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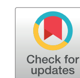
Research article

Phenolic acids and flavonoids from *Salvia plebeia* and HPLC-UV profiling of four *Salvia* species



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ABSTRACT

We isolated and purified phenolic acids and flavonoids from the ethanolic extract of *Salvia plebeia* using silica gel and a Sephadex LH-20 column chromatography. Spectroscopy revealed the isolated compounds were caffeic acid, rosmarinic acid, hispidulin, luteolin, jaceosidin, nepitrin, homoplantaginin, 6-hydroxyluteolin 7-*O*-glucoside, 6-methoxynaringenin 7-*O*-glucoside, naasanone, and cosmosiin. Quantitative analyses, using high-performance liquid chromatography coupled with UV (HPLC-UV), revealed that the major flavonoid from *S. plebeia* was 6-hydroxyluteolin 7-*O*-glucoside (100.63 mg/g) and the most abundant phenolic acid was rosmarinic acid (47.73 mg/g). Furthermore, among four other *Salvia* species, *S. officinalis* contained the highest overall phenolic acid and flavonoid level but these were still lower than *S. plebeia*. These results can help assess the potential of phenolic acids and flavonoids as potent sources of pharmacological ingredients from different *Salvia* species extracts.

1. Introduction

Plants from traditional Chinese medicine (TCM) are important sources of natural products to develop new drugs and drug intermediates (Ren et al., 2014). Therefore, separation, isolation, characterization, and quantification of such bioactive compounds from plant source materials are vital. *Salvia plebeia* has been widely used in TCM as it contains diverse chemical components. It is a medically important plant of the genus *Salvia* that is commonly distributed throughout China, India, Japan, and Korea (Lee et al., 2018). Traditional prescriptions of this plant include treating nephritis, hepatitis, bronchitis, the common cold, flu, cough, and rheumatoid arthritis (Liang et al., 2020; Choi et al., 2015). Importantly, these traditional applications have been scientifically confirmed and validated. *S. plebeia* displayed strong antibacterial, antioxidant, anti-inflammatory, and antiviral effects according to literatures (Wang et al., 2018). Furthermore, recent studies on the plants' biological activities indicated anti-atopic dermatitis, anti-asthma, anti-cancer, anti-obesity, anti-oxidant, and anti-influenza effects (Kil et al., 2020; Liang et al., 2020).

Approximately 75 phenolic acids and 50 flavonoids have been isolated from 23 and 51 *Salvia* species, respectively (Lu and Foo, 2002). These phytochemicals are considered major bioactive components of *S. plebeia*. Aside from phenolic acids and flavonoids, diverse classes of compounds such as diterpenoids, phenylpropanoids, sesquiterpenoids, and phytosterols have also been identified (Liang et al., 2020). Considerable efforts have been expended to the development of functional food ingredients from *S. plebeia* as its biological effects are mostly attributed to its high contents of flavonoids. Nepetin, hispidulin, apigenin, luteolin, and their glycosides are the major flavonoid constituents present in *S. plebeia*. These types of flavones are major phytochemicals responsible

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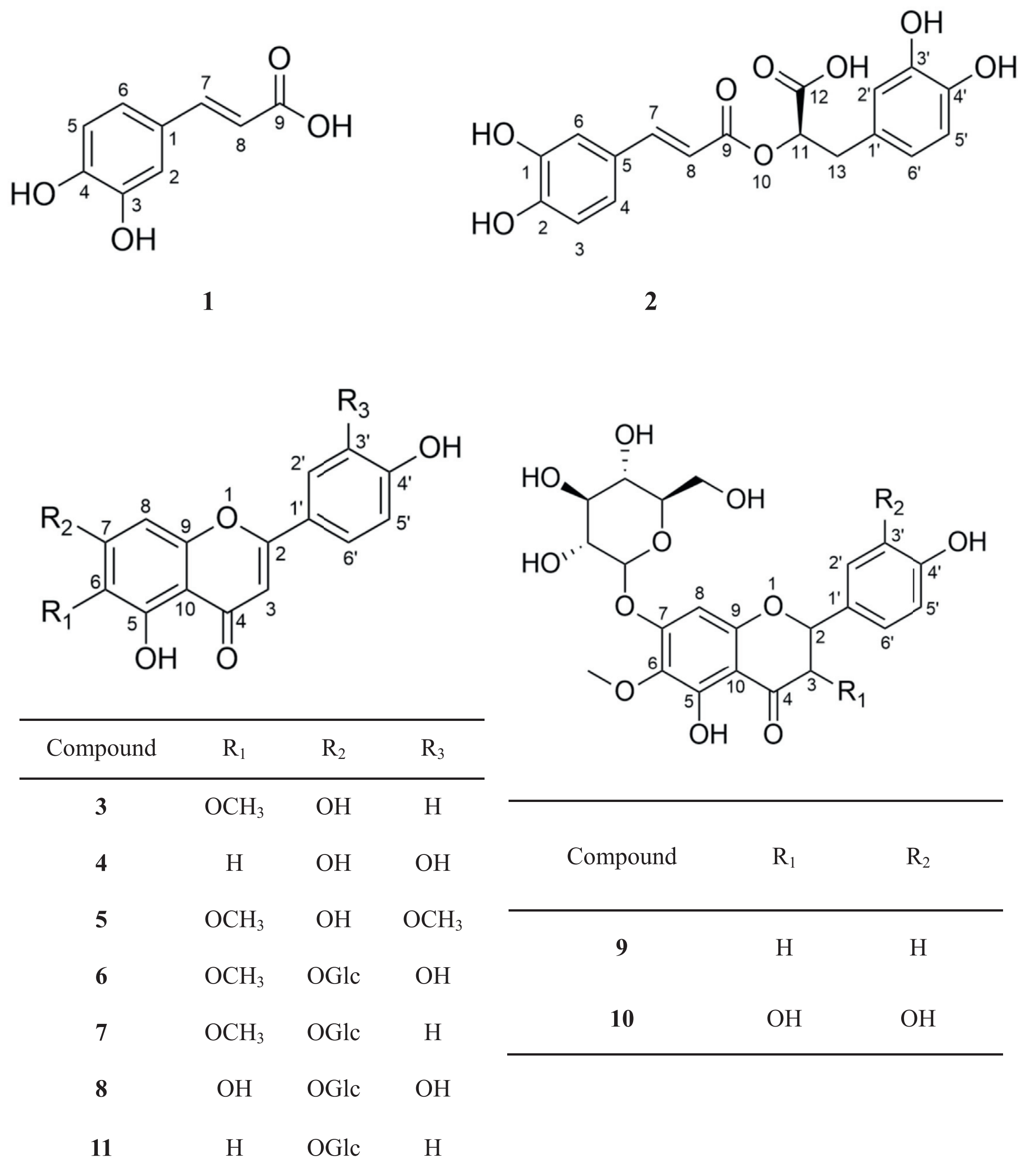
for the potent biological activity of the plant (Lee et al., 2018). Therefore, studies that isolate and quantify these marker compounds are warranted to help characterize their biological potency.

This study isolated compounds from the ethanol (EtOH) extract of *S. plebeia* (SPE) using open column chromatography. Structure elucidation using nuclear magnetic resonance (NMR) and mass spectroscopy (MS) was performed to identify the compounds. Quantitative analyses of SPE, *S. plebeia* methanol (MeOH) extract (SPM), and *S. plebeia* water (DW) extract (SPW), with MeOH extracts from three different *Salvia* species, *S. japonica* (SJM), *S. splendens* (SSM), and *S. officinalis* (SOM) were also conducted using high-performance liquid chromatography coupled with UV (HPLC-UV). By further isolating and quantifying compounds from *S. plebeia* and performing a chemical profiling of compounds from different *Salvia* species, we assessed their potential as sources of pharmacological ingredients.

2. Materials and methods

2.1. Plant materials

The aerial parts of *S. plebeia* were collected with support from Gimpo Agricultural Extension Center (2019), Gimpo, Korea. A voucher specimen was deposited at the Department of Plant Science and Technology Herbarium, Chung-Ang University, Korea. SJM, SSM, and SOM were



Compound	R_1	R_2	R_3
3	OCH_3	OH	H
4	H	OH	OH
5	OCH_3	OH	OCH_3
6	OCH_3	OGlc	OH
7	OCH_3	OGlc	H
8	OH	OGlc	OH
11	H	OGlc	H

Compound	R_1	R_2
9	H	H
10	OH	OH

Figure 1. Chemical structures of phenolic acids and flavonoids from SPE. caffeic acid (**1**), rosmarinic acid (**2**), hispidulin (**3**), luteolin (**4**), jaceosidin (**5**), nepitrin (**6**), homoplantaginin (**7**), 6-hydroxyluteolin 7-*O*-glucoside (**8**), 6-methoxynaringenin 7-*O*-glucoside (**9**), naasanone (**10**), and cosmosiin (**11**).

Table 1. ^{1}H-NMR spectroscopy data for compounds **3–11** from *S. plebeia*.

No.	3	4	5	6	7	8	9	10	11
2	-	-	-	-	-	-	5.46 (dd, 13.0, 3.0)	5.41 (dd, 5.0, 2.5)	-
3	6.59 (s)	6.67 (s)	6.89 (s)	6.73 (s)	6.86 (s)	6.69 (s)	-	-	6.86 (s)
3α	-	-	-	-	-	-	3.15 (m)	3.16 (m)	-
3β	-	-	-	-	-	-	2.71 (dd, 17.5, 3.0)	2.70 (m)	-
4	-	-	-	-	10.43 (s)	-	-	-	-
5	-	-	-	-	-	-	-	-	-
6	-	6.18 (d, 2.0)	-	-	-	-	-	-	6.44 (s)
7	-	-	-	-	-	-	-	-	-
8	6.23 (s)	6.43 (d, 2.0)	6.71 (s)	6.97 (s)	7.02 (s)	6.96 (s)	6.31 (s)	6.31 (s)	6.83 (s)
9	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
1'	-	-	-	-	-	-	-	-	-
2'	7.85 (d, 9.0)	7.39 (d, 2.5)	7.42 (d, 2.0)	7.41 (d, 2.0)	7.95 (d, 9.0)	7.39 (d, 2.0)	7.32 (d, 8.5)	6.76 (s)	7.94 (d, 8.0)
3'	6.89 (d, 8.5)	-	-	-	6.93 (d, 8.5)	-	6.79 (d, 8.5)	9.10 (s)	6.92 (d, 8.5)
4'	-	-	-	-	-	-	9.61 (s)	9.05 (s)	10.53 (s)
5'	6.89 (d, 8.5)	6.90 (d, 8.5)	6.84 (d, 8.5)	6.89 (d, 8.0)	6.93 (d, 8.5)	6.88 (d, 8.0)	6.79 (d, 8.5)	6.87 (s)	6.92 (d, 8.5)
6'	7.85 (d, 9.0)	7.42 (dd, 8.5, 2.5)	7.45 (dd, 2.0, 8.0)	7.44 (dd, 8.0, 2.0)	7.95 (d, 9.0)	7.42 (dd, 2.0, 9.0)	7.32 (d, 8.5)	6.76 (s)	7.94 (d, 8.0)
G-1	-	-	-	5.13 (d, 7.5)	5.12 (d, 14.0)	5.01 (d, 7.5)	4.98 (d, 7.0)	5.04 (d, 4.0)	5.40 (s)
G-2'	-	-	-	3.17–3.72 (m)	3.19–3.72 (m)	3.19–3.74 (m)	-	3.26–3.64 (m)	3.71 (d, 11.5)
G-3'	-	-	-	3.17–3.72 (m)	3.19–3.72 (m)	3.19–3.74 (m)	-	3.26–3.64 (m)	3.18–3.72 (m)
G-4	-	-	-	3.17–3.72 (m)	3.19–3.72 (m)	3.19–3.74 (m)	-	3.26–3.64 (m)	3.18–3.72 (m)
G-5	-	-	-	3.17–3.72 (m)	3.19–3.72 (m)	3.19–3.74 (m)	-	3.26–3.64 (m)	3.18–3.49 (m)
G-6	-	12.98 (s)	13.02 (s)	3.17–3.72 (m)	3.19–3.72 (m)	3.19–3.74 (m)	-	3.26–3.64 (m)	3.18–3.49 (m)
5-OH	12.96 (s)	-	3.73 (s)	13.02 (s)	12.97 (s)	12.76 (s)	11.97 (s)	11.97 (s)	12.99 (s)
6-OCH_3	3.68 (s)	-	3.93 (s)	3.76 (s)	3.76 (s)	-	3.68 (s)	3.68 (s)	-
3'-OCH_3	-			-	-	-	-	-	-

acquired from Korea Research Institute of Bioscience & Biotechnology, Daejeon, Korea.

2.2. Reagents and instruments

The solvents MeOH, EtOH, *n*-butanol (*n*-BuOH), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and *n*-hexane were purchased from Samchun Pure Chemicals Co., Pyeongtaek, Korea. HPLC-grade solvents such as acetonitrile (ACN), acetic acid, MeOH, and DW were obtained from J.T. Baker Chemicals (Avantor, Radnor, PA, USA). For open column chromatography, a column was packed with silica gel (60–200 μm, Merck Co., Darmstadt, Germany) and Sephadex LH-20 (Sigma-Aldrich, St. Louis, MO, USA). Thin-layer chromatography (TLC) analyses were conducted on a glass pre-coated with silica gel 60 (Merck Co., Darmstadt, Germany). NMR spectra were recorded on an AVANCE NMR spectrometer (Rheinstetten, Germany) operating at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. MS spectra were recorded using a JEOL-MS spectrometer (Tokyo, Japan). Quantitative analyses were conducted using an HPLC Agilent 1260 Infinity II LC system (Santa Clara, CA, USA) equipped with a pump, auto-sampler, and UV detector. All standards were isolated from *S. plebeia* (Figure 1).

2.3. Extraction, fractionation, and isolation

Dried aerial parts of *S. plebeia* (1.6 kg) were ground to obtain a powder. The powder was then extracted under reflux in 95% ethanol at 80 °C for 3 h and repeated three times using a soxhlet extractor. The resulting extract solution was filtered and concentrated using a rotary evaporator to obtain crude EtOH extract (SPE, 360 g). SPE (340 g) was suspended in DW and sequentially partitioned with equal volumes of *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH to obtain *n*-hexane (21 g), $CHCl_3$ (5 g), EtOAc (17 g), and *n*-BuOH fractions (22 g), respectively. The EtOAc fraction (10.0 g) of SPE was subjected to silica gel column chromatography and was eluted with a step-wise gradient system of $CHCl_3$:MeOH (100:0 to 0:100 *v/v*) and collected to obtain fractions. The fractions were analyzed using TLC and combined which resulted further into six sub-fractions. Sub-fraction 1 was subjected to purification using Sephadex LH-20 column and was eluted with a step-wise gradient of H_2O:MeOH (5:1 to 1:5 *v/v*) to obtain the compound **3**. Sub-fraction 3 was also purified in Sephadex LH-20 column using the previously described method to obtain compounds **4** and **5**. Compound **9** was isolated by purifying the vials in sub-fraction 4 using the MeOH recrystallization method. Compound **1** was obtained from the first 5 vials of sub-fraction 5. Vials 20–30 were subjected to MeOH recrystallization to obtain compound **10**. The remaining vials were re-chromatographed in an Sephadex LH-20 column eluted with H_2O:MeOH which resulted in obtaining crystals. The crystals were then analyzed using TLC and divided into 2 sub-fractions (5a and 5b). Sub-fraction 5a yielded compounds **2** and **11** was obtained from sub-fraction 5b. Compound **6** was obtained from sub-fraction 6 by MeOH recrystallization. The *n*-BuOH fraction (12.0 g) of SPE was chromatographed on a silica gel column and was eluted with a gradient system of $CHCl_3$:MeOH (100:0 to 0:100 *v/v*) in a step-wise manner to afford 4 sub-fractions. Sub-fraction 2 was subjected to MeOH recrystallization to obtain compound **7**. Sub-fraction 4 was re-chromatographed in the Sephadex LH-20 column eluted with a gradient system of increasing MeOH in H_2O to afford compound **8**.

Compound **1**: White powder; $C_9H_8O_4$; EI-MS: *m/z* 180 $[M]^+$ (100), 163 (39.0), 134 (49.0), 89 (25.0), 77 (13.0); ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.40 (1H, d, J = 15.5 Hz, H-7), 7.01 (1H, d, J = 2.0 Hz, H-2), 6.95 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.75 (1H, d, J = 8.0 Hz, H-5), 6.16 (1H, d, J = 16.0 Hz, H-8); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 167.9 (C-9), 148.1 (C-4), 145.5 (C-3), 144.5 (C-7), 125.7 (C-1), 121.1 (C-6), 115.7 (C-5), 114.6 (C-8), 115.1 (C-2).

Compound **2**: Yellow orange powder; $C_{18}H_{16}O_8$; FAB-MS $[M+1]^+$: *m/z* 361; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.46 (1H, d, J = 15.5 Hz, H-7′), 7.05 (1H, d, J = 2.0 Hz, H-2′), 7.01 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.76 (1H, d, J = 8.0 Hz, H-5′), 6.67 (1H, d, J = 2.0 Hz, H-2), 6.63 (1H, d, J = 8.0 Hz, H-5), 6.52 (1H, dd, J = 8.0, 1.5 Hz, H-6), 6.23 (1H, d, J = 16.0 Hz, H-8′), 5.03 (1H, dd, J = 8.5, 4.5 Hz, H-8), 2.90 (1H, dd, J = 14.0, 8.5 Hz, H-7a), 2.98 (1H, dd, J = 14.5, 4.0 Hz, H-7b); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 170.8 (C-9), 165.9 (C-9′)), 148.6 (C-4′), 145.9 (C-7′), 145.6 (C-3′), 144.9 (C-3), 144.0 (C-4), 127.2 (C-1), 125.3 (C-1′), 121.6 (C-6′), 120.0 (C-6), 116.7 (C-2), 115.7 (C-5′), 115.4 (C-8′), 114.9 (C-5), 113.2 (C-2′), 72.8 (C-8), 36.1 (C-7).

Compound **3**: Yellow orange powder; $C_{16}H_{12}O_6$; EI-MS: *m/z* 300 $[M]^+$ (100), 285 (64.0), 257 (55.0), 167 (14.0), 139 (16.0), 119 (17.0), 84 (70.0), 66 (72.0); ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **4**: Yellow powder; $C_{15}H_{10}O_6$; EI-MS: *m/z* 286 $[M]^+$ (100), 258 (12.0), 229 (5.0), 180 (2.0), 153 (19.0), 129 (10.0), 106 (1.0), 88 (1.5), 69 (4.0); ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **5**: Yellow powder; $C_{17}H_{14}O7$; EI-MS: *m/z* 330 $[M]^+$ (55.0), 315 (45.0), 301 (14.0), 287 (29.0), 181 (11.0), 153 (28.0), 135 (9.0), 84 (100), 66 (98.0); ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Table 2. ^{13}C-NMR spectroscopy data for compounds **3**–**11** from *S. plebeia*.

No.	3	4	5	6	7	8	9	10	11
2	162.9	163.9	164.4	164.6	164.6	164.3	78.8	78.9	163.5
3	102.1	102.8	102.4	102.5	102.6	102.4	42.2	42.3	102.9
4	181.3	181.6	182.0	182.1	182.3	182.2	197.9	197.8	181.9
5	153.0	161.5	152.6	152.5	152.4	146.6	154.4	158.4	156.9
6	132.0	98.8	131.8	132.4	132.5	130.4	130.1	130.1	99.8
7	157.4	164.2	158.5	156.4	156.4	151.3	159.0	158.6	164.3
8	94.8	93.8	91.4	94.2	94.3	93.2	95.6	94.4	94.8
9	152.4	157.3	152.1	152.1	152.1	150.1	158.9	158.0	162.9
10	104.2	103.7	105.0	105.7	105.7	105.8	903.2	106.0	105.3
1′	121.3	121.5	121.0	121.0	120.9	121.3	129.7	121.1	121.0
2′	128.2	113.3	113.1	113.2	128.6	113.3	128.8	114.4	128.6
3′	116.0	145.7	146.0	145.8	116.0	145.8	115.2	145.8	116.1
4′	161.2	149.7	150.0	151.0	161.0	149.0	158.6	145.2	161.1
5′	116.0	116.0	115.9	115.9	116.0	115.9	115.2	115.2	116.1
6′	128.2	119.0	119.2	119.2	128.6	119.0	128.8	118.0	128.6
G-1	-	-	-	100.1	100.2	100.9	100.0	103.2	99.5
G-2	-	-	-	73.2	73	73.2	73.1	73.1	73.1
G-3	-	-	-	77.2	69.5	77.3	78.7	77.3	77.1
G-4	-	-	-	69.5	77.3	69.6	69.6	69.5	69.6
G-5	-	-	-	76.7	76.7	75.8	77.2	76.6	76.4
G-6	-	-	-	60.6	60.6	60.6	60.5	60.5	60.6
6-OCH_3	59.5	-	60.0	60.3	60.3	-	60.3	60.3	-
3′-OCH_3	-	-	56.4	-	-	-	-	-	-

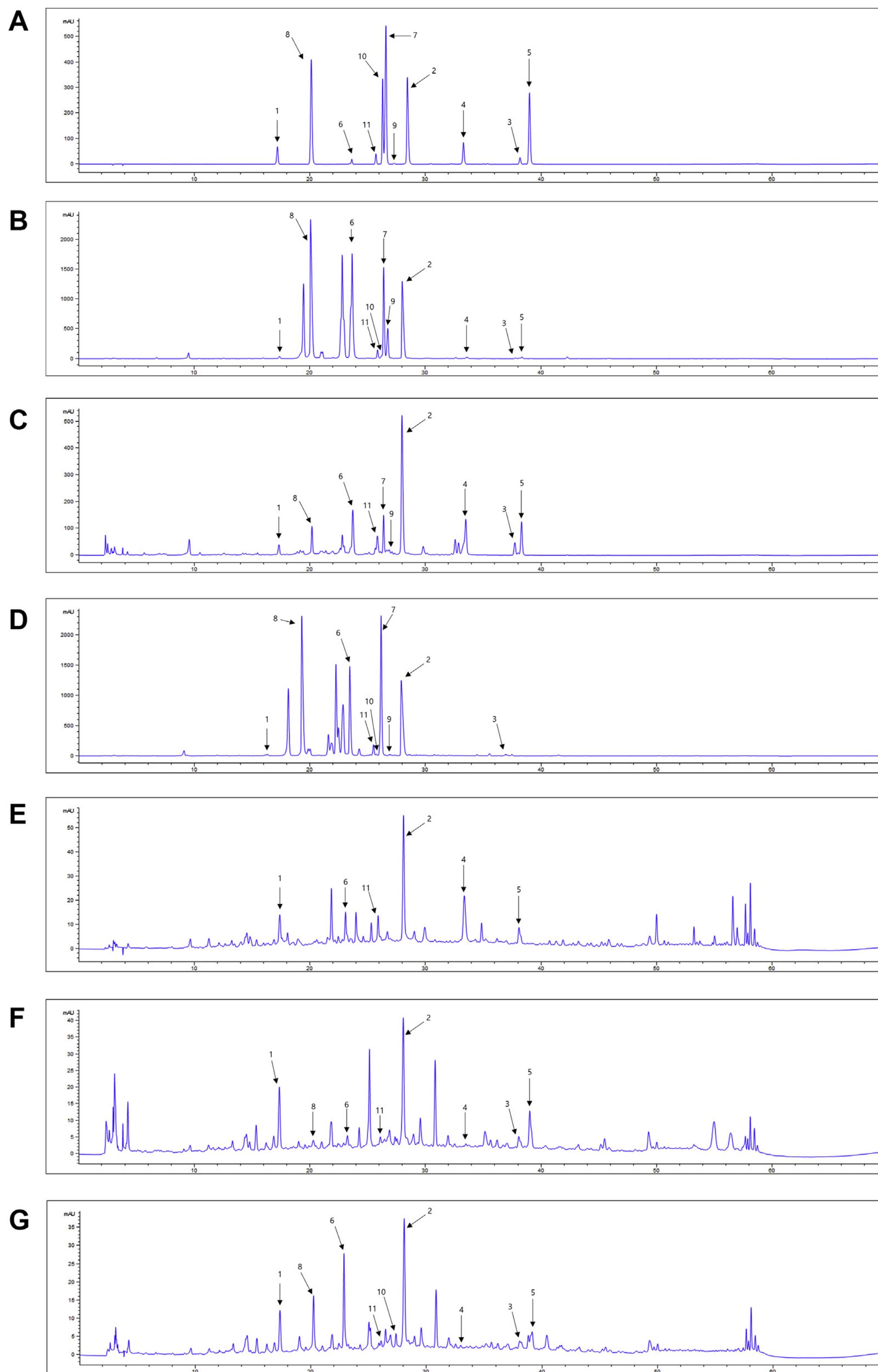


Figure 2. HPLC chromatograms of compounds **1**–**11** (**A**), SPE (**B**), SPW (**C**), SPM (**D**), SJM (**E**), SSS (**F**), and SOM (**G**) caffeic acid (**1**), rosmarinic acid (**2**), hispidulin (**3**), luteolin (**4**), jaceosidin (**5**), nepitrin (**6**), homoplantaginin (**7**), 6-hydroxyluteolin 7-*O*-glucoside (**8**), 6-methoxynaringenin 7-*O*-glucoside (**9**), naasanone (**10**), and cosmosiin (**11**).

Compound **6**: Yellow powder; $C_{22}H_{22}O_{12}$; FAB-MS: *m/z* 479 $[M+1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **7**: White yellow powder; $C_{22}H_{22}O_{11}$; FAB-MS: *m/z* 463 $[M+1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **8**: Yellow powder; $C_{21}H_{20}O_{12}$; FAB-MS: *m/z* 465 $[M+1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **9**: White yellow powder; $C_{22}H_{24}O_{11}$; FAB-MS: *m/z* 465 $[M+1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **10**: Yellow powder; $C_{22}H_{24}O_{12}$; FAB-MS: *m/z* 481 $[M+1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

Compound **11**: Yellow powder; $C_{21}H_{20}O_{10}$; FAB-MS: *m/z* 433 $[M+1]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6): see Tables 1 and 2.

2.4. Sample preparation and HPLC analysis

SPW was obtained from ground SP extracted in water (85 °C, 8 h), filtered, and dried using a spray dryer. SPW was cultivated in Gyeongsangbuk-do, Korea and provided by FromBio (Gyeonggi-do, Korea). SPE, SPM, SJM, SOM, SPM, and SPW were dissolved in MeOH (20 mg/mL). Phenolic acid and flavonoid standard compounds were also dissolved in MeOH (1 mg/mL). All samples were sonicated for 20 min and filtered through a syringe filter (0.45-μm) prior to analyses. The chromatographic separation of individual components was conducted using an YMC Pro C18 column (250 × 4.6 mm, 5μm). The mobile phase comprised a mixture of 0.5% acetic acid in water (0.5:95.5, *v/v*) (solvent A) and ACN (solvent B). Gradient elution started with 95% of solvent A and decreased to 75% after 20 min. Solvent A was decreased to 50% at 45 min and further decreased to 10% at 55 min. It was then increased to 65% at 65 min and maintained until 70 min. The injection volume was 10 μL and the flow rate was 1.0 mL/min. The UV detection wavelength was 280 nm.

2.5. Calibration curves

Standard compounds were serially diluted to prepare different concentrations (0.1–1.0 mg/mL). A calibration curve for each standard was constructed by plotting concentration (x, μg/mL) versus peak area (y). Linearity was assessed from the correlation coefficient value (r^2).

3. Results and discussion

Open column chromatography using silica gel was performed and a total of 2 phenolic acids and 9 flavonoids were isolated from the EtOAc and *n*-BuOH fractions of SPE. Flavonoids in SP have been used as marker compounds to evaluate its medicinal value and are mainly responsible for its bioactivity. We isolated two classes of phenolic acids and flavonoids (Figure 1). NMR and MS spectroscopy elucidated the structures of the isolated compounds and the results are shown in Table 1 for ^{1}H-NMR and Table 2 for ^{13}C-NMR data. The HPLC-UV chromatogram of standard compounds are shown in Figure 2. Quantitative analyses were performed in four different *Salvia* species and separation of individual components was achieved using HPLC-UV analysis (Figures 2B-2G).

Compounds **1** and **2** were identified as caffeic acid and rosmarinic acid, respectively, based on previous literature (Kil et al., 2020). Caffeic acid (**1**) is a hydroxycinnamic derivative present in many food sources. It acts as a carcinogenic inhibitor and possesses antioxidant and antibacterial activity (Magnani et al., 2014). Rosmarinic acid (**2**) possesses platelet anti-aggregation, hepatoprotection, nitric oxide inhibition, anti-oxidation and vaso-relaxation (Nugroho et al., 2012).

Structures **3–8** and **11** were identified as flavone compounds such as hispidulin (**3**), luteolin (**4**), jaceosidin (**5**), nepitrin (**6**), homoplantaginin (**7**), 6-hydroxyluteolin 7-*O*-glucoside (**8**) and cosmosiin (**11**) by comparing their MS, ^{1}H-, and ^{13}C-NMR data with previous studies (Lee et al., 2010, 2021; Ren et al., 2014; Kil et al., 2020; Cuong et al., 2019; Yuan et al., 2016). Hispidulin (**3**) is a naturally occurring flavone with anti-fungal, antioxidant, anti-inflammatory, anxiolytic, and anticonvulsive properties (Lin et al., 2010; Kavvadias et al., 2004). A previous study reported that luteolin (**4**) could be used to treat influenza A virus, H1N1, due to its enzymatic inhibition of H1N1 neuraminidase (Bang et al., 2016). Jaceosidin (**5**) exhibits various pharmacological activities including antibacterial, anti-allergic, anti-inflammatory, and anti-cancer (Nageen et al., 2021). Nepitrin (**6**) has significant anti-inflammatory, anti-pyretic, and anti-arthritic activity (Agarwal, 1982). Homoplantaginin (**7**) exhibits anti-inflammatory, antioxidant, and anti-influenza activities (Bang et al., 2016). 6-Hydroxyluteolin 7-*O*-glucoside (**8**) exerts anti-inflammatory effects (Williams et al., 1999) while cosmosiin (**11**) exhibited anti-cancer and anti-diabetes activities (Rao et al., 2011).

The ^{1}H- and ^{13}C-NMR spectra of compounds **9** and **10** depicted flavanone structures and were elucidated to be 6-methoxynaringenin 7-*O*-glucoside (**9**) and naasanone (= 5,7,3′,4′-tetrahydroxy-6-methoxyflavanone 7-*O*-glucoside) (**10**), respectively, based on the previous studies (Ren et al., 2014; Lee et al., 2010). Naasanone (**10**) is a newly isolated compound from *S. plebeia*.

The quantitative analyses of phenolic acids and flavonoids from *S. plebeia* showed their levels varied depending on the type of extract used. Our results showed that the main flavonoid in SPE was 6-hydroxyluteolin 7-*O*-glucoside (**8**) which exhibited the highest peak (100.63 mg/g) in the HPLC chromatogram (Figure 2B). High levels of nepitrin (**6**) and homoplantaginin (**7**) were also detected at 59.15 and 27.83 mg/g, respectively (Table 3). These were previously considered to be the major flavone compounds in SPE (Lee et al., 2018).

Table 3. Contents of compounds **1–11** in different *Salvia* species.

Compound	Content (mg/g)					
	SPE	SPW	SPM	SJM	SSM	SOM
1	0.56 ± 0.05	0.61 ± 0.02	0.26 ± 0.06	0.18 ± 0.02	0.27 ± 0.06	0.18 ± 0.00
2	47.70 ± 0.19	18.10 ± 0.01	48.22 ± 0.61	2.13 ± 0.04	1.67 ± 0.03	1.58 ± 0.03
3	0.56 ± 0.02	0.75 ± 0.01	0.29 ± 0.03	0.15 ± 0.00	0.08 ± 0.00	0.03 ± 0.01
4	1.18 ± 0.01	7.58 ± 0.19	0.63 ± 0.12	1.34 ± 0.08	0.12 ± 0.01	0.22 ± 0.01
5	0.03 ± 0.01	2.37 ± 0.05	0.58 ± 0.06	-	0.24 ± 0.01	0.04 ± 0.01
6	59.15 ± 0.65	10.64 ± 0.10	30.02 ± 0.69	0.89 ± 0.19	0.39 ± 0.00	1.87 ± 0.02
7	27.83 ± 0.26	2.29 ± 0.05	28.10 ± 0.20	-	-	-
8	100.63 ± 0.29	3.66 ± 0.04	99.26 ± 0.92	-	0.62 ± 0.00	1.04 ± 0.02
9	10.51 ± 0.18	0.39 ± 0.02	0.98 ± 0.08	-	0.25 ± 0.00	0.27 ± 0.02
10	0.58 ± 0.09	0.12 ± 0.02	2.87 ± 0.33	-	0.03 ± 0.03	0.10 ± 0.01
11	4.61 ± 0.01	2.16 ± 0.12	5.14 ± 0.22	0.31 ± 0.02	1.16 ± 0.03	0.06 ± 0.00

The most abundant phenolic acid was rosmarinic acid (**2**) having contents of 47.70 mg/g and 18.10 mg/g in SPE and SPW, respectively. As presented in the HPLC chromatogram (Figure 2C), peak number 2 was the highest in SPW indicating that the major constituent was rosmarinic acid (**2**). SPE showed an overall higher content of major flavonoids. However, while SPW contained relatively high rosmarinic acid (**2**) levels, they were not as high as SPE. These findings agreed with a recent study where water extract contained large amounts of rosmarinic acid (**2**) (Kil et al., 2020). The higher content of rosmarinic acid (**2**) in *S. plebeia* suggests it as a potential functional ingredient in cosmetics (Lee et al., 2018). Additionally, the contents of caffeic acid (**1**), luteolin (**4**), and jaceosidin (**5**) were higher in SPW suggesting the use of water as a solvent for extraction will obtain a higher yield of these compounds (Figures 2B and 2C).

In a previous report, the content of nepitrin (**6**) and 6-hydroxyluteolin 7-*O*-glucoside (**8**) in the MeOH extract (27.75 and 17.96 mg/g, respectively) was much lower to that of EtOH extract found in our study (Nugroho et al., 2012). Likewise, the compounds hispidulin (**3**), nepitrin (**6**), and homoplantaginin (**7**) had higher concentration in SPE (Jin et al., 2011).

The major flavanone previously detected in SP was naasanone (**10**) with a content of 938.3 mg/100 g DW and 6-methoxynaringenin 7-*O*-glucoside (**9**) was not detected (Lee et al., 2018). In contrast, the glycosidic flavanone 6-methoxynaringenin 7-*O*-glucoside (**9**) showed the highest content in the EtOH extract (10.51 mg/g) and the lowest in the DW extract (0.39 mg/g). These differences could be attributable to several factors such as agro-ecological variations in plant growth, extraction procedures, and analytical quantification methods (Lee et al., 2010; Bang et al., 2016; Kim et al., 2016). Although they exhibited significant pharmacological activities, flavanone and other sub-classes of flavonoids are rarely reported (Cabrera et al., 2007). Therefore, further studies are required regarding the isolation of flavanones from *S. plebeia* so they can be utilized for biological assays.

Phenolic acid and flavonoid contents in SJM, SSM, and SOM were also quantified. Among the four *Salvia* species, the SPM extract contained the highest content of phenolic acids and flavonoids (Table 3). Rosmarinic acid (**2**) was the major phenolic acid in SJM (2.13 mg/g), SSM (1.67 mg/g), and SOM (1.58 mg/g) with SJM having the highest content. As shown in Figures 2D-2G, peak 2 was the highest in all HPLC chromatograms of three *Salvia* species indicating that rosmarinic acid (**2**) was the major constituent. The major flavonoid was 6-hydroxyluteolin 7-*O*-glucoside (**8**) in the SPM extract (99.26 mg/g). This extract also contained slightly higher amounts of rosmarinic acid (**2**), homoplantaginin (**7**), and cosmosiin (**11**), when compared with SPE.

Alcoholic extracts from *S. officinalis* and several *Salvia* species are rich in phenolic compounds, particularly rosmarinic acid (**2**) (Ghorbani and Esmaeilizadeh, 2017). The flavonoid nepitrin (**6**) was highest in SOM (1.87 mg/g) whereas luteolin was highest in SJM (1.34 mg/g). This suggests that SOM contained a higher amount of these two marker compounds and could be utilized for its bioactivity. In SJM, only major flavonoids and phenolic acids such as caffeic acid (**1**), rosmarinic acid (**2**), hispidulin (**3**), luteolin (**4**), and cosmosiin (**11**) were detected. Homoplantaginin (**7**) was not detected in the three *Salvia* species suggesting barely detectable levels. SSM contained the highest amount of cosmosiin (**11**) at 1.16 mg/g.

Several *Salvia* species have been intensively studied as they possess a wide range of bioactive components, with their antioxidant properties primarily attributed to rosmarinic acid (**2**) (Fatma et al., 2017). Among the three *Salvia* species studied here, *S. officinalis* was the best documented. It is native to the Middle East and Mediterranean areas and has been used as a folk medicine to treat different types of health problems. The plant contains a plethora of bioactive constituents such as waxes, fatty acids, carbohydrates, alkaloids, phenolic compounds (e.g., flavonoids, coumarins, and tannins), steroids, poly acetylenes, and terpenoids (e.g., monoterpenoids, sesquiterpenoids, diterpenoids, and triterpenoids) (Ghorbani and Esmaeilizadeh, 2017). Pharmacological findings include antioxidant, anti-microbial, anti-cancer, anti-nociceptive, anti-inflammatory, anti-mutagenic, anti-dementia, hypolipidemic, and hypoglycemic effects (Garcia et al., 2016).

Overall, our findings suggest that the *Salvia* species studied here are good sources of rosmarinic acid (**2**). SOM had higher amounts of biomarker compounds as compared to the other *Salvia* species. However, SJM had a higher content of rosmarinic acid (**2**) but also had lower amounts of other flavonoids.

4. Conclusions

In total, 11 compounds were isolated and characterized from SPE. The majority were flavonoids which are the major phytochemical constituents of this plant. These compounds are reportedly responsible for its several bioactivities. The compound 6-hydroxyluteolin 7-*O*-glucoside (**8**) had the highest content in the SPE and SPM, whereas rosmarinic acid (**2**) was highest in SPW. The major flavonoids in SPE were similar to SPM. Furthermore, SPE and SPM could be a viable source of abundant flavonoids. Rosmarinic acid (**2**) was the major compound found in SJM, SSM, and SOM and could be a potential marker compound to evaluate the medicinal value of this plant.

Declarations

Author contribution statement

Leo Adrianne Paje: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

Jungwon Choi, Hak-Dong Lee, Juree Kim, A Ram Yu, Min-Jung Bae: Performed the experiments.

Paul John L. Geraldino: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sanghyun Lee: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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