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국내 재배 품종별 커피나무 잎 추출물의 주요 성분 비교

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Comparison of Major Compounds of Coffee Tree Leaf Extract by Varieties Cultivated in Korea

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ABSTRACT

Background: To identify suitable varieties of coffee for Korea, various varieties of coffee trees are being cultivated. In relation to this process, it is necessary to develop the analysis methods to confirm and compare the characteristics of each variety.
Methods and Results: In this study, ethanol extracts of coffee tree leaves of four varieties, *Coffea arabica* var. bourbon (Bourbon), *C. arabica* var. maragogype (Maragogipe), *C. arabica* var. typica (Typica), and *C. arabica* var. geisha (Geisha), were targeted. The liquid chromatography (LC) profile of each extract was analyzed using a ultra-violet detector. Sixteen major peaks were selected from the LC profile analysis results, and mass spectrometry (MS) was performed using an LC-MS/MS system with the same separation conditions. The MS results confirmed that the major peaks were caffeoyl quinic acid, procyanidin, dicaffeoyl quinic acid, mangiferin, cinchonain I isomer, iriflophenone 3-C-glucoside, caffeine, and theobromine. The quantities of these major compounds were compared for each variety through additional multiple reaction monitoring mode MS analysis.
Conclusions: Differences in the major compound contents of the leaves of different domestic coffee tree varieties were confirmed, and LC-MS/MS analysis was found to be useful for the comparative analysis of compounds between varieties.

Key Words: *Coffea arabica*, Coffee Leaves, Compound Profile, Liquid Chromatography, Mass Spectrometry, Varieties

INTRODUCTION

Coffee is a crop grown in tropical and subtropical climates called the Coffee Zone, located between latitudes 22 degrees north and latitude 26 degrees south. Since Korea is located at 33 to 43 degrees north latitude, the temperature is relatively cold compared to the existing coffee growing regions. Therefore, although production is difficult in Korea's natural environment, cultivation is gradually increasing using facility cultivation methods in Jeju, Jeonnam, and Gyeongnam areas (Moon *et al*., 2019; Kim, 2020).

The most representative coffee tree varieties worldwide are *Coffea arabica*, *C. canephora*, and *C. liberica* (Davis *et al*., 2006). Among them, the varieties belonging to *C. arabica* are known to be more suitable for the cultivation environment of Korea. In particular, *C. arabica* varieties suitable for high-altitude areas such as *C. arabica* var. typica (Typica) and *C. arabica* var. bourbon (Bourbon) are considered preferentially (WCR, 2019).

The value of coffee tree leaves is ignored or of little interest because of the value possessed by the coffee beans that are mainly used. However, it has been traditionally used for the treatment of various diseases or alleviation of symptoms, mainly in the major coffee growing regions (Ngamsuk *et al*.,

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2019). In addition, research on various compounds present in the leaves of coffee trees and studies to increase the utility are continuously being conducted (Chen *et al*., 2018; Chen *et al*., 2019; de Almeida *et al*., 2019; Monteiro *et al*., 2020; Cangeloni *et al*., 2022 Maxiselly *et al*., 2022).

Various studies on the compounds and activity of coffee beans are being conducted in Korea (Nam and Kang, 2015; Kim and Lee, 2018; Lee *et al*., 2018; Shin, 2019). In addition, some studies on the activity and compounds of coffee tree leaves grown in Korea were also conducted (Im and Doo, 2021a; Im and Doo, 2021b; Lee *et al*., 2022). Except for these few studies, there are still few studies on the physiological activity or compounds of coffee trees grown in Korea.

The compound profile of a plant varies depending on the plant cultivar, growing region, climate and vegetation stage along with the cultivation process (Cangeloni *et al*., 2022). As far as is known, coffee leaves contain numerous compounds such as caffeine, chlorogenic acid, and mangiferin (Campa *et al*., 2012; Chen *et al*., 2019; Mendes *et al*., 2019; Monteiro *et al*., 2020). Various compounds, including these active compounds, show different contents depending on the environment such as the variety or growing area. Therefore, it is necessary to continuously study the compound analysis of coffee trees grown in a new environment in Korea.

Compound profile analysis is a method used when it is not possible to specify a index compound or for quality control of herbal medicines. As an analysis performed without a standard compound, the difference between samples is confirmed by comparing the specific LC chromatogram pattern of each plant. The plant extract used for this profile analysis is mixed with at least hundreds of compounds. Therefore, even if the separation method is optimized, there is a limit in the general liquid chromatography (LC) system to completely separate all compounds present in the extract. In addition, it is practically difficult to prepare standards for chromatographic analysis for all compounds. Mass spectrometry (MS) is increasingly being used as an analysis equipment that can overcome these problems (Lee *et al*., 2022).

LC-MS/MS having a triple quadrupole structure is capable of multiple reaction monitoring (MRM) or selected reaction monitoring (SRM) mode analysis as well as scan and selected ion monitoring (SIM) mode analysis that can be performed in general LC-MS. MRM or SRM mode is an analysis that selectively detects a specific product ion generated from a specific molecular ion (Na *et al*., 2020). It is possible to minimize the influence of adjacent compounds in general LC analysis, and it is possible to analyze compounds with overlapping retention times (Im and Lee, 2020).

On the other hand, high-resolution MS such as time of flight (TOF) MS, which have superior qualitative analysis capability than tandem MS having such a triple quadrupole structure, are being used. However, the use of tandem MS is often considered first due to the initial cost or relative ease of operation. In particular, tandem MS can be effective in the search and quantitative analysis of compounds known through various existing studies.

In this study, the LC profiles of major compounds present in extracts of coffee tree leaf samples were compared to determine the differences between coffee tree varieties grown in Korea. For the coffee tree leaf sample, 4 types that are widely cultivated in the same area were selected. MS analysis in scan mode was performed on the major compound peaks identified in the LC profile analysis, and each compound was identified with reference to existing studies. In addition, by setting the conditions for MS analysis in MRM mode, the applicability of the method for rapid comparative analysis of major compounds in coffee leaves was confirmed.

MATERIALS AND METHODS

1. Plant materials

Coffee tree leaves of 4 varieties *Coffea arabica* var. bourbon (Bourbon), *C. arabica* var. maragogype (Maragogipe), *C. arabica* var. typica (Typica), and *C. arabica* var. geisha (Geisha) were collected in 2022 from those grown in Hwasun, Jeollanam-do (Fig. 1).

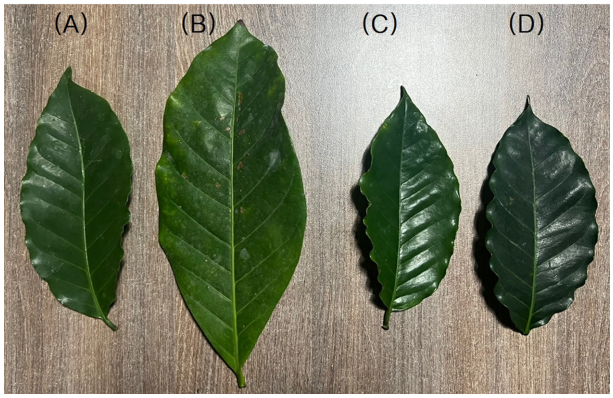

Fig. 1. Coffee tree leaf images by varieties. (A) Bourbon, (B) Maragogipe, (C) Typica, (D) Geisha.

The age of each coffee tree variety was between 13 and 15 years, and only mature leaves free from pests and diseases were collected. The samples were dried at 50℃ for 48 hours. The dried sample was pulverized to a size of 0.25 ㎜ or less using a blender and used for extraction.

2. Preparation of extraction

Extraction was performed by mixing 200 ㎖ of ethanol with 10 g of a coffee tree leaf sample for each variety. Shaking extraction was performed at a speed of 180 rpm for 24 hours.

Extraction for each sample was performed three times separately and used for analysis. After extraction, it was filtered with a syringe filter (0.45 ㎛) and stored at 4℃ for use in the experiment.

3. LC profile analysis

LC-30A (Shimadzu, Kyoto, Japan) liquid chromatography and Kinetex C18 (2.1 ㎜ × 100 ㎜, 1.7 ㎛, Phenomenex, Torrance, CA, USA) column were used to analyze the LC profile of coffee tree leaf extract.

The sample injection volume was set at 1 ㎕, and the column oven was set at 40℃. 0.1% formic acid (A) and methanol (B) were used as mobile phases, and the flow rate was maintained at 0.3 ㎖/min. Gradient program for profile analysis: from 0.0 to 5.0 min 5% B (isocratic), from 5.0 to 25.0 min 5 - 40% B (linear), from 25.0 to 32.0 min 40 - 100% B (linear), From 32.0 to 38 min 100% B (isocratic), from 38.0 to 38.5 min 100 - 5% B (linear), from 38.5 to 45.0 min 5% B (isocratic).

The compounds for mass spectrometry were selected based on the 280 ㎚ analysis results showing the most diverse compound profile.

4. LC-MS/MS analysis of major compounds

LC-30A (Shimadzu Co., Kyoto, Japan) liquid chromatography and LCMS-8050 (Shimadzu Co., Kyoto, Japan) triple quadrupole mass spectroscopy were used for mass spectrometry of major compounds of coffee tree leaf extract.

The mobile phase and separation conditions used in the LC profile analysis were applied. An electro-spray ionization (ESI) device was used for ionization for mass spectrometry of major compounds. Scan mode analysis was performed in both positive and negative polarity, and multiple reaction monitoring (MRM) mode analysis conditions were set by applying polarity with excellent sensitivity or specificity for each compound. The collision-induced dissociation gas (argon) pressure was set 270 kPa. The nebulizing gas, the drying gas, and the heating gas flow were set to 3 ℓ/min, 10 ℓ/min, and 10 ℓ/min, respectively.

The mass spectra were scanned 100 to 1,200 *m/z*. Other MS conditions included interface temperature 300℃, desolvation line temperature 250℃, heat block temperature 350℃.

RESULTS AND DISCUSSION

1. LC profile of coffee tree leaf extract by varieties

In order to confirm the profile of various compounds present in the coffee tree leaf extract, LC analysis was performed under 200 ㎚ - 400 ㎚ UV detector conditions. The compound profiles of 4 coffee tree leaf extracts by varieties were compared based on the result chromatogram of 280 ㎚, in which the compound profile was varied and clearly identified among the detector wavelength conditions (Fig. 2).

In the analysis results, there were compound peaks that were common to all varieties, but also compounds with very low or no content were identified in some varieties. In particular, it was confirmed that the compound profile of Maragogipe variety (Fig. 2C) was significantly different from other varieties.

2. LC-MS analysis of major compound peaks

In the LC profile analysis result, the main peaks from 1 to 16 were selected from the Typica variety extract, which showed the most diverse compound peaks, and mass spectrometry of the peaks was performed (Fig. 3). For mass spectrometry, the same separation conditions as for LC profile analysis were applied, and the mass spectrometry results of each peak were reviewed based on the retention time of the LC profile analysis results.

In general, in a mass spectrometer based on an electrospray ionization (ESI) device, the molecular weight can be estimated by comparing $[M+H]^+$, which is a molecular ion pattern in positive mode, and $[M-H]^-$, a molecular ion pattern, shown in negative mode (Lee *et al*., 2022). On the other hand, the molecular ion pattern may appear selectively in the positive or negative mode due to the structural characteristics of the compound.

By comparing the main peak of the LC profile analysis result with the peak of the mass spectrometry result, the molecular weight of each peak was measured as shown in Table 1. 1 and 5 peak, which molecular ion was confirmed only in positive mode, were analyzed by MS^2 in positive mode to confirm product ions. Except for peaks 1 and 5, the product

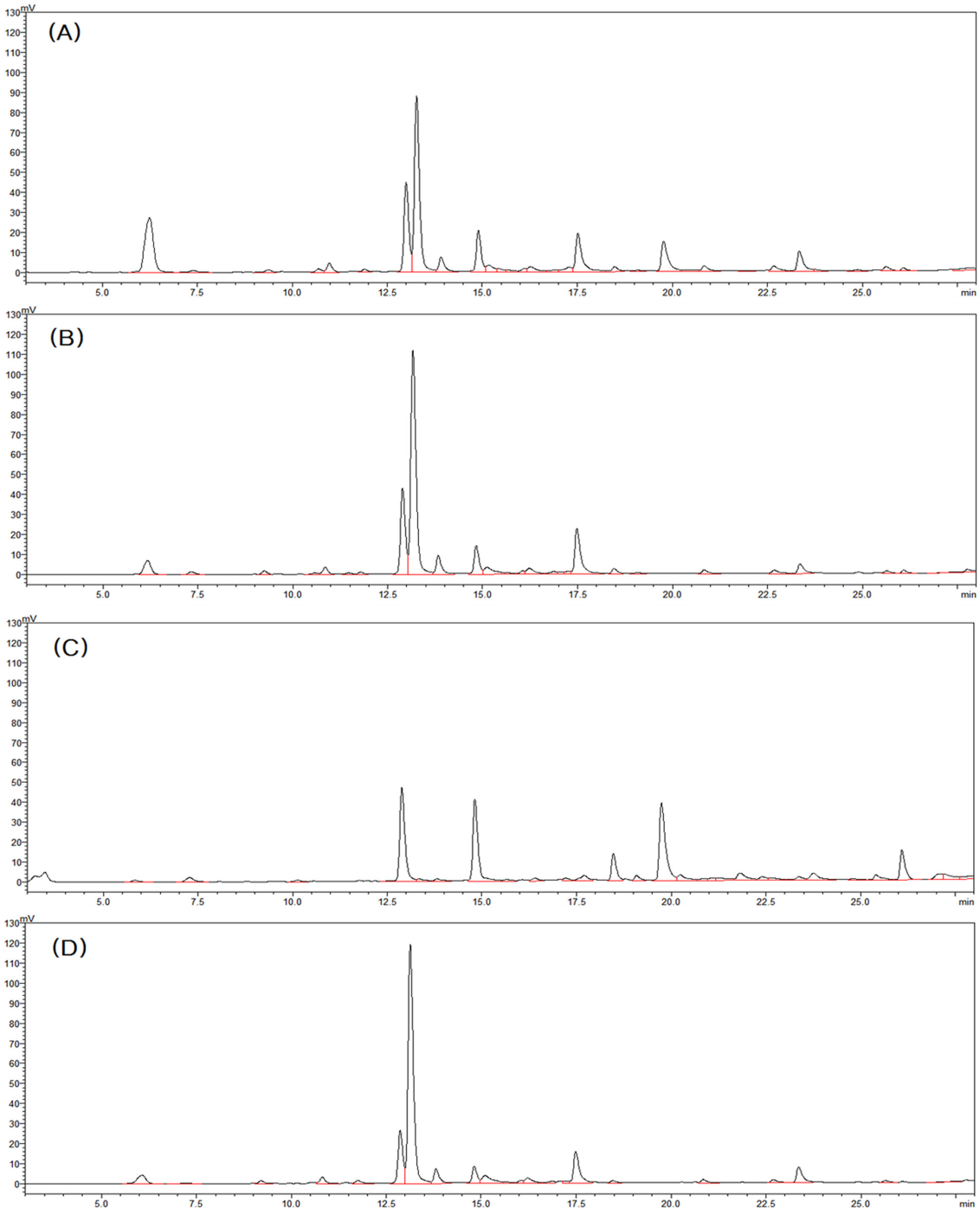

Fig. 2. LC profile of coffee tree leaf extract by varieties. (A) Typica, (B) Bourbon, (C) Maragogipe, (D) Geisha.

ions were confirmed by MS^2 analysis in negative mode.

In the MS spectra of peak 1 presented in Table 1, 181 *m/z* in the form of $[M+H]^+$ was confirmed only in positive mode. Therefore, it was estimated as a compound with a molecular weight of 180, and product ions were confirmed in positive mode. The product ions generated from 181 *m/z* in positive mode were 163, 138, 67, and 42 *m/z*. This MS spectra was found to be in the form of theobromine by referring to the existing literature (Mendes *et al*., 2019).

Peak 2 was confirmed to be a compound with a molecular

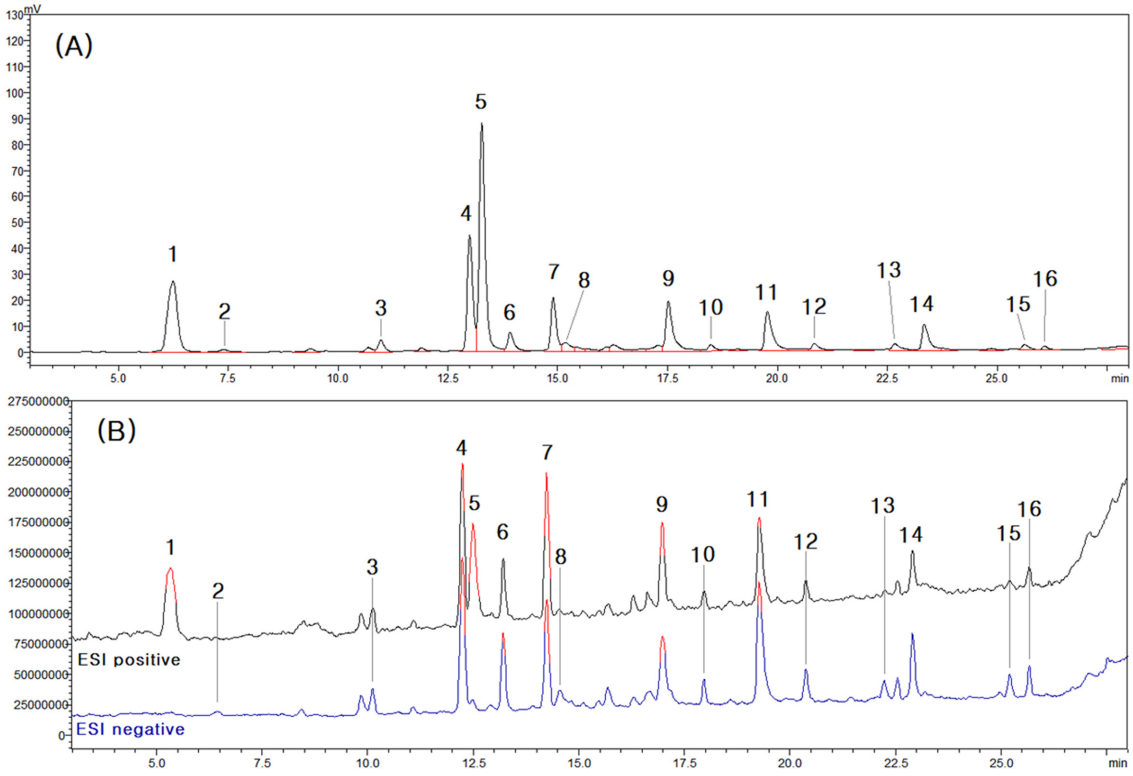

Fig. 3. LC profile (A) and LC-MS total ion chromatogram (B) of extracts from coffee tree leaves of the Typica variety. Different superscripts (1 - 16) are major compounds.

weight of 354 as 353 *m/z* in the form of $[M-H]^-$ in the negative mode and 355 *m/z* in the form of $[M+H]^+$ in the positive mode. The product ions generated from 353 *m/z* in negative mode were 191, 179, and 135 *m/z*. This MS spectra was found to be a typical form of caffeoyl quinic acid-based compounds by referring to the existing literature (Bajko *et al*., 2016). The MS spectra of peak 4 also showed MS and MS^2 spectra of a pattern similar to that of peak 2, so it could be estimated that it was a caffeoyl quinic acid-based compounds. By reviewing previous research reports, it was confirmed that peaks 2 and 4 were 5-caffeoyl quinic acid and 3-caffeoyl quinic acid, respectively (Zhang *et al*., 2015; Chen *et al*., 2018; Kim *et al*., 2020; Lee *et al*., 2022). Caffeoyl quinic acid is a major compound of coffee and has been reported as a compound with antioxidant activity in various studies (Monteiro *et al*., 2020; Lee *et al*., 2022).

Peak 3 was confirmed to be a compound with a molecular weight of 408 as 407 *m/z* in the form of $[M-H]^-$ in the negative mode and 409 *m/z* in the form of $[M+H]^+$ in the positive mode. The product ions generated from 407 *m/z* in negative mode were 317, 287, 245, and 193 *m/z*. It was confirmed that iriflophenone 3-C-glucoside was compared with the existing literature related to the compound of coffee tree leaves (Cangeloni *et al*., 2022).

In the MS spectra of peak 5, 195 *m/z* in the form of $[M+H]^+$ was confirmed only in positive mode. Therefore, it was estimated as a compound with a molecular weight of 194, and product ions were confirmed in positive mode. The product ions generated from 195 *m/z* in positive mode were 138, 123, 110, and 83 *m/z*. This MS spectra was found to be a typical form of caffeine by referring to the existing literature (Mendes *et al*., 2019).

In the MS spectra of peaks 6 and 12, 577 *m/z* in the form of $[M-H]^-$ in the negative mode and 579 *m/z* in the form of $[M+H]^+$ in the positive mode were confirmed, so it was estimated as a compound with a molecular weight of 578. The product ions generated from 577 *m/z* in negative mode were 425, 407, 289, and 125 *m/z*. This MS spectra is a form that appears in the dimer structure of catechin or epicatechin. Compared with the existing literature on mass spectrometry studies of procyanidin compounds, it was confirmed that it was a B-type procyanidin dimer (Gu *et al*., 2003; Bakhytkyzy *et*

al., 2018; Rue *et al*., 2018; Cangeloni *et al*., 2022). Procyanidin exhibits strong antioxidant activity of catechin or epicatechin, which constitutes the structure (Murakami *et al*., 2006; Calderón *et al*., 2009).

Peak 7 was confirmed to be a compound with a molecular weight of 290 as 289 *m/z* in the form of $[M-H]^-$ in the negative mode and 291 *m/z* in the form of $[M+H]^+$ in the positive mode. The product ions generated from 289 *m/z* in negative mode were 245, 203, 123, and 109 *m/z*. This MS spectra was found to be a form that appears in the structure of catechin or epicatechin, and compared with the existing literature on mass spectrometry studies on catechin compounds, epicatechin was confirmed (Murakami *et al*., 2006; Calderón *et al*., 2009; Nemes *et al*., 2018).

Peak 8 was confirmed to be a compound with a molecular weight of 864 as 863 *m/z* in the form of $[M-H]^-$ in the negative mode and 865 *m/z* in the form of $[M+H]^+$ in the positive mode. The product ions generated from 863 *m/z* in negative mode were 711, 573, 451, 411, and 289 *m/z*. Such MS spectra appear in structures derived from trimer forms of catechin or epicatechin. Compared with the existing literature on mass spectrometry of procyanidin compounds, it was confirmed that it was cinnamtannin B-1 (Rue *et al*., 2018; Rush *et al*., 2018; Lee *et al*., 2022). Cinnamtannin B-1 has been reported to have high antioxidant activity (Kim *et al*., 2016; Woo *et al*., 2017; Lee *et al*., 2022).

Peak 9 was confirmed to be a compound with a molecular weight of 422 as 421 *m/z* in the form of $[M-H]^-$ in the negative mode and 423 *m/z* in the form of $[M+H]^+$ in the positive mode. The product ions generated from 421 *m/z* in negative mode were 331, 301, 271, and 259 *m/z*. This MS spectra was found to be a form of mangiferin present in coffee leaves (Campa *et al*., 2012; Chen *et al*., 2019; Ngamsuk *et al*., 2019; Monteiro *et al*., 2020). Mangiferin is a compound that has been reported for anticancer-related activity as well as antioxidant activity (Lee *et al*., 2018; Morozkina *et al*., 2021)

In the MS spectra of peaks 10 and 16, 451 *m/z* in the form of $[M-H]^-$ in the negative mode and 453 *m/z* in the form of $[M+H]^+$ in the positive mode were confirmed, so it was estimated as a compound with a molecular weight of 452. The product ions generated from 451 *m/z* in negative mode were 341, 217, 189, and 177 *m/z*. It was confirmed that it is a cinchonain I isomer by comparing the MS spectra and the existing literature related to the study of coffee leaves (Cangeloni *et al*., 2022). Cinchonain I isomer is known to have antidiabetic, antioxidant and hepatoprotective effects (Gomes *et al*., 2017; Sobeh *et al*., 2017).

Peak 11 was confirmed to be a compound with a molecular weight of 576 as 575 *m/z* in the form of $[M-H]^-$ in the negative mode and 577 *m/z* in the form of $[M+H]^+$ in the positive mode. The product ions generated from 575 *m/z* in negative mode were 449, 423, 289, and 285 *m/z*. This MS spectra is confirmed in the form shown in the A-type procyanidin dimer (Bakhytkyzy *et al*., 2018).

In the MS spectra of peaks 13, 14 and 15, 515 *m/z* in the form of $[M-H]^-$ in the negative mode and 517 *m/z* in the form of $[M+H]^+$ in the positive mode were confirmed, so it was estimated as a compound with a molecular weight of 516. The product ions generated from 517 *m/z* in negative mode were 353, 335, 191, and 179 *m/z*. Such MS spectra are shown in compounds having a dicaffeoyl quinic acid structure in which two caffeoyl groups and quinic acid are combined (Clifford *et al*., 2005). By reviewing existing studies on the components of coffee leaves, it was confirmed that peaks 13, 14, and 15 were 3,4-dicaffeoyl quinic acid, 3,5-dicaffeoyl quinic acid, and 4,5-dicaffeoyl quinic acid, respectively (Campa *et al*., 2012; Chen *et al*., 2018; Monteiro *et al*., 2020). It has been reported that three types of dicaffeoyl quinic acid have anti-inflammatory activity as well as antioxidant activity (Chen *et al*., 2019; Kim *et al*., 2020).

3. Multiple reaction monitoring condition setting

Mass spectrometry in multiple reaction monitoring (MRM) mode selectively detects only specific ions generated from specific molecular ion. MRM mode analysis is useful for the analysis of samples in which hundreds of compounds are mixed, such as plant extracts (Na *et al*., 2020; Im and Lee, 2020).

Based on the results of LC-MS analysis of the major peaks presented in Table 1, molecule ions and product ions were selected. For qualitative and quantitative analysis, 2 MRM conditions were set for each compound as shown in Table 2.

In order to confirm the usefulness of the established MRM mode analysis method, comparative analysis was performed on the ethanol extract of coffee tree leaves by varieties. In the chromatogram of the MRM mode presented in Figure 4, the difference in the content of each compound was clearly confirmed for each extract. Since the detection of compounds other than the compounds to be analyzed was excluded, a simple and clear comparison was possible compared to the

Table 1. Assignment of identified compounds in LC-MS/MS chromatograms of coffee leaf extracts.

Peaks	Compound	Retention time (min)	$[M-H]^-$ (*m/z*)	$[M+H]^+$ (*m/z*)	MS^2		References
					Polarity	Product ions (*m/z*)	
1	Theobromine	5.33	-	181	+	163 - 138 - 67 - 42	Mendes *et al*., 2019
2	5-Caffeoyl quinic acid	6.42	353	355	-	191 - 179 - 135	Kim *et al*., 2020
3	Iriflophenone 3-C-glucoside	10.11	407	409	-	317 - 287 - 245 - 193	Cangeloni *et al*., 2022
4	3-Caffeoyl quinic acid	12.23	353	355	-	191 - 179 - 135	Kim *et al*., 2020
5	Caffeine	12.48	-	195	+	138 - 123 - 110 - 83	Mendes *et al*., 2019
6	Procyanidin dimer B-type 1	13.20	577	579	-	425 - 407 - 289 - 125	Rue *et al*., 2018
7	Epicatechin	14.22	289	291	-	245 - 203 - 123 - 109	Nemes *et al*., 2018
8	Cinnamtannin B-1	14.53	863	865	-	711 - 573 - 451 - 411 - 289	Rue *et al*., 2018
9	Mangiferin	16.96	421	423	-	331 - 301 - 271 - 259	Campa *et al*., 2012
10	Cinchonain I isomer 1	17.95	451	453	-	341 - 217 - 189 - 177	Cangeloni *et al*., 2022
11	Procyanidin dimer A-type	19.26	575	577	-	449 - 423 - 289 - 285	Bakhytkyzy *et al*., 2018
12	Procyanidin dimer B-type 2	20.37	577	579	-	425 - 407 - 289 - 125	Rue *et al*., 2018
13	3,4-Dicaffeoyl quinic acid	22.22	515	-	-	353 - 335 - 191 - 179	Campa *et al*., 2012
14	3,5-Dicaffeoyl quinic acid	22.89	515	-	-	353 - 191 - 179 - 135	Campa *et al*., 2012
15	4,5-Dicaffeoyl quinic acid	25.19	515	-	-	353 - 191 - 179 - 135	Campa *et al*., 2012
16	Cinchonain I isomer 2	25.66	451	453	-	341 - 217 - 189 - 177	Cangeloni *et al*., 2022

Table 2. Multiple reaction monitoring conditions of LC-MS/MS for analysis of major compounds.

Peaks	Compound	Polarity	Molecular ion (*m/z*)	Product ion (*m/z*)	
				1	2
1	Theobromine	+	181.1	67.1	138.1
2	5-Caffeoyl quinic acid	-	353.1	191.1	179.1
3	Iriflophenone 3-C-glucoside	-	407.1	287.1	317.1
4	3-Caffeoyl quinic acid	-	353.1	179.1	135.1
5	Caffeine	+	195.1	138.1	110.1
6	Procyanidin dimer B-type 1	-	577.1	425.1	289.1
7	Epicatechin	-	289.1	245.1	109.1
8	Cinnamtannin B-1	-	863.1	711.1	411.1
9	Mangiferin	-	421.1	301.1	271.1
10	Cinchonain I isomer 1	-	451.1	341.1	189.1
11	Procyanidin dimer A-type	-	575.1	289.1	449.1
12	Procyanidin dimer B-type 2	-	577.1	289.1	425.1
13	3,4-Dicaffeoyl quinic acid	-	515.1	353.1	179.1
14	3,5-Dicaffeoyl quinic acid	-	515.1	353.1	191.1
15	4,5-Dicaffeoyl quinic acid	-	515.1	353.1	179.1
16	Cinchonain I isomer 2	-	451.1	341.1	189.1

chromatogram using the ultra violet (UV) detector of general LC analysis.

4. Comparative analysis of extracts by varieties

Table 3 presents the results of comparative analysis of major components of coffee tree leaf extracts by varieties. Relative content results were calculated by comparing the peak areas of each compound for each varieties based on the peak area of the Typica variety extract.

Based on the content of the Typica variety, procyanidin

Table 3. Relative contents of major compounds in coffee leaf extracts.

Compound	Relative content (% of peak area of Typica)			
	Typica	Bourbon	Maragogipe	Geisha
Theobromine	100.00±1.52[1)]	26.50±0.54	-	19.98±0.09
5-Caffeoyl quinic acid	100.00±1.75	130.22±1.66	220.67±4.07	57.46±0.11
Iriflophenone 3-C-glucoside	100.00±0.89	84.17±1.42	-	77.16±0.94
3-Caffeoyl quinic acid	100.00±2.09	124.04±2.21	130.27±2.72	64.06±0.91
Caffeine	100.00±1.93	121.02±1.75	0.08±0.01	127.51±2.08
Procyanidin dimer B-type 1	100.00±1.44	128.04±1.96	9.64±0.03	97.67±1.85
Epicatechin	100.00±2.42	72.01±1.16	174.31±4.11	44.16±1.52
Cinnamtannin B-1	100.00±4.10	108.80±3.29	41.92±0.71	155.34±4.76
Mangiferin	100.00±0.94	111.34±1.74	0.06±0.00	85.44±0.98
Cinchonain I isomer 1	100.00±1.56	99.51±0.93	693.96±8.49	34.85±0.79
Procyanidin dimer A-type	100.00±2.61	0.71±0.05	180.27±2.95	1.14±0.02
Procyanidin dimer B-type 2	100.00±1.83	70.80±0.87	11.10±0.09	62.46±0.59
3,4-Dicaffeoyl quinic acid	100.00±2.11	66.44±1.62	16.36±0.02	61.74±1.17
3,5-Dicaffeoyl quinic acid	100.00±1.05	50.16±0.44	12.44±0.04	78.90±0.96
4,5-Dicaffeoyl quinic acid	100.00±1.19	54.71±0.61	10.38±0.01	44.71±0.75
Cinchonain I isomer 2	100.00±0.74	106.95±1.29	779.96±12.71	35.95±0.81

[1)]Values are expressed as percentages for each compound based on peak area of Typica variety extract, and represent means ± standard deviation (n=3).

dimer type A and theobromine in the extract of the Bourbon variety showed low relative content of 0.71% and 26.5%, respectively. In Geisha variety, 6 compounds showed a big difference of more than 50%. Similar to the Bourbon variety, procyanidin dimer type A was very low at 1.14%, and theobromine also showed a relatively low content at 19.98%. In addition, epicatechin, 4,5-dicaffeoyl quinic acid, cinchonain I isomer 1 and 2 showed a low relative content of less than 50%.

Compared to the Typica variety, the Maragogipe variety showed the most different compound relative content pattern. Specifically, theobromine and iriflophenone 3-C-glucoside were not detected in the extract of Maragogipe variety. Caffeine and mangiferin, which appeared in similar contents in extracts of other varieties, were confirmed only at trace levels. In addition, cinnamtannin B-1, 3,4-dicaffeoyl quinic acid, 3,5-dicaffeoyl quinic acid, 4,5-dicaffeoyl quinic acid, procyanidin dimer B-type 1 and 2 showed a low relative content of less than 50%. On the other hand, the content of cinchonain I isomer 1 and 2 were high around 700%, and epicatechin, procyanidin dimer A-type, and 5-caffeoyl quinic acid were found to be high at 174 - 220%.

In Korea, the cultivation of coffee trees is increasing by using the facility cultivation method. Therefore, various studies are needed to develop coffee-related products. The major compounds identified in coffee leaves grown in Korea through this study are 16 compounds, including caffeine and 3-caffeoylquinic acid. Most of the identified main componunds are compounds that exhibit physiological activities such as antioxidant activity (Calderón *et al*., 2009; Kim *et al*., 2016; Gomes *et al*., 2017; Sobeh *et al*., 2017; Woo *et al*., 2017; Lee *et al*., 2018; Chen *et al*., 2019; Kim *et al*., 2020; Morozkina *et al*., 2021; Lee *et al*., 2022).

By setting the MRM mode analysis conditions, the main compound analysis method of the coffee tree leaf extract using LC-MS/MS was established. The usefulness of the MRM mode analysis method was confirmed by performing comparative analysis of coffee tree leaf extracts by varieties. There were differences in some compounds depending on the variety, and the Maragogipe variety showed a significantly different compound content pattern compared to other varieties. In addition, differences in compounds occur in plants depending on conditions such as varieties, cultivation regions, and harvesting times. Therefore,

the analysis method for these compounds will also need to be continuously improved.

These results can be used as basic data for domestic coffee tree cultivation related varieties and characteristics by production area, product development and quality control in related industries such as food, cosmetics, and pharmaceuticals.

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