Published in final edited form as:

J Sep Sci. 2018 June ; 41(11): 2331–2346. doi:10.1002/jssc.201800037.

Ultra-fast liquid chromatography coupled with electrospray ionization/time-of-flight mass spectrometry for rapid phenolic profiling of red maple (*Acer rubrum*) leaves

Chunting Li, Navindra P. Seeram

Bioactive Botanical Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA

Abstract

The red maple (*Acer rubrum*) species is economically important to North America because of its sap which is used to produce maple syrup. In addition, various other red maple plant parts, including leaves, were used as a traditional medicine by the Native Americans. Currently, red maple leaves are being used for nutraceutical and cosmetic applications but there are no published analytical methods for comprehensive phytochemical characterization of this material. Herein, a rapid and sensitive method using liquid chromatography-electrospray ionization/time-of-flight tandem mass spectrometry was developed to characterize the phenolics in a methanol extract of red maple leaves and a proprietary phenolic-enriched red maple leaves extract (Maplifa™). Time-of-flight mass spectrometry and tandem MS experiments led to the identification of 106 phenolic compounds in red maples leaves with the vast majority of these compounds also detected in Maplifa™. The compounds included 68 gallotannins, 25 flavonoids, gallic acid, quinic acid, catechin, epicatechin, and 9 other gallic acid derivatives among which 11 are potentially new and 75 are being reported from red maple for the first time. The developed method to characterize red maple leaves phenolics is rapid and highly sensitive and could aid in future standardization and quality control of this botanical ingredient.

Keywords

Acer rubrum; Maplifa™; Phenolics; Red maple; Tandem mass spectrometry

1 INTRODUCTION

The red maple (*Acer rubrum*) species is native to eastern North America where it is widely regarded as an economically important agricultural crop mainly for its sap which is used to produce the natural sweetener, maple syrup. Although maple sap/syrup are widely consumed as a food product, various parts of the red maple species, including its buds, flowers, leaves,

Correspondence: Professor, Navindra P. Seeram, Bioactive Botanical Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, 7 Greenhouse Road, Kingston, RI 02881, USA, nseeram@uri.edu; Tel: +1 401-874-9367; Fax: +1 401-874-5787.

CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

twigs, and barks, were used as a folk medicine by the Native Americans for the treatment of a variety of ailments [1]. More recently, extracts purified from these red maple plant parts are being investigated for their potential nutraceutical and cosmetic applications based on a wide range of biological properties including antioxidant, α -glucosidase enzyme inhibitory, blood-glucose lowering, and skin lightening cosmetic effects [2–6]. These biological effects have largely been attributed to the phenolic constituents found in the red maple species, in particular, anhydro-1,5-glucitol-core containing gallotannins which are only produced by certain members of the maple (*Acer*) genus in the entire higher plant kingdom [7]. Our group has conducted extensive previous isolation and structure elucidation studies (by TOF-MS and NMR spectroscopic data) of the leaves, flowers, twigs, and barks of the red maple species which yielded a series of new compounds containing different numbers of galloyl substituents located at different positions on the anhydro-1,5-glucitol-core [8–10]. In addition, the buds of the red maple species have also been reported to contain a variety of phenolic sub-classes, including anhydro-1,5-glucitol-core containing gallotannins [3].

The utilization of well-established analytical methodologies, such as liquid chromatography-electrospray ionization/time-of-flight tandem mass spectrometry (LC-ESI-TOF-MS/MS) have been extensively applied for the structure elucidation of target and/or non-target compounds in botanical extracts [11–15]. Given limited phytochemical compositional data available on the leaves of the red maple species, which are necessary for its future advancement as a botanical raw material or ingredient for dietary supplement and cosmetic applications, herein, we developed a rapid and sensitive ultra-fast liquid chromatography-electrospray ionization/time-of-flight tandem mass spectrometry (UFLC-ESI-TOF-MS/MS) method to identify its phenolic compounds. We also used UFLC-ESI-TOF-MS/MS to evaluate the phenolics present in a proprietary phenolic-enriched red maple leaves extract (Maplifa™) which has been previously studied for its cosmetic applications [5].

2 MATERIALS AND METHODS

2.1 Chemicals

Reference standards for maplexin A, maplexin B, maplexin C, maplexin D, ginnalin A, ginnalin B, ginnalin C, 3,6-di-*O*-galloyl-1,5-anhydroglucitol, catechin, epicatechin, gallic acid, gallic acid methyl ester, 5-methyl gallic acid methyl ester, and ellagic acid, used for ESI-TOF-MS/MS characterization, were previously isolated and identified by our laboratory [8, 9, 16]. LC-MS grades of acetonitrile and methanol were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Sample preparation

Leaves of the red maple species were collected in the summer and botanically authenticated with a deposited voucher specimen as previously reported by our laboratory [8–10]. Finely powdered air-dried red maple leaves (1.0 g) was extracted by sonication with methanol (10 mL) for 1 hour under room temperature. The extract was stored at 4 °C and filtered (0.22 μ m) before analyses.

2.3 Ultra-fast Liquid Chromatography Mass Spectrometry Conditions

UFLC-ESI-TOF-MS/MS analyses were performed on a SHIMADZU Prominence UFLC system (Marlborough, MA, USA) consisting of two LC-20AD pumps, a DGU-20A degassing unit, SIL-20AC auto sampler, CTO-20AC column oven and CBM-20A communication bus module. Chromatographic separation was performed on a 150 mm × 4.6 mm i.d., 5 µm, Prodigy ODS column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid/acetonitrile (A) and 0.1% aqueous formic acid (B) with a gradient elution of 5% A from 0 to 5 min, 5–16% A from 5 to 16 min, and 16–36% A from 16 to 76 min. The column temperature was 40 °C, flow rate was 0.50 mL/min, and injection volume was 4.00 µL. Mass spectrometry was performed using a Triple TOF 4600 system from Applied Biosystems/MDS Sciex (Framingham, MA, USA) coupled with an electrospray ionization (ESI) interface. Nitrogen was used in all cases. In this study, the parameters were optimized as follows: ESI voltage, –4500 V; nebulizer gas, 40; auxiliary gas, 50; curtain gas, 25; turbo gas temperature, 450 °C; declustering potential, –80V; collision energy, 50 eV. Instrument calibration was carried out according to the manufacturer's instructions. The mass range was set from m/z 100 to 1150. The data were acquired and processed using Analyst TF 1.7 Software.

3 RESULTS AND DISCUSSION

Given previous reports that the phytochemical constituents present in the red maple species are predominantly phenolics, including gallotannins [8–10], methanol was used as the extracting solvent of choice to prepare a phenolic-enriched extract of red maple leaves. A qualitative characterization of the phenolic compounds present in the methanol extract of the red maple leaves was performed in ESI negative ionization mode and all of the compounds showed $[M - H]^-$ ions (Table 1). Accurate mass data were then acquired in full scan analysis and product ion mass data were acquired via the Information Dependent Acquisition (IDA) method. A Prodigy ODS column was used and formic acid was introduced into the mobile phase (0.1%) to alleviate peak tailing and to produce better peak shapes. The acidic conditions did not significantly affect the ionization efficiency of the compounds in negative mode.

A total of 106 phenolic compounds, including 68 gallotannins, 25 flavonoids, gallic acid, quinic acid, catechin, epicatechin and 9 other gallic acid derivatives, were tentatively characterized (Table 1). Among these, 11 are potentially new compounds and 75 compounds are being reported from the red maple species for the first time (further described below). The general chemical structural skeletons of the compounds are shown in Fig. 1 and the total ion chromatograms (TIC) profiles of the compounds are shown in Fig. 2 (with peaks numbered as shown in Table 1). Accurate mass measurements, retention times (t_R), formula, errors and main MS/MS product ions for all of the phenolic compounds are summarized in Table 1. For ease of discussion, the red maple leaves phenolics are classified into general chemical structural classes, namely, gallotannins, flavonoids, and other gallic acid derivatives, as discussed below.

3.1 Characterization of gallotannins

Gallotannins are a subgroup of hydrolyzable tannins composed of galloyl units or their meta-depsidic derivatives bound to diverse polyol-, catechin-, or triterpenoid units [17]. In the present study, 68 gallotannins were identified in red maple leaves including 16 galloyl hexosides, 32 galloyl deoxyhexosides (consisting of 5 galloyl deoxyhexosides, 6 digalloyl deoxyhexosides, 1 methyl digalloyl deoxyhexoside, 6 trigalloyl deoxyhexosides, 3 methyl trigalloyl deoxyhexosides, 6 tetragalloyl deoxyhexosides, 3 methyl tetragalloyl deoxyhexosides, and 2 pentagalloyl deoxyhexosides), 9 galloyl catechins, 7 galloyl quinic acid derivatives and 4 galloyl shikimic acid derivatives. The characteristic product ions at m/z 169 ([gallic acid – H][–]) and 125 ([gallic acid – H – CO₂][–]), as well as the loss of 152 Da in the MS/MS spectra supported the presence of galloyl groups in the compounds.

Compound **9** (t_R = 7.13 min) exhibited an [M – H][–] ion at m/z 169.0142 (C₇H₆O₅). In the MS/MS spectra, it gave a characteristic product ion at m/z 125 corresponding to the loss of CO₂ (44 Da). By comparison of the t_R , high resolution MS data, and MS/MS spectra data with those of an authentic standard, compound **9** was identified as gallic acid which has previously been reported from the red maple species [8].

3.1.1 Galloyl hexosides—Compounds **2** (t_R = 4.85 min), **5** (t_R = 5.49 min), **6** (t_R = 6.17 min), **10** (t_R = 8.18 min), **12** (t_R = 9.01 min) and **14** (t_R = 10.83 min) all gave the same [M – H][–] ion at m/z 331, with molecular formula C₁₃H₁₆O₁₀ provided by TOF-MS. Their MS/MS fragmentation ions at m/z 125 (typical fragment ion for gallic acid due to loss of CO₂) and m/z 169 [M – H – 162][–] indicated the existence of a galloyl moiety and loss of a hexose moiety. Thus, compounds **2**, **5**, **6**, **10**, **12** and **14** were tentatively identified as galloyl hexosides. Notably, 1-*O*-galloyl-glucoside was previously reported from the red maple species [18].

Compounds **15** (t_R = 11.47 min) and **19** (t_R = 13.07 min) gave the same [M – H][–] ion at m/z 345, with molecular formula C₁₄H₁₈O₁₀ provided by TOF-MS. Their MS/MS fragmentation ions at m/z 183 [M – H – 162][–] suggested the existence of a methylgalloyl moiety and the loss of a hexose moiety. The daughter ions at m/z 168 [methyl gallic acid – H – 15][–] and m/z 124 [methyl gallic acid – H – 15 – 44][–] were attributed to loss of a •CH₃ and a CO₂ from a methyl gallic acid moiety, respectively. Thus, compounds **15** and **19** were tentatively identified as methylgalloyl hexosides. A methylgalloyl hexoside was previously reported from the red maple species [10]. Similarly, compound **22** (t_R = 14.16 min) was tentatively identified as dimethyl galloyl hexoside based on the [M – H][–] ion at m/z 359 and MS/MS daughter ion at m/z 197 [M – H – 162][–] indicating the existence of a dimethyl galloyl moiety and the loss of a hexose moiety. Due to the sequential losses of two methyl free radicals and a CO₂, it formed the MS/MS daughter ions at m/z 182, m/z 167 and m/z 123.

Compounds **20** (t_R = 13.74 min), **23** (t_R = 14.57 min), **26** (t_R = 15.21 min), **30** (t_R = 16.85 min), **32** (t_R = 17.68 min), **37** (t_R = 18.90 min) and **41** (t_R = 20.43 min) were tentatively identified as digalloyl hexosides based on their [M – H][–] ions at m/z 483 and MS/MS fragment ions at m/z 331 [M – H – 152][–] due to the loss of a galloyl group and m/z 169 [M – H – 152 – 162][–] due to the loss of a hexose moiety. The typical MS/MS fragmentation ions of the galloyl moiety at m/z 169 and m/z 125 were also observed.

3.1.2 Galloyl deoxyhexosides—Compounds **4** ($t_R = 5.23$ min), **7** ($t_R = 6.90$ min), **13** ($t_R = 9.46$ min), **17** ($t_R = 11.83$ min) and **18** ($t_R = 12.37$ min) all gave $[M - H]^-$ ions at m/z 315 with molecular formula of $C_{13}H_{16}O_9$. Their MS/MS fragmentation ions at m/z 169 $[M - H - 146]^-$ indicated the existence of a galloyl group and the loss of a deoxyhexose moiety. The typical MS/MS fragmentation ions of galloyl at m/z 169 and m/z 125 were also observed. Thus, compounds **4**, **7**, **13**, **17** and **18** were tentatively identified as galloyl deoxyhexosides. The identification of compounds **4**, **7**, **17** and **18** were further confirmed as maplexin A, maplexin B, ginnalin B and ginnalin C, respectively, by comparison to corresponding authentic reference standards previously isolated from the red maple species by our laboratory [8, 9, 16].

Compounds **29** ($t_R = 16.79$ min), **36** ($t_R = 18.55$ min), **38** ($t_R = 19.09$ min), **39** ($t_R = 19.60$ min), **43** ($t_R = 21.27$ min) and **45** ($t_R = 21.97$ min) exhibited $[M - H]^-$ ions at m/z 467. They were tentatively identified as digalloyl deoxyhexosides based on their MS/MS fragment ions at m/z 315 $[M - H - 152]^-$ and m/z 169 $[M - H - 152 - 146]^-$ due to the loss of galloyl and deoxyhexose moieties, respectively. The identities of compounds **29**, **36**, **39**, and **45** were confirmed as maplexin C, 3,6-di-*O*-galloyl-1,5-anhydroglucitol, maplexin D, and ginnalin A, respectively, by comparison to authentic reference standards previously isolated from the red maple species by our laboratory [8, 9].

Compound **47** ($t_R = 23.57$ min) displayed a $[M - H]^-$ ion at m/z 481. In the MS/MS spectra, the daughter ion at m/z 315 $[M - H - 166]^-$ indicated the loss of a methylgalloyl moiety and the daughter ion at m/z 169 $[M - H - 166 - 146]^-$ indicated the loss of a deoxyhexose moiety. The diagnostic fragmentation ions at m/z 169 and m/z 125 confirmed the existence of galloyl moieties. Thus, compound **47** was tentatively identified as galloyl methylgalloyl deoxyhexoside which is potentially a new compound.

Compounds **46**, **48**, **49**, **58**, **61** and **62** with t_R of 22.87, 23.73, 24.44, 28.09, 29.18 and 29.85 min, respectively, were tentatively identified as isomers of trigalloyl deoxyhexosides. These compounds all gave identical m/z 619 $[M - H]^-$ ions with MS/MS product ions at m/z 467 $[M - H - 152]^-$ and m/z 315 $[M - H - 152 - 152]^-$, indicating the loss of galloyl moieties. All of the six compounds exhibited typical fragment ions at m/z 125 and m/z 169 $[M - H - 152 - 152 - 146]^-$ suggesting the existence of galloyl moieties and the loss of a deoxyhexose moiety. Maplexin E [8] and maplexin F, two trigalloyl deoxyhexosides, were previously reported from the red maple species by our laboratory [9]. These compounds contain 3 galloyl groups located at 3 different positions on the 1,5-anhydro-glucitol core. Since two other trigalloyl deoxyhexosides have previously been reported from *Acer ginnala* [19], there are at least two potentially new trigalloyl deoxyhexosides among the six trigalloyl deoxyhexosides identified herein.

Compounds **72** ($t_R = 35.20$ min), **75** ($t_R = 35.87$ min) and **79** ($t_R = 36.73$ min) displayed $[M - H]^-$ ions at m/z 633. In the MS/MS spectra, the daughter ions at m/z 467 $[M - H - 166]^-$, m/z 315 $[M - H - 166 - 152]^-$ and m/z 169 $[M - H - 166 - 152 - 146]^-$ indicated the loss of methylgalloyl, galloyl, and deoxyhexose moieties, respectively. The diagnostic fragmentation ions at m/z 169 and m/z 125 indicated the existence of galloyl moieties. Thus, compound **72**, **75**, and **79** were tentatively identified as digalloyl methylgalloyl

dexoyhexosides. Our laboratory has previously isolated and identified three new digalloyl methylgalloyl dexoyhexosides, namely, maplexin G, maplexin H and maplexin I, from the red maple species [9]. Given that these new compounds have not been previously analyzed by MS/MS, a proposed fragmentation pattern is being proposed for maplexin I (Figure 3). In the MS/MS spectrum of maplexin I, fragment ions at m/z 467 $[M - H - 166]^-$, 315 $[M - H - 166 - 152]^-$ and m/z 169 $[M - H - 166 - 152 - 146]^-$ corresponded to sequential losses of methylgalloyl, galloyl and 1,5-anhydroglucitol groups, respectively. A daughter ion at m/z 297 $[M - H - 166 - 170]^-$ was formed from the $[M - H]^-$ ion by the loss of a methylgalloyl and a gallic acid and the typical fragment ion at m/z 125 was a product of m/z 169 corresponding to loss of carbon dioxide. Thus, the fragmentation pattern of a digalloyl methylgalloyl dexoyhexoside is being reported herein for the first time.

Compounds **67**, **71**, **74**, **78**, **80** and **81** produced pseudomolecular ions $[M - H]^-$ at m/z 771 with different t_R of 32.99, 34.75, 35.52, 36.22, 37.53 and 38.56 min, respectively. These compounds were tentatively identified as isomers of tetragalloyl deoxyhexosides on the basis of their MS/MS fragment ions at m/z 619 $[M - H - 152]^-$, m/z 467 $[M - H - 152 - 152]^-$, m/z 315 $[M - H - 152 - 152 - 152]^-$, m/z 169 $[M - H - 152 - 152 - 152 - 146]^-$, and m/z 125, suggesting the loss of three galloyl groups, a deoxyhexose, and a CO_2 , respectively. While tetragalloyl deoxyhexosides have not been previously reported from the red maple species, three tetragalloyl deoxyhexosides have been reported from *Acer ginnala* [19]. Therefore, among the six tetragalloyl deoxyhexosides tentatively identified herein, there are at least three potentially new compounds. Notably, this is the first evidence of the presence of tetragalloyl deoxyhexosides in the red maple species.

Compounds **90** ($t_R = 42.05$ min) and **93** ($t_R = 43.36$ min) displayed $[M - H]^-$ ions at m/z 785. In the MS/MS spectra, the daughter ions at m/z 633 $[M - H - 152]^-$, m/z 467 $[M - H - 152 - 166]^-$, m/z 315 $[M - H - 152 - 166 - 152]^-$ and m/z 169 $[M - H - 152 - 166 - 152 - 146]^-$ indicated the loss of methylgalloyl, two galloyls and deoxyhexose moieties, respectively. The diagnostic fragmentation ions at m/z 169 and m/z 125 indicated the existence of a galloyl moiety. Thus, compounds **90** and **93** were tentatively identified as trigalloyl methylgalloyl dexoyhexosides which are both potentially new compounds.

Compound **98** ($t_R = 50.70$ min) gave a $[M - H]^-$ ion at m/z 799. In the MS/MS spectra, the daughter ions at m/z 633 $[M - H - 166]^-$, m/z 467 $[M - H - 166 - 166]^-$, m/z 315 $[M - H - 166 - 166 - 152]^-$ and m/z 169 $[M - H - 166 - 166 - 152 - 146]^-$ indicated the loss of two methylgalloyls, a galloyl and deoxyhexose moieties, respectively. The diagnostic fragmentation ions at m/z 169 and m/z 125 indicated the existence of a galloyl moiety. Thus, compound **98** was tentatively identified as a digalloyl di-methylgalloyl dexoyhexoside which is potentially a new compound.

Compounds **86** ($t_R = 40.96$ min) and **89** ($t_R = 42.01$ min) gave the same $[M - H]^-$ ion at m/z 923. Based on the MS/MS daughter ions formed by sequential losses of galloyl moieties at m/z 771 $[M - H - 152]^-$, m/z 619 $[M - H - 152 - 152]^-$, m/z 467 $[M - H - 152 - 152 - 152]^-$ and m/z 315 $[M - H - 152 - 152 - 152 - 152]^-$, as well as the typical daughter ions at m/z 169 $[M - H - 152 - 152 - 152 - 152 - 146]^-$, and m/z 125, indicating the loss of a deoxyhexose and a CO_2 , respectively, compounds **86** and **89** were tentatively identified as

isomers of pentagalloyl deoxyhexosides. Since a pentagalloyl deoxyhexoside has never been previously reported, compounds **86** and **89** are potentially new compounds.

3.1.3 Galloyl catechins—Compounds **33** ($t_R = 18.00$ min) and **42** ($t_R = 20.95$ min) gave the same $[M - H]^-$ ion at m/z 289 with a molecular formula of $C_{15}H_{14}O_6$ and were identified as catechin and epicatechin by comparing their t_R , high resolution MS data and MS/MS spectra data with those of authentic reference standards. Their MS/MS spectra showed typical fragment ions at m/z 245 $[M - H - CO_2]^-$, m/z 203 $([M - H - CO_2 - C_2H_2O]^-)$ and m/z 205 $([M - H - 2C_2H_2O]^-)$. Catechin and epicatechin have previously been reported from the red maple species [16].

Compounds **57** ($t_R = 27.81$ min) and **59** ($t_R = 28.60$ min) gave $[M - H]^-$ ions at m/z 441 with a molecular formula of $C_{22}H_{18}O_{10}$. In their MS/MS spectra, fragmentation ions at m/z 289 $([M - H - 152]^-)$ and m/z 245 $([M - H - 152 - 44]^-)$ indicated the loss of a galloyl moiety and the existence of a (epi)catechin moiety. The diagnostic daughter ions at m/z 169 and 125 also indicated the galloyl moiety. Thus, compounds **57** and **59** were tentatively identified as galloyl (epi)catechins. While the (epi)catechin unit cannot be identified as either catechin or epicatechin based on MS data, galloyl epicatechin has previously been isolated and identified (by NMR) from the red maple species [16].

Compounds **69** ($t_R = 34.30$ min) gave a $[M - H]^-$ ion at m/z 455. In the MS/MS spectra, fragmentation ions at m/z 289 $[M - H - 166]^-$ and m/z 245 $[M - H - 166 - 44]^-$ indicated the loss of a methylgalloyl moiety and the existence of a (epi)catechin moiety. The diagnostic daughter ions at m/z 169 and 125 also indicated the galloyl moiety thus compound **69** was tentatively identified as methylgalloyl (epi)catechin.

Compounds **68**, **77**, **82**, **85** and **88**, with t_R of 33.50, 36.19, 39.80, 40.93 and 41.31 min, respectively, were tentatively identified as isomers of digalloyl-(epi)catechins. These compounds all gave identical m/z 593 $[M - H]^-$ ions with MS/MS product ions at m/z 441 $[M - H - 152]^-$, m/z 289 $[M - H - 152 - 152]^-$ and m/z 271 $[M - H - 170]^-$, respectively, indicating the loss of two galloyl moieties. All of these five compounds exhibited diagnostic fragment ions at m/z 125 and m/z 169 confirming the galloyl moiety, as well as typical daughter ions at m/z 289 and m/z 245 corresponding to the (epi)catechin moiety.

Compounds **95** ($t_R = 45.82$ min) displayed a $[M - H]^-$ ion at m/z 607. In the MS/MS spectra, the daughter ions at m/z 455 $[M - H - 152]^-$, m/z 289 $[M - H - 152 - 166]^-$ and m/z 245 $[M - H - 152 - 166 - 44]^-$ indicated the loss of galloyl, methylgalloyl and (epi)catechin moieties, respectively. Therefore, compound **95** was tentatively identified as galloyl methylgalloyl (epi)catechin.

3.1.4 Galloyl quinic acids—Compound **1** ($t_R = 3.48$ min) gave a $[M - H]^-$ ion at m/z 191.0568 ($C_7H_{12}O_6$). In the MS/MS spectra, it gave product ions at m/z 173 due to the loss of H_2O (18 Da) and m/z 127 $[M - H - CO - 2H_2O]^-$. Therefore, compound **1** was identified as quinic acid [14] and this is the first report of its occurrence in the red maple species.

Compounds **3** ($t_R = 4.98$ min), **8** ($t_R = 7.06$ min) and **11** ($t_R = 8.44$ min), all gave deprotonated ions at m/z 343, with a molecular formula of $C_{14}H_{16}O_{10}$. In the MS/MS spectra, they showed diagnostic [quinic acid – H] $^-$ ions at m/z 191 [M – H – 152] $^-$ and loss of 152 Da (galloyl), as well as diagnostic fragment ions at m/z 125 and m/z 169 for a galloyl moiety suggesting that these compounds are galloyl quinic acids. Based on the intense characteristic [quinic acid – H – H₂O] $^-$ fragmental ion at m/z 173 [20], compound **11** was identified as 4-*O*-galloyl quinic acid. The presence of this compound in the red maple species is in agreement with the previous report [10].

Compounds **21**, **27** and **31** produced a pseudomolecular ion [M – H] $^-$ at m/z 495 with different t_R at 13.97, 16.02 and 17.39 min, respectively. In their MS/MS spectra, fragmentation ions at m/z 343 [M – H – 152] $^-$ and m/z 191 [M – H – 152 – 152] $^-$ indicated the loss of two galloyl moieties. The diagnostic fragment ion at m/z 191 indicated the existence of quinic acid. Thus, compounds **21**, **27** and **31** were tentatively identified as digalloyl quinic acid isomers.

Compound **44** ($t_R = 21.71$ min) gave a [M – H] $^-$ ion at m/z 647. Based on the MS/MS daughter ions formed by sequential losses of three galloyl moieties at m/z 495 [M – H – 152] $^-$, m/z 343 [M – H – 152 – 152] $^-$ and m/z 191 [M – H – 152 – 152 – 152] $^-$, as well as typical daughter ions at m/z 191, m/z 169 and m/z 125 indicating the existence of quinic acid and galloyl moieties, compound **44** was tentatively identified as trigalloyl quinic acid.

3.1.5 Galloyl shikimic acid derivatives—Compounds **16**, **24** and **28** all produced the same pseudomolecular ion [M – H] $^-$ at m/z 325 (molecular formula $C_{14}H_{14}O_9$ provided by TOF-MS) with different t_R at 11.60, 14.61 and 16.18 min, respectively. These compounds were tentatively identified as isomers of galloyl shikimic acid derivatives based on their product ions in MS/MS at m/z 173 [M – H – 152] $^-$ indicating the loss of 152 Da and the existence of a shikimic acid moiety, as well as diagnostic galloyl daughter ions at m/z 169 and m/z 125. These MS data were in agreement with the presence of monogalloyl shikimic acid derivatives [13].

Compound **40** ($t_R = 19.92$ min) gave a deprotonated ion at m/z 477, with a molecular formula of $C_{21}H_{18}O_{13}$. The MS/MS product ions at m/z 325 [M – H – 152] $^-$ and m/z 173 [M – H – 152 – 152] $^-$ indicated the loss of two galloyl moieties and the existence of a shikimic acid moiety. The compound also exhibited the diagnostic daughter ions at m/z 169 and m/z 125 for a galloyl moiety and thus, was tentatively identified as digalloyl shikimic acid.

3.2 Characterization of flavonoids

In the present study, 25 flavonoids were identified based on previously reported MS/MS fragmentation patterns for flavonoids [15]. The compounds constituted 19 flavonoids based on the quercetin aglycon and 6 flavonoids based on the kaempferol aglycon. The flavonoids included flavonoid glycosides and flavonoid galloyl glycosides.

Compound **70** ($t_R = 34.33$ min) displayed a pseudomolecular ion [M – H] $^-$ at m/z 447. The characteristic fragment ions of quercetin at m/z 301, m/z 255 and m/z 151 [15] were

observed in their MS/MS spectra. The loss of 146 Da was associated with a deoxyhexose moiety and thus, compound **70** was tentatively identified as quercetin deoxyhexoside. According to the previous study [21], quercetin flavonoids substituted at the 3-OH position should yield a high intensity radical aglycone ion at m/z 300. The intense typical product ion at m/z 300 was observed in the MS/MS spectra of compound **70** suggesting that it was quercetin-3-*O*-deoxyhexoside. Notably, quercetin-3-*O*-rhamnopyranoside was previously reported from the red maple species [16].

Compound **64** (t_R = 31.90 min) displayed a deprotonated ion at m/z 433 and was tentatively identified as quercetin-3-*O*-pentoside based on the characteristic fragment ions of quercetin at m/z 301, m/z 255 and m/z 151, the diagnostic daughter ion of 3-OH substituted quercetin at m/z 300, and the loss of 132 Da corresponding to a pentose moiety. Notably, quercetin-3-*O*-arabinoside has previously been reported from the red maple species [3].

Compounds **50** (t_R = 24.86 min) and **55** (t_R = 27.42 min) gave $[M - H]^-$ ions at m/z 595 and 609. Their MS/MS spectra showed the intense quercetin aglycone ions at m/z 301, suggesting the loss of pentosyl-hexosyl (294 Da) and deoxyhexosyl-hexosyl (308 Da) groups, respectively. A high intensity ion at m/z 300 was observed in the MS/MS spectra of compound **55**. Thus, compounds **50** and **55** were identified as quercetin pentosyl hexoside and quercetin-3-*O*-deoxyhexosyl hexoside, respectively. Notably, quercetin-3-*O*-rhamnoglucoside was previously reported from the red maple species [22].

Compounds **92**, **96** and **97** all produced the same $[M - H]^-$ ion at m/z 599 with different t_R of 42.75, 46.52 and 47.31 min, respectively. In their MS/MS spectra, they all showed a similar fragment ion at m/z 447 which was produced by the loss of a galloyl group (152 Da). In addition, the characteristic fragment ions of quercetin at m/z 301, m/z 255, and m/z 151 were observed. Thus, these compounds were tentatively identified as quercetin galloyl deoxyhexosides. A high intensity radical aglycone ion at m/z 300 was found in the MS/MS spectra of compound **92** indicating that it was quercetin-3-*O*-galloyl deoxyhexoside. Notably, quercetin-3-*O*-(3''-*O*-galloyl)-rhamnopyranoside and quercetin-3-*O*-(2''-*O*-galloyl)-rhamnopyranoside were previously reported from the red maple species [16].

Compounds **51** (t_R = 25.66 min), **53** (t_R = 26.49 min), **60** (t_R = 29.18 min) and **63** (t_R = 29.97 min) all exhibited $[M - H]^-$ ions at m/z 615. Based on the MS/MS daughter ions corresponding to loss of a galloyl moiety and a hexose moiety at m/z 463 $[M - H - 152]^-$ and m/z 301 $[M - H - 152 - 162]^-$, respectively, as well as the typical daughter ions at m/z 301, m/z 255 and m/z 151, indicating the existence of a quercetin moiety, compounds **51**, **53**, **60** and **63** were tentatively identified as quercetin galloyl hexosides. Compounds **51** and **53** were further tentatively identified as quercetin-3-*O*-galloyl hexosides by their high intensity radical aglycone ion at m/z 300 in MS/MS spectra. Notably, a quercetin-*O*-galloyl-hexoside was previously reported in the red maple species [3].

Compounds **73** (t_R = 35.36 min) and **87** (t_R = 41.05 min), produced the same $[M - H]^-$ ions at m/z 585 and were tentatively identified as quercetin galloyl pentosides by their MS/MS product ions at m/z 433 $[M - H - 152]^-$ and m/z 301 $[M - H - 152 - 132]^-$ indicating the loss of a galloyl and a pentose moiety, as well as the typical daughter ions at m/z 301, m/z

255 and m/z 151, indicating the existence of a quercetin moiety. Compound **87** was further tentatively identified as quercetin-3-*O*-galloyl pentoside based on the intense MS/MS product ion at m/z 300.

Compounds **99** (t_R = 53.04 min), **101** (t_R = 53.83 min) and **103** (t_R = 55.14 min) all gave $[M - H]^-$ ions at m/z 751. The MS/MS product ions at m/z 599 $[M - H - 152]^-$, m/z 447 $[M - H - 152 - 152]^-$ and m/z 301 $[M - H - 152 - 152 - 146]^-$, suggested the loss of two galloyl moieties and a deoxyhexose moiety. The typical daughter ions at m/z 301, m/z 255 and m/z 151 indicated the existence of a quercetin moiety. Thus, compounds **99**, **101** and **103** were tentatively identified as quercetin digalloyl deoxyhexosides.

Compound **83** (t_R = 39.96 min) gave a deprotonated ion at m/z 737. The MS/MS product ions at m/z 585 $[M - H - 152]^-$, m/z 433 $[M - H - 152 - 152]^-$ and m/z 301 $[M - H - 152 - 152 - 132]^-$ indicated the loss of two galloyls and a pentose moiety, respectively. It also exhibited diagnostic daughter ions at m/z 301, m/z 255 and m/z 151 corresponding to quercetin. Thus, compound **83** was tentatively identified as quercetin digalloyl pentoside.

Compound **106** (t_R = 60.13 min) gave a deprotonated ion at m/z 765. The MS/MS product ions at m/z 599 $[M - H - 166]^-$, m/z 447 $[M - H - 166 - 152]^-$ and m/z 301 $[M - H - 166 - 152 - 146]^-$ indicated the loss of a methylgalloyl, a galloyl and a deoxyhexose moiety, respectively. It also exhibited the diagnostic quercetin daughter ions at m/z 301, m/z 255 and m/z 151. Thus, compound **106** was tentatively identified as quercetin galloyl methylgalloyl deoxyhexoside.

Compound **104** (t_R = 57.86 min) displayed an $[M - H]^-$ ion at m/z 903. This compound was tentatively identified as quercetin trigalloyl deoxyhexoside based on the MS/MS daughter ions formed by sequential losses of three galloyls and a deoxyhexose moiety at m/z 751 $[M - H - 152]^-$, m/z 599 $[M - H - 152 - 152]^-$, m/z 447 $[M - H - 152 - 152 - 152]^-$ and m/z 301 $[M - H - 152 - 152 - 152 - 146]^-$, respectively, as well as the typical daughter ions at m/z 301, m/z 255 and m/z 151 indicating the existence of quercetin moiety [15].

Compound **84** (t_R = 40.86 min) displayed a pseudomolecular ion $[M - H]^-$ at m/z 431. The characteristic fragment ions of kaempferol [15] at m/z 285, m/z 255 and m/z 227 were observed in the MS/MS spectra and the loss of 146 Da was associated with a deoxyhexose moiety. Thus, compound **84** was tentatively identified as kaempferol deoxyhexoside. The diagnostic daughter ion of 3-OH substituted kaempferol at m/z 284 was observed in the MS/MS spectra suggesting that compound **84** was kaempferol-3-*O*-deoxyhexoside. Notably, kaempferol-3-*O*-rhamnoside was previously reported from the red maple species [22].

Compounds **65** (t_R = 32.19 min) and **76** (t_R = 36.04 min) displayed deprotonated ions at m/z 447 and m/z 417. In their MS/MS spectra, the characteristic fragment ions at m/z 285, m/z 284, m/z 255 and m/z 227 indicated a 3-OH substituted kaempferol moiety, and the loss of 162 Da and 132 Da suggested the existence of a hexose moiety for compound **65** and a pentose moiety for compound **76**, respectively. Therefore, compounds **65** and **76** were tentatively identified as kaempferol-3-*O*-hexoside and kaempferol-3-*O*-pentoside,

respectively. Notably, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-galactoside were previously reported from the red maple species [22].

Compounds **100** (t_R = 53.51 min) and **102** (t_R = 54.47 min) both gave similar deprotonated ion at m/z 583. The MS/MS product ions at m/z 431 $[M - H - 152]^-$ and m/z 285 $[M - H - 152 - 146]^-$ indicated the loss of a galloyl and a deoxyhexose moiety, respectively. The compounds also exhibited diagnostic daughter ions for kaempferol at m/z 285, m/z 255 and m/z 227 and thus, compounds **100** and **102** were tentatively identified as kaempferol galloyl deoxyhexosides.

Compound **105** (t_R = 59.55 min) displayed a $[M - H]^-$ ion at m/z 735 and was tentatively identified as kaempferol digalloyl deoxyhexoside based on the MS/MS daughter ions formed by losses of two galloyls and a deoxyhexose moiety at m/z 583 $[M - H - 152]^-$, m/z 431 $[M - H - 152 - 152]^-$ and m/z 285 $[M - H - 152 - 152 - 146]^-$, respectively, as well as the typical daughter ions at m/z 285, m/z 255 and m/z 227 indicating the existence of kaempferol moiety.

3.3 Characterization of other gallic acid derivatives

Compound **54** (t_R = 27.04 min) gave a $[M - H]^-$ ion at m/z 300.9996, with a molecular formula of $C_{14}H_5O_8$ provided by TOF-MS and was identified as ellagic acid. In its MS/MS spectrum, the characteristic fragment ions at m/z 284, 257, 229, 201, 185, 173 and 145 were observed, which were identical with an authentic reference standard of ellagic acid. Notably, ellagic acid was previously reported in the red maple species [22].

Compounds **25** (t_R = 15.15 min) and **35** (t_R = 18.42 min) exhibited deprotonated $[M - H]^-$ ions at m/z 183, with a molecular formula of $C_8H_8O_5$ provided by TOF-MS. In the MS/MS spectrum, the characteristic fragment ions at m/z 168 $[M - H - 15]^-$ and m/z 124 $[M - H - 15 - 44]^-$ were due to the loss of a methyl free radical and a CO_2 , respectively. These compounds were tentatively identified as isomers of methyl gallic acid. Notably, two methyl gallic acids were previously reported from the red maple [8, 16]. The identification of compound **35** was further confirmed as gallic acid methyl ester after comparing its data with that of a corresponding authentic reference standard.

Compounds **52** (t_R = 25.95 min) and **56** (t_R = 27.68 min) gave $[M - H]^-$ ions at m/z 197, with a molecular formula of $C_9H_{10}O_5$ provided by TOF-MS. In the MS/MS spectrum, the characteristic fragment ions at m/z 182 $[M - H - 15]^-$, m/z 167 $[M - H - 15 - 15]^-$ and m/z 123 $[M - H - 15 - 15 - 44]^-$ were due to the loss of two methyl radicals and a CO_2 . They were tentatively identified as isomers of di-methyl gallic acid which were in agreement with the presence of di-methyl gallic acid from the red maple species [8]. By comparison of t_R , high resolution MS data and MS/MS spectra data with those of an authentic standard, compound **56** was identified as 5-methyl gallic acid methyl ester.

Similarly, according to this fragmentation pathway, compounds **34**, **66**, **91**, and **94** with t_R of 18.04, 32.51, 42.43 and 44.31 min, respectively, were tentatively identified as digallate, methyl digallate, methyl trigallate and di-methyl digallate, respectively. Notably, a digallate and a di-methyl digallate were previously reported from the red maple species [22].

3.4 LC-ESI-TOF-MS/MS analyses of Maplifa™

Maplifa™ is a proprietary phenolic-enriched extract of red maple leaves which has previously been studied for its cosmetic potential [5]. As shown in Table 1, LC-ESI-TOF-MS/MS analyses revealed that Maplifa™ was very similar to the methanol extract of red maple leaves and contained the vast majority of the identified compounds except for compounds **15, 21, 22, 25, 44, 69, 81, 83, 90, 91, 93, 95, 98, 104 and 106**.

4 CONCLUDING REMARKS

In summary, a rapid and reliable method employing UFLC-ESI-TOF-MS/MS was developed for the identification of phenolic compounds in red maple leaves. Based on accurate mass measurement and characteristic fragmentation ions, 106 phenolic compounds, including 68 galloyl tannins, 25 flavonoids, gallic acid, quinic acid, catechin, epicatechin and 9 other gallic acid derivatives, were identified. Among these, 11 compounds are potentially new and 75 compounds are being reported in the red maple species for the first time. The UFLC-ESI-TOF-MS/MS method established herein could be used for future characterization and standardization of this botanical material used as an ingredient in nutraceutical and/or cosmetic formulations.

ACKNOWLEDGEMENT

Research reported in this publication was made possible by the use of equipment and services available through the RI-INBRE Centralized Research Core Facility which is supported by the Institutional Development Award (IDeA) Network for Biomedical Research Excellence from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103430.

Non-standard abbreviations

UFLC	ultra-fast liquid chromatography
IDA	information dependent acquisition
TIC	total ion chromatogram
t_R	retention time

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galloyl groups
R = OH/OCH₃

flavonoids
R = H/OH/hexose/
deoxyhexose/pentose

(epi)catechin derivatives
R = OH/galloyl group(s)

galloyl hexosides/
galloyl deoxyhexosides
R = H/OH/galloyl
group(s)

quinic acid derivatives
R = OH/galloyl group(s)

shikimic acid derivatives
R = OH/galloyl group(s)

Figure 1.

General structures and substitution patterns of phenolic compounds identified in a methanol extract of red maple leaves.

Figure 2.

Total ion chromatogram of a methanol extract of red maple leaves (with peaks numbered as shown in Table 1).

Figure 3.
Proposed fragmentation pattern of maplexin I.

Table 1.

Characterization of phenolic compounds in a methanol extract red maple leaves and Maplifa™ by LC-ESI-TOF-MS/MS

Number	t _R (min)	Formula	HR-ESI(-)-MS [M-H] ⁻ (m/z exptl (theor))	Error (ppm)		Proposed compounds	Presence	
							Extract	Maplifa™
1	3.48	C ₇ H ₁₂ O ₆	191.0568 (191.0561)	3.6017	173, 155, 137, 127, 109	quinic acid	+	+
2	4.85	C ₁₃ H ₁₆ O ₁₀	331.0680 (331.0670)	2.8073	313, 271, 211, 169, 125	galloyl hexoside	+	+
3	4.98	C ₁₄ H ₁₆ O ₁₀	343.0687 (343.0670)	4.7485	191, 169, 125	galloyl quinic acid	+	+
4	5.23	C ₁₃ H ₁₆ O ₉	315.0729 (315.0721)	2.3615	169, 125	maplexin A	+	+
5	5.49	C ₁₃ H ₁₆ O ₁₀	331.0679 (331.0670)	2.5052	271, 211, 169, 125	galloyl hexoside	+	+
6	6.17	C ₁₃ H ₁₆ O ₁₀	331.0686 (331.0670)	4.6196	271, 211, 169, 125	galloyl hexoside	+	+
7	6.90	C ₁₃ H ₁₆ O ₉	315.0735 (315.0721)	4.2658	169, 125	maplexin B	+	+
8	7.06	C ₁₄ H ₁₆ O ₁₀	343.0686 (343.0670)	4.4580	191, 169, 125	galloyl quinic acid	+	+
9	7.13	C ₇ H ₆ O ₅	169.0145 (169.0142)	1.4970	125	gallic acid	+	+
10	8.18	C ₁₃ H ₁₆ O ₁₀	331.0684 (331.0670)	4.0155	313, 241, 211, 169, 125	galloyl hexoside	+	+
11	8.44	C ₁₄ H ₁₆ O ₁₀	343.0682 (343.0670)	3.2921	191, 173, 169, 125	4- <i>O</i> -galloyl quinic acid	+	+
12	9.01	C ₁₃ H ₁₆ O ₁₀	331.0687 (331.0670)	4.9217	313, 241, 169, 125	galloyl hexoside	+	+
13	9.46	C ₁₃ H ₁₆ O ₉	315.0729 (315.0721)	2.3615	169, 125	galloyl deoxyhexoside	+	+
14	10.83	C ₁₃ H ₁₆ O ₁₀	331.0682 (331.0670)	3.4114	169, 125	galloyl hexoside	+	+
15	11.47	C ₁₄ H ₁₈ O ₁₀	345.0844 (345.0827)	4.8664	285, 225, 210, 183, 168, 138, 124	methylgalloyl hexoside	+	—
16	11.60	C ₁₄ H ₁₄ O ₉	325.0577 (325.0565)	3.6736	281, 173, 169, 125	galloyl shikimic acid	+	+
17	11.83	C ₁₃ H ₁₆ O ₉	315.0725 (315.0721)	1.0920	169, 125	ginnalin B	+	+
18	12.37	C ₁₃ H ₁₆ O ₉	315.0733 (315.0721)	3.6310	169, 125	ginnalin C	+	+
19	13.07	C ₁₄ H ₁₈ O ₁₀	345.0843 (345.0827)	4.5766	183, 168, 124	methylgalloyl hexoside	+	+
20	13.74	C ₂₀ H ₂₀ O ₁₄	483.0808 (483.0780)	5.7354	331, 313, 271, 211, 169, 125	digalloyl hexoside	+	+
21	13.97	C ₂₁ H ₂₀ O ₁₄	495.0803 (495.0780)	4.5865	343, 325, 191, 173, 169, 125	digalloyl quinic acid	+	—
22	14.16	C ₁₅ H ₂₀ O ₁₀	359.0998 (359.0983)	3.9801	197, 182, 167, 153, 138, 123	dimethyl galloyl hexoside	+	—
23	14.57	C ₂₀ H ₂₀ O ₁₄	483.0816 (483.0780)	7.3915	331, 271, 211, 169, 125	digalloyl hexoside	+	+
24	14.61	C ₁₄ H ₁₄ O ₉	325.0577 (325.0565)	3.6736	173, 169, 125	galloyl shikimic acid	+	+
25	15.15	C ₈ H ₈ O ₅	183.0305 (183.0298)	3.2942	168, 124	methyl gallic acid	+	—
26	15.21	C ₂₀ H ₂₀ O ₁₄	483.0810 (483.0780)	6.1494	331, 313, 271, 211, 169, 125	digalloyl hexoside	+	+

Number	t_R (min)	Formula	HR-ESI(-)-MS [M-H] ⁻ (m/z exptl (theor))	Error (ppm)		Proposed compounds	Presence	
							Extract	Maplifa™
27	16.02	C ₂₁ H ₂₀ O ₁₄	495.0817 (495.0780)	7.4143	343, 191, 169, 125	digalloyl quinic acid	+	+
28	16.18	C ₁₄ H ₁₄ O ₉	325.0583 (325.0565)	5.5194	173, 169, 125	galloyl shikimic acid	+	+
29	16.79	C ₂₀ H ₂₀ O ₁₃	467.0842 (467.0831)	2.3236	315, 297, 169, 125	maplexin C	+	+
30	16.85	C ₂₀ H ₂₀ O ₁₄	483.0816 (483.0780)	7.3915	465, 331, 313, 271, 211, 169, 125	digalloyl hexoside	+	+
31	17.39	C ₂₁ H ₂₀ O ₁₄	495.0806 (495.0780)	5.1924	343, 191, 173, 169, 125	digalloyl quinic acid	+	+
32	17.68	C ₂₀ H ₂₀ O ₁₄	483.0812 (483.0780)	6.5635	465, 331, 313, 271, 241, 169, 125	digalloyl hexoside	+	+
33	18.00	C ₁₅ H ₁₄ O ₆	289.0722 (289.0717)	1.5154	245, 205, 203	catechin	+	+
34	18.04	C ₁₄ H ₁₀ O ₉	321.0259 (321.0252)	2.1627	169, 125	digallate	+	+
35	18.42	C ₈ H ₈ O ₅	183.0313 (183.0298)	7.6550	168, 124	gallic acid methyl ester	+	+
36	18.55	C ₂₀ H ₂₀ O ₁₃	467.0862 (467.0831)	6.6055	315, 297, 169, 125	3,6-di- <i>O</i> -galloyl-1,5-anhydroglucitol	+	+
37	18.90	C ₂₀ H ₂₀ O ₁₄	483.0813 (483.0780)	6.7705	465, 331, 313, 169, 125	digalloyl hexoside	+	+
38	19.09	C ₂₀ H ₂₀ O ₁₃	467.0865 (467.0831)	7.2477	315, 169, 125	digalloyl deoxyhexoside	+	+
39	19.60	C ₂₀ H ₂₀ O ₁₃	467.0869 (467.0831)	8.1041	315, 297, 169, 125	maplexin D	+	+
40	19.92	C ₂₁ H ₁₈ O ₁₃	477.0704 (477.0674)	6.1530	325, 201, 173, 169, 125	digalloyl shikimic acid	+	+
41	20.43	C ₂₀ H ₂₀ O ₁₄	483.0812 (483.0780)	6.5635	331, 313, 169, 125	digalloyl hexoside	+	+
42	20.95	C ₁₅ H ₁₄ O ₆	289.0724 (289.0717)	2.2072	245, 205, 203	epicatechin	+	+
43	21.27	C ₂₀ H ₂₀ O ₁₃	467.0868 (467.0831)	7.8900	423, 315, 169, 152, 125	digalloyl deoxyhexoside	+	+
44	21.71	C ₂₈ H ₂₄ O ₁₈	647.0920 (647.0889)	4.6546	495, 343, 191, 173, 169, 125	trigalloyl quinic acid	+	—
45	21.97	C ₂₀ H ₂₀ O ₁₃	467.0856 (467.0831)	5.3209	315, 297, 211, 169, 125	ginnalin A	+	+
46	22.87	C ₂₇ H ₂₄ O ₁₇	619.0973 (619.0940)	5.2118	467, 315, 297, 169, 125	trigalloyl deoxyhexoside	+	+
47	23.57	C ₂₁ H ₂₂ O ₁₃	481.1002 (481.0987)	2.9832	315, 169, 125	galloyl methylgalloyl deoxyhexoside	+	+
48	23.73	C ₂₇ H ₂₄ O ₁₇	619.0975 (619.0940)	5.5348	467, 315, 169, 125	trigalloyl deoxyhexoside	+	+
49	24.44	C ₂₇ H ₂₄ O ₁₇	619.0973 (619.0940)	5.2118	467, 315, 297, 169, 125	trigalloyl deoxyhexoside	+	+
50	24.86	C ₂₆ H ₂₈ O ₁₆	595.1333 (595.1304)	4.7738	301, 300, 271, 255, 243, 227, 179, 151	quercetin hexosyl pentoside	+	+
51	25.66	C ₂₈ H ₂₄ O ₁₆	615.1035 (615.0991)	7.0577	463, 301, 300, 271, 255, 243,	quercetin-3- <i>O</i> -galloyl hexoside	+	+

Number	t _R (min)	Formula	HR-ESI(-)-MS [M-H] ⁻ (m/z exptl (theor))	Error (ppm)		Proposed compounds	Presence	
							Extract	Maplifa™
					227, 211, 179, 169, 151, 125			
52	25.95	C ₉ H ₁₀ O ₅	197.0460 (197.0455)	2.2982	182, 167, 123	di-methyl gallic acid	+	+
53	26.49	C ₂₈ H ₂₄ O ₁₆	615.1042 (615.0991)	8.1957	463, 301, 300, 271, 255, 243, 227, 211, 179, 169, 151, 125	quercetin-3- <i>O</i> - galloyl hexoside	+	+
54	27.04	C ₁₄ H ₅ O ₈	300.9996 (300.9989)	2.0235	284, 257, 229, 201, 185, 173, 145,	ellagic acid	+	+
55	27.42	C ₂₇ H ₃₀ O ₁₆	609.1496 (609.1461)	5.7309	301, 300, 271, 255, 243, 151	quercetin-3- <i>O</i> - hexosyl deoxyhexoside	+	+
56	27.68	C ₉ H ₁₀ O ₅	197.0461 (197.0455)	2.8057	182, 167, 123	5-methyl gallic acid methyl ester	+	+
57	27.81	C ₂₂ H ₁₈ O ₁₀	441.0857 (441.0827)	6.7545	289, 245, 169, 125	galloyl (epi)catechin	+	+
58	28.09	C ₂₇ H ₂₄ O ₁₇	619.0977 (619.0940)	5.8579	467, 315, 297, 169, 125	trigalloyl deoxyhexoside	+	+
59	28.60	C ₂₂ H ₁₈ O ₁₀	441.0858 (441.0827)	7.2079	289, 245, 169, 125	galloyl (epi)catechin	+	+
60	29.18	C ₂₈ H ₂₄ O ₁₆	615.1021 (615.0991)	7.7080	463, 313, 301, 273, 255, 245, 229, 211, 179, 169, 151, 125	quercetin galloyl hexoside	+	+
61	29.18	C ₂₇ H ₂₄ O ₁₇	619.0975 (619.0940)	5.5348	467, 315, 297, 169, 125	trigalloyl deoxyhexoside	+	+
62	29.85	C ₂₇ H ₂₄ O ₁₇	619.0978 (619.0940)	3.7266	467, 449, 315, 297, 169, 125	trigalloyl deoxyhexoside	+	+
63	29.97	C ₂₈ H ₂₄ O ₁₆	615.1039 (615.0991)	4.7817	463, 313, 301, 273, 255, 245, 229, 211, 179, 169, 151, 125	quercetin galloyl hexoside	+	+
64	31.90	C ₂₀ H ₁₈ O ₁₁	433.0808 (433.0776)	7.3074	301, 300, 271, 255, 243, 151	quercetin-3- <i>O</i> - pentoside	+	+
65	32.19	C ₂₁ H ₂₀ O ₁₁	447.0956 (447.0932)	5.1770	285, 284, 255, 227	kaempferol-3- <i>O</i> - hexoside	+	+
66	32.51	C ₁₅ H ₁₂ O ₉	335.0430 (335.0408)	6.3998	183, 168, 124	methyl digallate	+	+
67	32.99	C ₃₄ H ₂₈ O ₂₁	771.1099 (771.1050)	6.3128	619, 467, 449, 315, 297, 169, 125	tetragalloyl deoxyhexoside	+	+
68	33.50	C ₂₉ H ₂₂ O ₁₄	593.0966 (593.0936)	4.9243	441, 289, 271, 245, 203, 169, 125	digalloyl (epi)catechin	+	+
69	34.30	C ₂₃ H ₂₀ O ₁₀	455.0987 (455.0983)	0.7234	289, 245, 183, 168, 124	methylgalloyl (epi)catechin	+	—
70	34.33	C ₂₁ H ₂₀ O ₁₁	447.0953 (447.0932)	4.5060	301, 300, 271, 255, 243, 227, 151	quercetin-3- <i>O</i> - deoxyhexoside	+	+
71	34.75	C ₃₄ H ₂₈ O ₂₁	771.1108 (771.1050)	7.4799	619, 467, 315, 297, 169, 125	tetragalloyl deoxyhexoside	+	+
72	35.20	C ₂₈ H ₂₆ O ₁₇	633.1142 (633.1097)	7.0706	467, 315, 297, 169, 125	digalloyl methylgalloyl deoxyhexoside	+	+

Number	t _R (min)	Formula	HR-ESI(-)-MS [M-H] ⁻ (m/z exptl (theor))	Error (ppm)		Proposed compounds	Presence	
							Extract	Maplifa™
73	35.36	C ₂₇ H ₂₂ O ₁₅	585.0909 (585.0885)	3.9412	433, 301, 273, 255, 179, 151	quercetin galloyl pentoside	+	+
74	35.52	C ₃₄ H ₂₈ O ₂₁	771.1101 (771.1050)	6.5722	619, 467, 315, 297, 169, 125	tetragalloyl deoxyhexoside	+	+
75	35.87	C ₂₈ H ₂₆ O ₁₇	633.1113 (633.1097)	2.4901	467, 315, 297, 169, 125	digalloyl methylgalloyl deoxyhexoside	+	+
76	36.04	C ₂₀ H ₁₈ O ₁₀	417.0843 (417.0827)	3.7866	285, 284, 255, 227	kaempferol-3- <i>O</i> - pentoside	+	+
77	36.19	C ₂₉ H ₂₂ O ₁₄	593.0960 (593.0936)	3.9127	441, 423, 289, 271, 245, 203, 169, 125	digalloyl (epi)catechin	+	+
78	36.22	C ₃₄ H ₂₈ O ₂₁	771.1096 (771.1050)	5.9238	619, 467, 315, 297, 169, 125	tetragalloyl deoxyhexoside	+	+
79	36.73	C ₂₈ H ₂₆ O ₁₇	633.1140 (633.1097)	6.7547	467, 315, 297, 169, 125	digalloyl methylgalloyl deoxyhexoside	+	+
80	37.53	C ₃₄ H ₂₈ O ₂₁	771.1085 (771.1050)	4.4972	619, 467, 315, 297, 169, 125	tetragalloyl deoxyhexoside	+	+
81	38.56	C ₃₄ H ₂₈ O ₂₁	771.1098 (771.1050)	6.1831	619, 467, 315, 297, 169, 125	tetragalloyl deoxyhexoside	+	—
82	39.80	C ₂₉ H ₂₂ O ₁₄	593.0967 (593.0936)	5.0929	441, 289, 271, 245, 203, 169, 125	digalloyl (epi)catechin	+	+
83	39.96	C ₃₄ H ₂₆ O ₁₉	737.1038 (737.0995)	5.7620	585, 433, 301, 273, 255, 179, 151	quercetin digalloyl pentoside	+	—
84	40.86	C ₂₁ H ₂₀ O ₁₀	431.1001 (431.0983)	4.0112	285, 284, 255, 227	kaempferol-3- <i>O</i> - deoxyhexoside	+	+
85	40.93	C ₂₉ H ₂₂ O ₁₄	593.0969 (593.0936)	5.4301	441, 289, 271, 245, 203, 169, 125	digalloyl (epi)catechin	+	+
86	40.96	C ₄₁ H ₃₂ O ₂₅	923.1163 (923.1159)	0.3349	771, 619, 467, 315, 297, 169, 125	pentagalloyl deoxyhexoside	+	+
87	41.05	C ₂₇ H ₂₂ O ₁₅	585.0934 (585.0885)	8.2140	433, 301, 300, 271, 255, 179, 151	quercetin-3- <i>O</i> - galloyl pentoside	+	+
88	41.31	C ₂₉ H ₂₂ O ₁₄	593.0967 (593.0936)	5.0929	441, 289, 271, 245, 203, 169, 125	digalloyl (epi)catechin	+	+
89	42.01	C ₄₁ H ₃₂ O ₂₅	923.1230 (923.1159)	7.5929	771, 619, 467, 315, 297, 169, 125	pentagalloyl deoxyhexoside	+	+
90	42.05	C ₃₅ H ₃₀ O ₂₁	785.1248 (785.1206)	5.2446	633, 619, 467, 315, 297, 169, 125	trigalloyl methylgalloyl deoxyhexoside	+	—
91	42.43	C ₂₂ H ₁₆ O ₁₃	487.0547 (487.0518)	5.9243	335, 183, 168, 124	methyl trigallate	+	—
92	42.75	C ₂₈ H ₂₄ O ₁₅	599.1055 (599.1042)	2.0962	447, 315, 301, 300, 271, 255, 243, 227, 179, 151	quercetin-3- <i>O</i> - galloyl deoxyhexoside	+	+
93	43.36	C ₃₅ H ₃₀ O ₂₁	785.1257 (785.1206)	6.3911	633, 619, 467, 315, 297, 169, 125	trigalloyl methylgalloyl deoxyhexoside	+	—

Number	t _R (min)	Formula	HR-ESI(-)-MS [M-H] ⁻ (m/z exptl (theor))	Error (ppm)		Proposed compounds	Presence	
							Extract	Maplifa™
94	44.31	C ₁₆ H ₁₄ O ₉	349.0574 (349.0565)	2.5615	197, 182, 167, 123	di-methyl digallate	+	+
95	45.82	C ₃₀ H ₂₄ O ₁₄	607.1139 (607.1093)	7.5283	561, 455, 399, 289, 245, 203, 183, 125	galloyl methylgalloyl (epi)catechin	+	—
96	46.52	C ₂₈ H ₂₄ O ₁₅	599.1088 (599.1042)	7.6044	447, 301, 255, 179, 151	quercetin galloyl deoxyhexoside	+	+
97	47.31	C ₂₈ H ₂₄ O ₁₅	599.1078 (599.1042)	3.5559	447, 301, 255, 179, 151	quercetin galloyl deoxyhexoside	+	+
98	50.70	C ₃₀ H ₃₂ O ₂₁	799.1406 (799.1363)	5.3404	633, 467, 315, 297, 169, 125	digalloyl dimethylgalloyl deoxyhexoside	+	—
99	53.04	C ₃₅ H ₂₈ O ₁₉	751.1214 (751.1152)	8.2505	599, 447, 301, 255, 179, 151	quercetin digalloyl deoxyhexoside	+	+
100	53.51	C ₂₈ H ₂₄ O ₁₄	583.1144 (583.1093)	8.6956	431, 297, 285, 255, 227	kaempferol galloyl deoxyhexoside	+	+
101	53.83	C ₃₅ H ₂₈ O ₁₉	751.1208 (751.1152)	7.4517	599, 447, 301, 255, 179, 151	quercetin digalloyl deoxyhexoside	+	+
102	54.47	C ₂₈ H ₂₄ O ₁₄	583.1133 (583.1093)	6.8092	431, 297, 285, 255, 227, 169, 125	kaempferol galloyl deoxyhexoside	+	+
103	55.14	C ₃₅ H ₂₈ O ₁₉	751.1205 (751.1152)	7.0523	599, 447, 301, 255, 179, 151	quercetin digalloyl deoxyhexoside	+	+
104	57.86	C ₄₂ H ₃₂ O ₂₃	903.1316 (903.1261)	6.0217	751, 599, 447, 301, 255, 179, 151	quercetin trigalloyl deoxyhexoside	+	—
105	59.55	C ₃₅ H ₂₈ O ₁₈	735.1258 (735.1202)	7.4977	583, 431, 297, 285, 255, 227, 169, 125	kaempferol digalloyl deoxyhexoside	+	+
106	60.13	C ₃₆ H ₃₀ O ₁₉	765.1347 (765.1308)	3.8471	599, 447, 301, 255, 179, 151	quercetin galloyl methylgalloyl deoxyhexoside	+	—