Revista Brasileira de Farmacognosia 29 (2019) 695–701

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

Original Article

Anatomical characterization and LC-MS profiling of *Adenophora* roots from Korea

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ARTICLE INFO

Article history:
Received 26 April 2019
Accepted 8 July 2019
Available online 10 September 2019

Keywords:
Sasam
Inner morphological
Chemical analysis
Shashenoside I

ABSTRACT

"Sasam (沙参)" is a crude drug that is defined in the in Korean Herbal Pharmacopoeia as the root of *Adenophora triphylla* var. *japonica* (Regel.) Hara or *A. stricta* Miq., Campanulaceae. The dried roots of the *Adenophora* spp. are available in markets, and the roots of various species are similar to each other in shape, making it difficult to distinguish one from another using only the outer morphological appearance. Therefore, the present study aimed to establish quality control parameters for pharmacognostic evaluation and differentiation of five *Adenophora* species and two varieties grown in Korea. Inner morphological evaluation of the root of these plants was accomplished and preliminary chemical analysis was performed by liquid chromatography-mass spectrometry profiling. As a result, significant differences among samples were found in anatomical characteristics such as number and thickness of cork layer, existence of stone cell in cork layer, frequency of vessels, and area of intercellular space. Significant differences were found among the samples in the content of three components including shashenoside I and a new alkyl glycoside, adenophoroside I. These findings could provide the scientific criteria for the proper identification and establishment of standards for the use of "Sasam".

Introduction

The root of *Adenophora* sp., Campanulaceae, has been used as a folk medicine in Eastern Asia, including Korea, China and Japan, for the treatment of lung fever and the enrichment of 'Yin' in our body (Wiseman and Ellis, 1996). "Sasam (沙参)" is a crude drug that is defined in Korean Herbal Pharmacopoeia as the root of *Adenophora triphylla* var. *japonica* (Regel.) Hara or *A. stricta* Miq. (The Korean Herbal Pharmacopoeia, 2016). In the Chinese Pharmacopoeia, *A. tetraphylla* (Thunb.) Fisch. (a synonym of *Adenophora triphylla* var. *japonica*) or *A. stricta* is prescribed to be the botanical origin of "Nanshashen (南沙参)" (National Committee of Compendium for Drugs, 2015). *A. triphylla* var. *japonica* has been reported to have anti-obesity, hepatoprotective, antioxidant, and anti-cancer properties (Lee et al., 2000; Gum et al., 2007; Kim et al., 2009; Choi et al., 2010; Kang et al., 2013). Lupenone, lupeol and taraxerol derived from this plant are known to regulate the production and gene expression of airway MUC5AC mucin (Yoon et al., 2015). Polyhydroxylated alkaloids (Asano et al., 2000; Ikeda et al., 2000), triterpenoid saponins (Kang et al., 2013), phenolic glycosides (Kuang et al., 1991; Koike et al., 2010) and triterpenes (Konno et al., 1981; Yoon et al., 2015) have been isolated from these plants.

Adenophora species are perennial plants, and more than twenty species are distributed throughout Korea (Yoo, 1995). These species are categorized into four sections, Section Remotiflorae (Baranov) Ponomarchuk, Section Microdiscus Fed., Section Thysanthae (Fed.) Baranov and Section Platyphyllae (Borbas) Fed., by the characteristics of the petiole, leaves and flower. Additionally, the three sections are divided into two series, respectively (Yoo, 1995). Although the outer morphological studies on several *Adenophora* species

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https://doi.org/10.1016/j.bjp.2019.07.003

have been reported (Lee and Lee, 1994; Yoo and Lee, 1996), further characterization is required to provide a solid reference for authentication of the botanical origin of the root of *Adenophora* sp. when used as a traditional medicine. Moreover, since the dried roots of these plants are similar in shape and mixtures of more than two *Adenophora* species are sometimes sold, it is difficult to distinguish one species of root from another using only the outer morphological appearance. Therefore, the present study was designed to establish specific quality control parameters for pharmacognostic evaluation and differentiation by inner morphological study and by LC–MS profiling of *Adenophora* species grown in Korea. Five locally occurring *Adenophora* species and two varieties that represent each section and series of *Adenophora* species were studied.

Materials and methods

General experimental procedures

A Bruker DRX-500 spectrometer (Germany) was used for nuclear magnetic resonance (NMR) spectra including ^{1}H-^{1}H COSY, HMQC and HMBC. ESI-MS spectra were obtained with Finnigan MAT LCQ ion-trap mass spectrometer (San Jose, CA, USA). HRFAB-MS spectral data were recorded on a JEOL JMS-700 (Akishima, Japan). Preparative high-pressure liquid chromatography (HPLC) was performed on a YMC J'sphere column (4.6 × 250 mm, 4 μm i.d.) (YMC Co. Ltd., Kyoto, Japan). TLC was done on a silica gel 60 F_{254} (Merck, Damstadt, Germany). Column chromatography was performed on silica gel 60 (0.063–0.43 mm; Merck KGaA, Damstadt, Germany) and Diaion HP-20 (Supelco, MO, USA). The other chemicals were extra grade.

Plant material

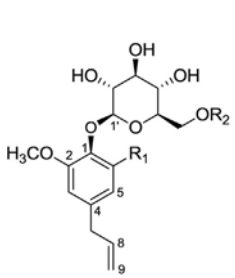
Seven *Adenophora* species were collected in Mt. Seorak, Mt. Jiri, Jeju Island and Busan of Korea from July to August from 1990 to 2001 and in 2016 (Table S1). For the isolation of secondary metabolites, fresh root of *A. triphylla* var. *japonica* (Regel.) Hara was purchased from Seobu traditional market in Jinju of Korea in 2016. The samples were identified by Dr. Jong Hee Park, College of Pharmacy, Pusan National University, and the voucher specimens were deposited in the herbarium of the College of Pharmacy, Gyeongsang National University (PGSC411–PGSC418).

Anatomical assays

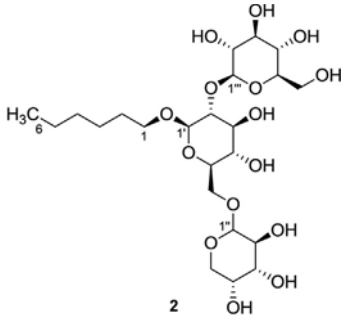
Freshly collected samples of the root of *Adenophora* species were stored in 70% ethanol. Transverse sections of the sample were obtained from the middle part of each root by free hand using commercial razor blades or a hand slicer. The sections were cleared by eau de javel solution (Sigma, MN, USA) and stained with methylene blue (Samchun Pure Chemical Co. Ltd., Korea). All sections were mounted in glycerinated water (50%) on a glass slide. The observations were performed by a photomicroscope, Olympus BX53 (Olympus, Japan), and the microphotographs were obtained with image processing software (IMT i-Solution Inc., BC, Canada) coupled to a video camera (PixeLINK, ON, Canada) for image capture.

Extraction and isolation

Fresh roots (950 g) of *A. triphylla* var. *japonica* were chopped and extracted three times with 100% methanol at room temperature for 1 h. Ultrasonication was applied to raise the extraction efficiency. The methanolic extract was concentrated through a rotary evaporator to give a crude extract (87.6 g). This extract was then suspended in water and partitioned successively with hexane and *n*-butanol to give hexane Fr. (5 g), *n*-BuOH Fr. (22 g) and water Fr. (60 g), respectively. The *n*-butanol fraction was subjected to Diaion HP-20 column chromatography (CC) with a gradient elution of water and methanol mixture (100:0 → 0:100) to give six fractions (Fr.1-Fr.6). The Fr.3 was applied on silica gel CC using a mixture of chloroform, methanol and water mixture (50:5:1 → 6:5:1) as an eluting solvent to give seven subfractions, fr.3a–fr.3 g. Compounds **1** (3.6 mg, t_R = 16.6 min) and **2** (3.0 mg, t_R = 17.4 min) were isolated from fr.3d and fr.3e, respectively by preparative HPLC with a gradient elution of acetonitrile and water as the developing solvent (88:12 → 65:35) for 30 min. Compound **3** (1.3 mg, t_R = 21.6 min) was isolated from fr.3c by preparative HPLC with the same mobile condition.



1 R_1 = *O*-β-D-glc; R_2 = H
3 R_1 = H; R_2 = α-L-ara



2

Adenophoroside I (**2**): A pale yellow powder; $[\alpha]_D^{25}$: -46.5° (*c* = 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz): δ 4.62 (1H, d, *J* = 7.9 Hz, H-1‴), 4.42 (1H, d, *J* = 7.7 Hz, H-1′), 4.33 (1H, d, *J* = 6.8 Hz, H-1″), 4.11 (1H, m, H-6′a), 3.90 (2H, t, *J* = 7.0 Hz, H-1), 3.88 (1H, m, H-6‴a), 3.85 (1H, m, H-5″a), 3.83 (1H, m, H-5‴), 3.74 (1H, m, H-6′b), 3.71 (1H, m, H-5″b), 3.61 (1H, m, H-2″), 3.57 (1H, m, H-3′), 3.55 (1H, m, H-6‴b), 3.54 (1H, m, H-4‴), 3.53 (1H, m, H-4′), 3.47 (1H, m, H-2′), 3.44 (1H, m, H-5′), 3.39 (1H, m, H-3‴), 3.34 (1H, m, H-3″), 3.29 (1H, m, H-4″), 3.25 (1H, m, H-2″), 1.62 (2H, quint, *J* = 7.0 Hz, H-2), 1.41 (2H, quint, *J* = 7.0 Hz, H-3), 1.34 (2H, quint, *J* = 7.0 Hz, H-4), 1.32 (2H, sext, *J* = 7.0 Hz, H-5), 0.93 (3H, t, *J* = 7.0 Hz, H-6); ^{13}C NMR (CD_3OD, 125 MHz): δ 103.8 (C-1″), 103.5 (C-1‴), 101.6 (C-1′), 81.3 (C-2′), 76.9 (C-4″), 76.3 (C-3′, C-3‴), 75.3 (C-5′), 74.5 (C-2‴), 72.8 (C-4′), 71.0 (C-2″), 70.1 (C-4‴), 70.0 (C-3″), 69.8 (C-1), 68.1 (C-5‴, C-6′), 65.3 (C-6‴), 61.3 (C-5″), 31.5 (C-5), 29.4 (C-2), 25.4 (C-3), 22.3 (C-4), 13.0 (C-6); HRFAB-MS *m*/*z* 581.2425 $[M+Na]^+$ (calcd for $C_{23}H_{42}O_{15}Na$, 581.2421)

Sample preparation

Methanol was added to the root of seven *Adenophora* species and extraction was done under sonication for 90 min, three times each. The extract was filtered through filter paper (Whatman No. 2, New York, NY, USA), and then filtered through a 0.45 μm PTFE syringe filter (Whatman, New York, NY, USA) before HPLC analysis. The final volume of methanol was adjusted to 100 ml per 10 g dried weight. Stock solutions for standard compounds (**1**-**3**) were prepared with HPLC-grade methanol as the solvent. Working calibration solutions were prepared by successive serial dilutions of the stock solution with methanol, and the final concentrations were 60, 6, 0.6 and 0.06 μg/ml.

HPLC-DAD-MS analysis

An Agilent 1260 infinity HPLC system equipped with an autosampler, a column oven, a binary pump and a degasser (Agilent Technologies, Palo Alto, CA, USA) was used for analysis. An aliquot (10 μl) of sample solution was directly injected on a Jemini 5 μ C18 (4.6 × 150 mm, 4 μm, Phenomenex, Torrance, CA, USA) equipped with compatible guard column. Components were resolved by gradient elution using an acetonitrile and water solvent system as follows: 10% acetonitrile for the first 30 min, 10%–40% for next 25 min, and then 95% for another 5 min. A conditioning phase was then used to return the column to the initial state for 5 min. The flow rate was 0.25 ml/min, and the column temperature was 30 °C. The eluent was detected with DAD (UV 204, 230 and 254 nm) and the positive ion was monitored with Finnigan MAT LCQ ion-trap mass spectrometer (San Jose, CA, USA) in SIM (selective ion monitoring) mode. The source voltage was set to +5 kV, and the capillary temperature was set to 300 °C. Other conditions were as follows: capillary voltage, +10 V; interoctapole lens voltage, +10 V; sheath gas flow, 80 arbitrary units; auxiliary gas flow, 20 arbitrary units. Nitrogen (>99.999%) and He (>99.999%) were used as sheath and damping gas, respectively. The mass scale was calibrated in the positive-ion mode using a solution consisting of caffeine, the tetrapeptide MRFA, and Ultramark 1621 (Thermo, Rockford, IL, USA) solution. Xcalibur software (Thermo, Finnigan MAT) was used to operate this ESI-MS system.

Quantification of peaks **1**-**3** in mass spectra was accomplished by comparison of the peak area with that of each peak in standard solution using linear regression analysis. The molecular ion of *m/z* 527 (**1**) was selected with an isolation width of 2 *m/z* during 14–19 min, in SIM mode-1. In SIM mode-2, molecular ions of *m/z* 581 (**2**) and 481 (**3**) were selected with the same isolation width during 14–19 min and 19–24 min, respectively.

Statistical analysis

All data were expressed as the mean ± SD (standard deviation), and one-way ANOVA (analysis of variance) followed by the Dunnett's test was used for statistical analysis using Excel software (Ver. 2016, MS office, Redmond, WA, USA). Values of $p < 0.05$ were considered statistically significant.

Results and discussion

Anatomical characteristics

Inner morphological study was accomplished on the root of five *Adenophora* species and two varieties. Representative transverse sections were obtained from the root samples (Fig. 1). The whole shape of the transverse section was round except for AL, elliptical. The root possessed three to twelve cork cell layers on the outer surface. AR, AT and ATJ showed higher number of cork layers than others (Table 1). The cork cell was rectangular. For the thickness of the cork layer, AP and AS displayed a thinner cork layer than others. In particular, ATJ showed the thickest cork layer of 450 ± 50 μm among the samples, and stone cells were found in the cork layer of ATJ only. Cortex beneath the cork layer was composed of roundish parenchyma cells, and the number of parenchyma cell layers was highest in AR with 5–8 compared to 2–5 for others. In general, plants of the Campanulaceae family store their carbohydrate product of photosynthesis as inulin not starch (Lewis, 1984). The inulin grains were 40–50 μm in diameter and were found in the cortex of AS and ATH samples with the frequency of 55–95/mm^2. Intercellular space was also found in the cortex. While around one fifth or one seventh of the total transverse area consisted of intracellular space in AL, AR and ATJ, only three to seven percent of the area was the intracellular space in AP, AS and AT. The vessel in xylem with medullary ray was arranged in a radial manner, and the principle vessel was scalariform vessel. Particularly, AP and AR displayed much higher frequency of vessels with 120 ± 14 and 150 ± 12 in mm^2, respectively, while others showed only approximately thirty vessels in mm^2. In addition, the arrangement of vessels was highly curled in these two species AP and AR. For the vessel diameter, AS and ATJ showed bigger diameter than others. AL, AR and ATJ ranked second. AP and AT displayed the smallest vessel diameter. Although ATH and ATJ belong to same species, they showed significant differences in anatomical characteristics such as number and thickness of cork layer, existence of stone cell in cork layer and area of intercellular space.

LC–MS profiles of Adenophora *spp*

LC–MS was employed to compare phytochemical content of methanol extracts of the *Adenophora* spp. roots. One hundred percent methanol was selected with the highest extraction efficacy among 70% methanol, 70% ethanol, 100% methanol and 100% ethanol solvents (data not shown). While it was difficult to find characteristic peaks in LC chromatogram by UV detection, three characteristic spots were found at R_f values of 0.28, 0.35 and 0.56 on the TLC by elution with chloroform, methanol and water mixture (10:5:1, v/v), respectively. The compounds that correspond to each peak were isolated from Diaion HP-20 CC, silica gel CC and preparative HPLC from the butanol fraction of *A. triphylla* var. *japonica* root. The chemical structures of peaks **1** and **3** were determined to be 3-methoxy-5-(2-propen-1-yl)-1,2-phenylene bis-β-D-glucopyranoside (shashenoside I) (**1**) and 2-methoxy-4(2-propen-1-yl) phenyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside (eugenyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside) (**3**), respectively, from the comparison of the spectroscopic data with the previously published data (Kuang et al., 1991; Straubinger et al., 1999). Compound **1** is known to be isolated from *A. tetraphylla* and to have a selective cytotoxic activity against the acute myeloid leukemia cell line MV4-11 (Xiong et al., 2013). Compound **3** is the first reported component isolated from the Campanulaceae family.

The compound of peak **2** was isolated as a pale yellow powder and showed a $[M+Na]^+$ peak at *m/z* 581.2425 in HRFAB-MS, which was consistent with the molecular formula, $C_{23}H_{42}O_{15}$. The characteristic UV maxima were observed at 210, 225 and 278 nm. The ^{1}H and ^{1}H-^{1}H COSY NMR spectra of **2** showed the presence of an *n*-hexyl group at δ_H 0.93 (3H, t, *J* = 7.0 Hz, H-6), 1.32 (2H, sext, *J* = 7.0 Hz, H-5), 1.34 (2H, quint, *J* = 7.0 Hz, H-4), 1.41 (2H, quint, *J* = 7.0 Hz, H-3), 1.62 (2H, quint, *J* = 7.0 Hz, H-2) and 3.90 (2H, t, *J* = 7.0 Hz, H-1). The H-1 peak indicated that the *n*-hexyl group is attached to an oxygen atom. In addition, three anomeric proton peaks were found at δ_H 4.33 (1H, d, *J*=H-1″ -> H-1‴-1' -> H-1') and 4.62 (1H, d, *J*=7.9 Hz, H-1⁗). The other proton peaks were detected at δ_H 3.25-4.11. These results indicated the presence of three saccharide moieties attached to the *n*-hexyl group. The ^{13}C NMR spectrum of compound **2** displayed resonances for all 23 carbons in the molecule. Carbon peaks of five methylene (δ_C 68.8, 31.5, 29.4, 25.4 and 22.3) and one methyl carbons of *n*-hexyl group were identified by HMQC and DEPT spectra. Three anomeric carbon resonances at δ_C 101.6 (C-1'), 103.5 (C-1⁗) and 103.8 (C-1‴), and fourteen carbon peaks at δ_C 61.3–81.3 indicated one pentose and two hexose moieties, which was determined as an arabinose and two glucoses, respectively, from ^{13}C and HMQC spectra (Straubinger et al., 1999; Yuda et al., 1990). In the HMBC spectrum of compound **2**, the correlations of the anomeric proton at δ_H 4.33 (H-1‴) with C-6' (δ_C 68.1), and the other anomeric proton at δ_H 4.62 (H-1⁗) with C-2' (δ_C 74.5) indicated that an arabinose

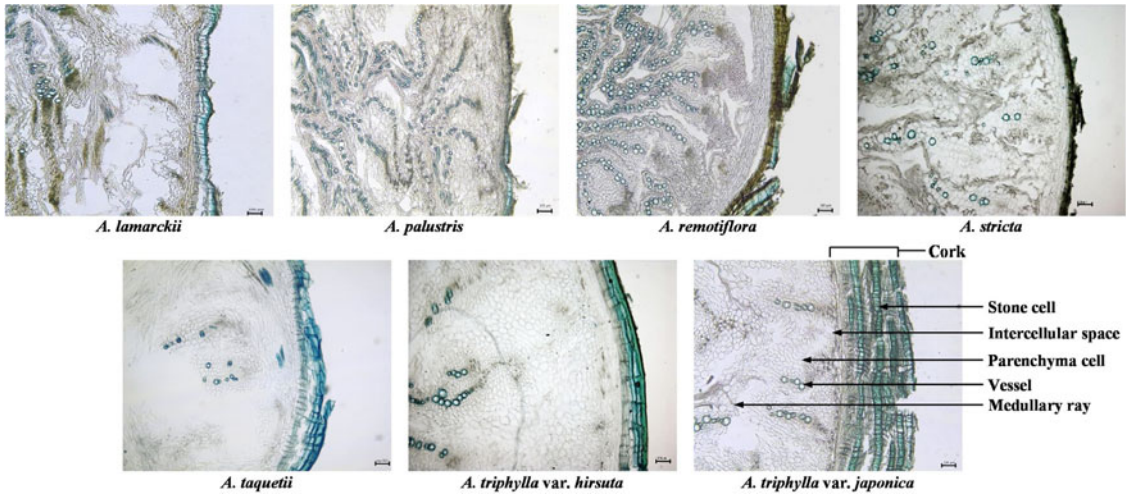


Fig. 1. Photomicrographs of transverse section from *Adenophora* spp. roots (×100). Black bars mean 100 μm.

Table 1
Microscopic data of roots from *Adenophora* spp.

Parameter	*A. lamarckii*	*A. palustris*	*A. remotiflora*	*A. stricta*	*A. taquetii*	*A. triphylla* var. *hirsuta*	*A. triphylla* var. *japonica*
Number of cork cell layer	5–9[b]	3–5[c]	8–10[a]	5–8[b]	8–12[a]	5–9[b]	8–10[a]
Thickness of cork layer (μm)	160 ± 35[b]	80 ± 10[c]	255 ± 30[b]	70 ± 10[c]	200 ± 30[b]	200 ± 15[b]	450 ± 50[a]
Size of cork cell (μm)	50–80 × 8–12[a]	25–55 × 5–10[c]	45–75 × 7–10[b]	50–80 × 8–12[a]	45–75 × 8–14[a]	55–90 × 10–16[a]	50–105 × 8–12[a]
Existence of stone cell	–	–	–	–	–	–	+
Number of parenchyma cell layer in cortex	2–4[b]	2–4[b]	5–8[a]	3–5[b]	2–5[b]	3–5[b]	2–4[b]
Diameter of parenchyma cell in cortex (μm)	40 ± 5[c]	50 ± 6[b]	75 ± 14[a]	75 ± 14[a]	65 ± 10[a]	80 ± 15[a]	75 ± 13[a]
Diameter of parenchyma cell in xylem (μm)	35 ± 4[c]	45 ± 5[b]	30 ± 7[c]	50 ± 4[a]	50 ± 4[a]	50 ± 7[a]	50 ± 5[a]
Area of intercellular space (%)	20 ± 5[a]	7 ± 3[c]	14 ± 5[a]	6 ± 3[c]	3 ± 1[d]	9 ± 5[b]	20 ± 6[a]
Frequency of vessels (mm^2)	30 ± 8[c]	120 ± 14[b]	150 ± 12[a]	35 ± 2[c]	30 ± 5[c]	30 ± 11[c]	30 ± 4[c]
Vessel diameter (μm)	30 ± 4[b]	20 ± 2[d]	40 ± 5[b]	45 ± 8[a]	25 ± 3[c]	40 ± 6[b]	50 ± 6[a]

Data are expressed as mean ± SD ($n = 5$).
Different upper letters in the same line indicate significant differences ($p < 0.05$) among samples.

moiety is attached to the 6′ position of a glucose moiety and that another glucose moiety is attached to the 2′ position of the glucose moiety (Fig. S10). The signal correlation of the anomeric proton at δ_H 4.42 (H-1′) with C-1 (δ_C 69.8) confirmed that the *n*-hexyl group is attached to position 1 of the glucopyranoside. Based on these spectroscopic data, the structure of compound **2** was determined to be *n*-hexyl *O*-β-D-glucopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→6)]-*O*-β-D-glucopyranoside and named adenophoroside I.

Significant differences in the content of these three compounds were found among the extracts by quantification with LC–MS in SIM modes. Three peaks corresponding to each compound (**1**-**3**) were found at 16.6, 17.4 and 21.6 min, respectively, on the LC–MS chromatograms (Fig. S27). Calibration curves showed good linearity, and the correlation coefficients were between 0.997 and 0.999 for the three compounds over the concentration ranges of quantification (Table S2). While the content of compounds **1** and **2** was the highest in AP with 856.4 ± 7.4 and 857.7 ± 35.0 μg/g DW (dry weight), ATH showed the highest content of compound **3** among the seven samples with 118.2 ± 4.1 μg/g DW (Table 2) (Figs. 2 and 3). All three compounds were not detected in AL and AR. Compound **3** was detected only in AT and ATH with the values of 19.6 ± 3.2 and 118.2 ± 4.1 μg/g DW, respectively. The RSD (relative standard deviation) values for intra- and interday analysis were below 16.5%.

Table 2
Contents of three compounds (**1**-**3**) in the methanolic extract of *Adenophora* spp.

Species	**1**	**2**	**3**
A. lamarckii	ND	ND	ND
A. palustris	856.4 ± 7.4[a]	857.7 ± 35.0[a]	ND
A. remotiflora	ND	ND	ND
A. stricta	55.1 ± 5.0[d]	394.0 ± 46.5[c]	ND
A. taquetii	472.2 ± 3.2[b]	416.9 ± 18.7[c]	19.6 ± 3.2[b]
A. triphylla var. *hirsuta*	ND	ND	118.2 ± 4.1[a]
A. triphylla var. *japonica*	216.2 ± 21.9[c]	584.9 ± 28.7[b]	ND

Contents were expressed as mean ± SD (μg/g dry weight) ($n = 4$).
Different upper letters in the column indicate significant differences ($p < 0.05$) among samples.
1, shashenoside I; **2**, adenophoroside I; **3**, eugenyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside.

Although ATH and ATJ belong to the same species, they showed significantly different inner-morphological properties and chemical profiles. While ATJ only showed stone cells in cork layer and wider intercellular space than ATH. In addition, while compounds

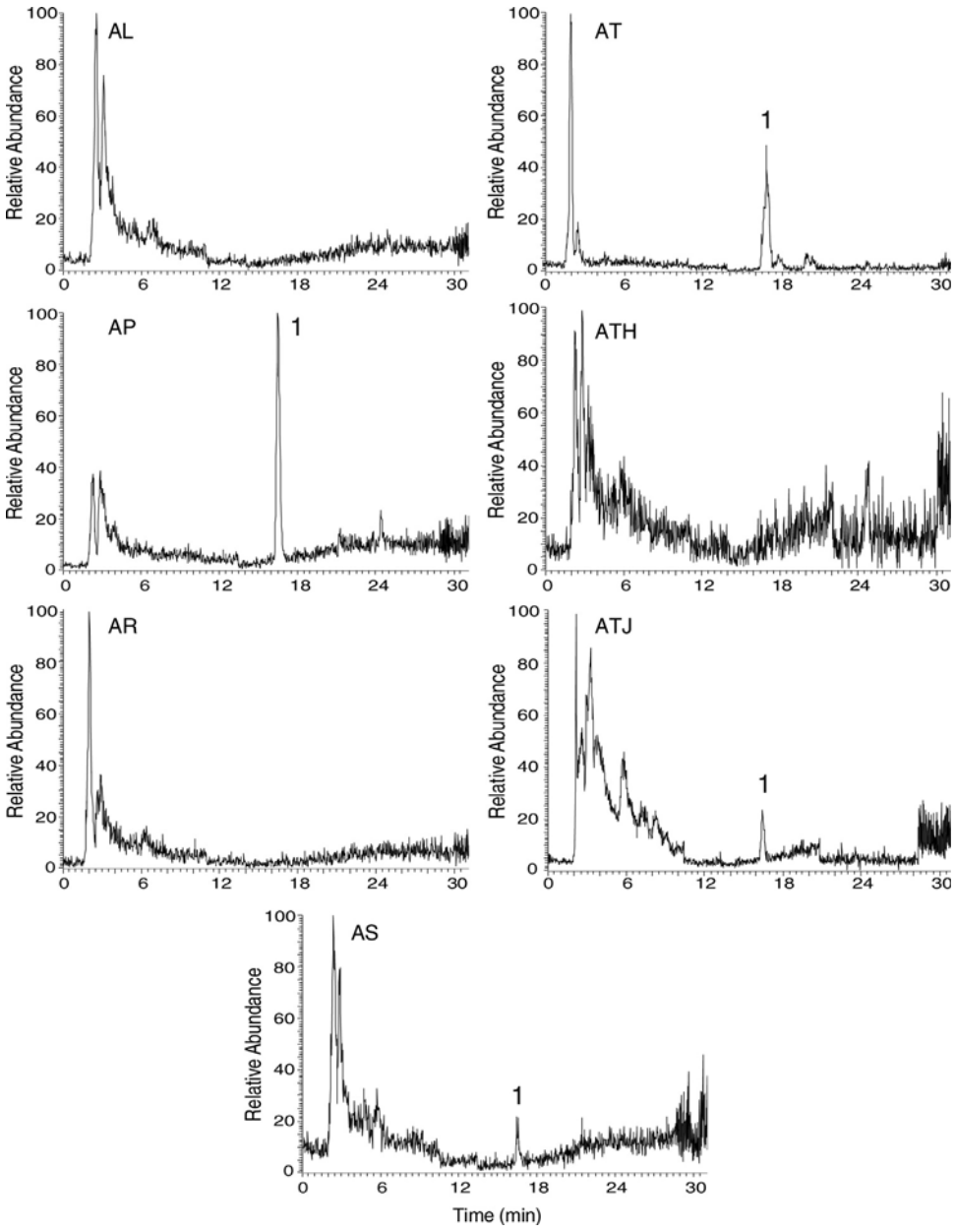


Fig. 2. LC–MS profiles of *Adenophora* spp. extracts in SIM mode-1. The molecular ion of *m/z* 527 was selected during 14–19 min.

1 and **2** were not detected in ATH, the contents were more than two hundred μg/g DW in ATJ. Meanwhile, although three characteristic compounds were not detected in both AL and AR, AR showed a thicker cork layer, more parenchyma cell layers in cortex and much higher frequency of vessels than AL in the anatomical study. Although compound **3** was isolated from commercial ATJ in this study, this secondary metabolite was not detected in a wild ATJ sample. This result can be ascribed to the fact that the content of compound **3** is lowest among the three compounds and detected in AT and ATH only.

Conclusion

A pharmacognostic evaluation and differentiation study was accomplished by systematic microscopic observations and secondary metabolite profiling to establish the parameters for five *Adenophora* spp. and two varieties collected from different locationswithin Korea. The microscopic data showed discriminative inner morphological characteristics such as number and thickness of cork layer, existence of stone cells in the cork layer, intercellular space, frequency and diameter of the vessel. Secondary metabolite profiles of root methanol extract were obtained from LC–MS analysis. The LC–MS profiles exhibited different contents of characteristic two phenyls and one alkyl glycoside. These findings could provide the scientific criteria for the proper identification and establishment of standards for the use of a crude drug “Sasam”.

Authors’ contributions

WSP and HJK extracted the samples and carried out the anatomical and LC–MS analysis with SJC. AAKK and KMA supervised the anatomical and LC–MS analysis, respectively. WSP, HJK and MJA wrote the manuscript. MCS and MJA created the project and supervised the laboratory work. MCS and HJC provided critical reading and insightful recommendations of the manuscript. JHP and MJA

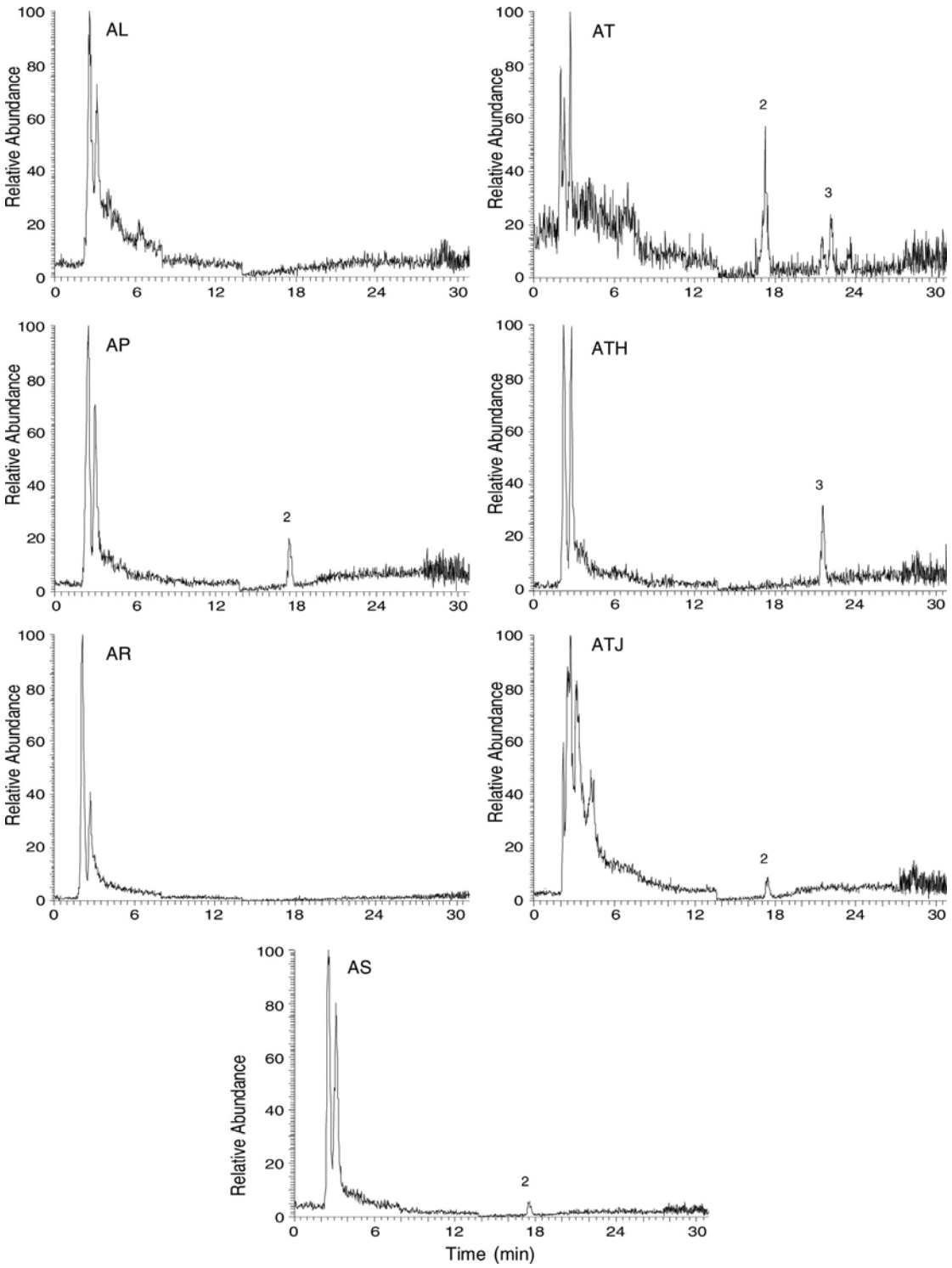


Fig. 3. LC–MS profiles of *Adenophora* spp. extracts in SIM mode-2. Molecular ions of *m/z* 581 and 481 were selected during 14–19 min and 19–24 min, respectively.

collected and identified the plant. All of the authors have read the final manuscript and approved the submission.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

This research was financially supported by the R&D Program for Forest Science Technology (Project No. 2017036A00-1719-BA01) provided by Korea Forest Service (Korea Forestry Promotion Institute).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bjp.2019.07.003.

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