305.0661	225.0 (88%)	6.08	R, O,[c] S,[c] B, T[c]
14	luteolin-7-*O*-glucoside[a]	$C_{21}H_{19}O_{11}^-$	447.0920	447.0927	285.0 (50%)	8.87	R,[c] O,[c] S,[c] B,[c] 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,[c] O,[c] S,[c] B,[c] T[c]
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,[c] S,[c] T[c]
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,[c] O,[c] S,[c] B,[c] T[c]
19	apigenin-7-*O*-rutinoside	$C_{27}H_{29}O_{14}^-$	577.1559	577.1557	269.0 (100%)	10.54	R,[c] O,[c] S,[c] B,[c] T[c]
20	rutin[a]	$C_{27}H_{29}O_{16}^-$	609.1473	609.1456	301.0 (100%)	10.59	R,[c] O,[c] B,[c] T
21	apigenin-7-*O*-glucoside[a]	$C_{21}H_{19}O_{10}^-$	431.0993	431.0978	269.1 (22%)	10.62	R,[c] O,[c] S,[c] B,[c] 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*-glucuronide	$C_{21}H_{17}O_{12}^-$	461.0725	461.0720	285.0 (100%)	12.15	R,[c] O,[c] S,[c] B,[c] T
24	luteolin-7-*O*-rutinoside	$C_{27}H_{29}O_{15}^-$	593.1533	593.1506	285.0 (28%)	14.09	R,[c] O,[c] S,[c] B,[c] T[c]
25	isorhamnetin	$C_{16}H_{11}O_7^-$	315.0489	315.0505	300.0 (100%)	14.80	R,[c] S,[c] T[c]
26	quercetin[a]	$C_{15}H_9O_7^-$	301.0334	301.0349	227.1 (10%), 151.1 (10%)	16.86	R,[c] O, S,[c]B,[c] T
27	apigenin[a]	$C_{15}H_9O_5^-$	269.0441	269.0450	158.9 (15%)	17.09	R, O,[c] S,[c] B, T
28	thymol[a]	$C_{10}H_{13}O^-$	149.0981	149.0966	131.0 (10%), 120.0 (25%)	17.30	T[c]
29	acacetin	$C_{16}H_{11}O_5^-$	283.0613	283.0606	268.0 (45%)	17.89	R, O,[c] B[c]
30	epirosmanol	$C_{20}H_{25}O_5^-$	345.1702	345.1702	301.2 (100%), 283.2 (25%)	17.93	R, O,[c] S, B,[c] T[c]
31	cirsimaritin	$C_{17}H_{13}O_6^-$	313.0700	313.0712	298.0 (55%), 283.0 (18%)	19.01	R, O,[c] S,[c] B,[c] T
32	methyl apigenin	$C_{16}H_{11}O_5^-$	283.0616	283.0606	268.0 (100%)	20.69	R,[c] O,[c] S,[c] B,[c] T[c]
33	hydroxybenzoic acid-*O*-hexoside	$C_{13}H_{15}O_8^-$	299.0752	299.0767	137.0 (100%)	22.64	R,[c] O,[c] S,[c] B,[c] T[c]
34	methyl carnosate	$C_{21}H_{29}O_4^-$	345.2054	345.2066	301.2 (100%), 286.2 (20%)	22.68	R, O,[c] S,[c] B,[c] T[c]
35	methoxycarnosol	$C_{21}H_{27}O_5^-$	359.1855	359.1858	329.2 (100%), 285.2 (40%)	22.70	R,[c] O,[c] S,[c] B,[c] T[c]
36	luteolin[a]	$C_{15}H_9O_6^-$	285.0392	285.0399	267.0 (60%)	22.95	R,[c] O,[c] S,[c] B,[c] T[c]
37	carnosol[a]	$C_{20}H_{25}O_4^-$	329.1747	329.1753	285.1 (40%)	23.09	R, O,[c] S, B,[c] T[c]
38	carnosic acid[a]	$C_{20}H_{27}O_4^-$	331.1903	331.1909	287.2 (100%), 244.2 (10%)	24.97	R, O,[c] S

[a] Identification confirmed using commercial standards. [b] R, rosemary; O, oregano; S, sage; B, basil; T, thyme. [c] Compounds characterized for the first time by LC-ESI-MS/MS.

mass spectra data were recorded on a negative ionization mode for a mass range from *m/z* 100 to 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 12−20 eV of energy with argon as the collision gas.

RESULTS AND DISCUSSION

A total of 38 polyphenols distributed in 4 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 have been assigned in **Table 1**. Because 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 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 of 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 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

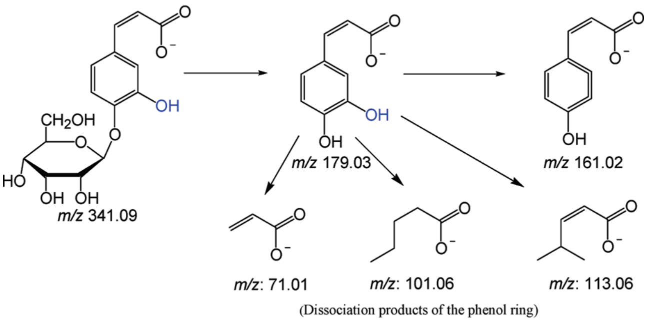

Figure 2. Schematic diagram of the production of fragments from caffeic acid hexoside (*m/z* 341.09) during CID analysis.

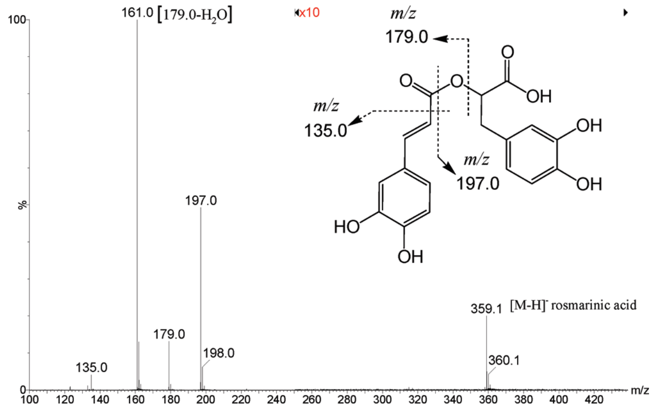

Figure 3. ESI-MS/MS spectrum of product ion scan of rosmarinic acid (*m/z* 359.10).

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 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 CID, characterized 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 precursor and fragment ions similar to those 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, 179.0, and 173.0. The product ions *m/z* 191.1 for quinic acid and *m/z* 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, 191.0, and 179.0 corresponding to the pseudomolecular ions of caffeoylquinic acid, quinic acid, and caffeic acid, respectively, in addition to the fingerprint 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**. A 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, 197.04, 167.04, 153.02, and 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, 123.0, 109.0, and 93.0, respectively, due to loss of CO_2 from their respective precursor ions. This pattern of fragmentation was a characteristic feature of hydroxybenzoic acid derivatives as for 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 a hexose moiety producing deprotonated 4-hydroxybenzoic acid at *m/z* 137.0. All of the hydroxybenzoic acid derivatives mentioned above were detected in all of the Lamiaceae spices examined by LC-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 of the spices studied by LC-MS. The eight flavonoids were apigenin, luteolin, apigenin-7-*O*-glucoside, luteolin-7-*O*-glucoside, gallocatechin, phloridzin, quercetin, and rutin. Furthermore, the fragmentation patterns of these flavonoids were 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 MS/MS data (**Table 1**). Acacetin found in rosemary, oregano, and basil; cirsimaritin and methyl apigenin found in all five 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 isorhamentin (*m/z* 315.0) eluting at 14.80 min lost one methyl group each, producing *m/z* 268.0, 268.0, and 300.0, respectively, whereas cirsimaritin (*m/z* 313.1) lost two consecutive methyl groups, resulting in fragment ions *m/z* 298.0 and 283.1. Despite the fact that acacetin and methylapigenin are isomers differing only in the position of the methyl group, they separated well in the reversed phase LC. Because acacetin is slightly more polar than methylapigenin, it eluted earlier in the LC separation. Justesen (*26*) described a 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 us, have noted three fragment ions from isorhamnetin, that is, *m*/*z* 300, 271, and 255, in fennel extracts by ESI-MS/MS analysis. The difference could probably be due to different sets 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 a deprotonated molecular ion of isorhamnetin, 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 detected in only the extracts of rosemary, sage, and thyme of the five spices examined (**Table 1**). A 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 of 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 extracts has been reported by Papageorgiou et al. (*28*) using reversed phase HPLC. However, only one glucuronide derivative of flavonoids could be detected in all of the spices examined. This compound eluting at 12.15 min was identified as luteolin-3-*O*-glucuronide (**Table 1**). Subsequent CID of luteolin-3-*O*-glucuronide showed the loss of a glucuronic 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 eight 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 LC-MS characteristics identical to those of the standards. Thymol, detected only in thyme, when subjected to CID produced fragments at *m*/*z* 131.0 and 120.0, corresponding to the loss of water and an ethyl ($-CH_2CH_3$) group (29 u) from the precursor ion (*m*/*z* 149.09). Carnosol, detected in all of 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 of the phenolic acids. Decarboxylated carnosic acid further fragmented, producing *m*/*z* 244.2 ions due to dissociation of a propyl group ($-CH_2CH_2CH_3$). Methylated carnosic acid and methoxycarnosol were also identified in all of 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 a methyl group, producing *m*/*z* 286.2 ions. This fragmentation pattern was in agreement with that reported by Herrero et al. (*17*) in analyzing the phenolic antioxidant compounds of rosemary extracts. The methoxycarnosol (*m*/*z* 359.17) eluting at 22.70 min also generated two major fragments in the MS/MS experiment: *m*/*z* 329.2 and 285.2, corresponding to loss of a methoxy group and subsequent loss of a carbon dioxide molecule. Epirosmannol, which has the same nominal mass as methyl carnosate, eluted 4.75 min earlier than the methyl carnosate in the LC separation (**Figure 1** and **Table 1**). In addition to the difference in elution time, the accurate mass measurement distinguished epirosmannol (calculated *m*/*z* 345.1702, observed *m*/*z* 345.1702) from methyl carnosate (calculated *m*/*z* 345.2066, observed *m*/*z* 345.2054). Furthermore, the MS/MS data from epirosmannol, unlike those from 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 lost ethylene and propyl groups from the precursor ions (*m*/*z* 343.20), resulting in fragment ions *m*/*z* 315.2 and *m*/*z* 300.2 respectively. There was only one phenolic lignan, namely, medioresinol, identified in the extracts of all Lamiaceae spices analyzed.

Application of the LC-ESI-MS/MS technique in the current study provided useful information to characterize 38 phenolic compounds in the extracts of 5 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 to be useful to differentiate compounds with the same nominal mass but dissimilar exact masses (**Table 1**). Equally, mass spectrometry was advantageous in the identification of polyphenols for those compounds that did not separate as different entities in the reversed phase column. Nonetheless, for isomeric polyphenols such as acacetin and methylapigenin, which posed challenges for MS, the LC was able to resolve the isomers. One inherent weakness of the low collision energy MS/MS studies was that they could not localize the position in the native phenolic ring that underwent modification. In such a 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 rosemary, 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 LC's ability to separate isomeric compounds provided a powerful tool in the identification of polyphenolic diversity in five species of the 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, 13.96, 23.5, and 24.45 min, respectively, could not be identified.

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