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Original Research Article

Taxonomical Study of *Chrysosplenium* L. (Saxifragaceae) in Korea Based on Chemical Composition

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Abstract - Components extracted from 7 species and 18 populations of *Chrysosplenium* in Korea were compared and analyzed using GC-MS analysis. 57 components (≥80% quality) were identified, of which neophytadiene, palmitic acid and phytol were found at all the taxa. Percentage composition of isolated extracts showed a clear difference in components type and GC-MS profile. On the basis of that result, data matrix was made and cluster analysis using UPGMA was conducted. From the result of cluster analysis, two groups were recombined; one with alternate leaves comprised *C. japonicum* in Ser. *Alternifolia* and *C. flagelliferum* in Ser. *Flagellifera* and the other with opposite leaves gradationally comprised *C. psuedofauriei* in Ser. Sinica, *C. ramosum* in Ser. *Oppositifolia* and *C. sphaerospermum*, *C. valdepilosum*, *C. flaviflorum* in Ser. *Pilosa*. These chemotaxonomic results agreed in general with those of existing studies on external morphology and molecular. In conclusion, chemical composition can be an useful characters in understanding the relation analysis among interspecific and intraspecific complex with the help of cluster analysis of 7 species and 18 populations of *Chrysosplenium* in Korea.

Key words - Saxifragaceae, *Chrysosplenium*, Chemotaxonomy, GC-MS, UPGMA

Introduction

About 57~65 species of *Chrysosplenium* L. in Saxifragaceae have been known to be distributed throughout the world (Hara, 1957; Pan, 2001). This genus except for 2 species distributed in South America is restrictively distributed at the northern hemisphere; 2 species in Northeastern America, 4 species in Northwestern America, 2 species in Europe and most of the remaining taxa in eastern Asia (35 species in China, 18 species in Japan) (Spongberg, 1972; Pan, 2001; Iwatsuki *et al*., 2001). This genus was established as taxon by Linne (1753) for the frist time, classified as 2 subgenus (*Gamosplenium, Dialyspelium*), according to arrangement type of sepals by Maximowicz (1877) and divided as 2 sections (*Gamosplenium, Dialyspelium*), according to arrangement type of leaves by Franchet (1890). On the other hand, Hara (1957) reclassified this genus 17 series, maintaining that the classification of Franchet wasn't nature classification. As the studies on *Chrysosplenium* L. in Korea, Forbes and Hemsley (1887) reported for the first time, and then Palibin (1898), Nakai (1909), Chung (1937, 1965), Park (1949, 1974), Lee (1976), Lee (1996a), Lee (1996b) reported in the plant checklist and illustrated book of flora. Additionally, Chung and Kim (1988) recorded this genus as 7 species and 1 variety, and Kim (2007) as 5 species and 3 varieties *C. flagelliferum* F. Shmidt, *C. alternifolium* var. *sibiricum* Seringe ex DC., *C. japonicum* (Maxim.) Makino, *C. ramosum* Maxim., *C. pseudofauriei* H. Lév., *C. flaviflorum* Ohwi, *C. pilosum* var. *fulvum* (Terracc.) Hara, *C. pilosum* var. *valdepilosum* Ohwi. Recently, Han *et al.* (2011) raised *C. pilosum* var. *fulvum* and *C. pilosum* var. *valdepilosum* to the status of species, *C. sphaerospermum* and *C. valdepilosum*, on the basis of taxonomic treatments such as ITS sequences and seed characteristics of *C. pilosum* complex faced with the different taxonomic views.

As the studies on the chemical compounds of *Chrysosplenium*, 77 components comprising Eicoane, Palmitic Acid ethyl ester, Dibutyl phthate, (z,z,z)-9,12,15-Octadecatrienoic acid, ethyl ester, 2,6-Butylated hydroxytoluene and 5,6,7,7α-Tetrahydro 4,4,7α-trimethyl-2 (4H)-benzofuranone were identified and isolated in *C. nudicaule* (Yang *et al.*, 2004), and 4 components chrysosplenol B, chrysosplenol D, chrysosplenoside B and

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chrysosplenoside D in *C. alternifolium* (Gudej and Czapski, 2009). As chemotaxonomic studies, Bohm and Collins (1979) reported that the arrangement of leaves was divided into alternate and opposite according to the containing degree of O-methylated flavones and supported the opinion of Franchet (1890) that *Chrysosplenium* should be classied into 2 sections, *Oppositifolia* and *Alternifolia*. But, studies on the components composition of *Chrysosplenium* in Korea are difficult to find and chemotaxonomic studies aren't carried out until now.

So, this study was conducted to determine the chemical components of *Chrysosplenium* in Korea and the interspecific chemical differences of it, and compare our findings with those of morphological and phylogenetic classification.

Materials and Methods

Plant Samples

18 populations representing *C. sphaerospermum*, *C. valdepilosum*, *C. flaviflorum*, *C. psuedofauriei*, *C. ramosum*, *C. japonicum* and *C. flagelliferum* were sampled in Korea from April to May, 2011. Information of taxa and sources used in this experiment was as Table 1. Voucher specimens were dried an kept at Herbarium of Semyung University (NMR).

Extraction and purification

The whole plants of collected samples were used as specimens for extraction after washing with distilled water, air-drying under shade for a week and then grinding. 200 mg of grinded specimens dissolved in 5 mL of 99.9% MeOH was extracted 3 times for 1 hour with the use of ultrasonic extraction. MeOH extracts from ultrasonic extraction were filtered with 0.45 μm nylon syringe filter and then used in GC-MS analysis.

GC-MS analysis

GC-MS analysis for the compositions of extracts was performed using same GC/MSD (HP6890GC/5973MSD, USA), equipped with Ultra 2 (Agilent 19091B-102, 315Max). The injection volume was 2 μL. The carrier gas used was helium, at a constant flow rate of 0.4 mL/min. The oven temperature was initially held at 120℃ for 5 minutes, then

Table 1. Collected materials used in this study

Taxa	Abbrev.	Source and voucher
C. sphaerospermum Maxim. 1	CSPH1	GB: Mt. Palgong, Gunwi-gun / NMR: H. J. Kim11041601
C. sphaerospermum Maxim. 2	CSPH2	GW: Mt. Chiak, Wonju-si / NMR: H. J. Kim11051801
C. sphaerospermum Maxim. 3	CSPH3	JN: Isl. Naro, Goheung-gun / NMR: H. J. Kim11043001
C. sphaerospermum Maxim. 4	CSPH4	JJ: Mt. Jeolmul, Jeju-si / NMR: H. J. Kim11051701
C. sphaerospermum Maxim. 5	CSPH5	JJ: Mt. Barime, Jeju-si / NMR: H. J. Kim11051702
C. valdepilosum (Ohwi) S.H. Kang & J.W. Han 1	CVAL1	GG: Mt. Bukbae, Gapyeoung-gun / NMR: H. J. Kim11050401
C. valdepilosum (Ohwi) S.H. Kang & J.W. Han 2	CVAL2	GW: Mt. Baekun, Wonju-si / NMR: H. J. Kim11050601
C. valdepilosum (Ohwi) S.H. Kang & J.W. Han 3	CVAL3	GW: Mt. Taebaek, Taebaek-si / NMR: H. J. Kim11052101
C. flaviflorum Ohwi 1	CFLV1	GB: Mt. Cheongyang, Bonghwa-gun / NMR: H. J. Kim11041701
C. flaviflorum Ohwi 2	CFLV2	GG: Mt. Myeongji, Gapyeoung-gun / NMR: H. J. Kim11050401
C. flaviflorum Ohwi 3	CFLV3	CB: Mt. Samdobong, Yeongdong-gun / NMR: H. J. Kim110513
C. pseudofauriei H.Lév 1	CPSE1	GW: Mt. Taebaek, Taebaek-si / NMR: H. J. Kim110521002
C. pseudofauriei H.Lév 2	CPSE2	GB: Mt. Yongmum, Yechon-gun / NMR: H. J. Kim11051102
C. ramosum Maxim.	CRAM1	GW: Mt. Taebaek, Taebaek-si / NMR: H. J. Kim11052103
C. japonicum (Maxim.) Makino 1	CJAP1	CB: Baegun-myeon, Jecheon-si / NMR: H. J. Kim11050101
C. japonicum (Maxim.) Makino 2	CJAP2	GB: Mt. Palgong, Gunwi-gun / NMR: H. J. Kim11041602
C. flagelliferum F.Shmidt 1	CFLG1	GG: Mt. Cheonma, Namyangju-si / NMR: H. J. Kim11050501
C. flagelliferum F.Shmidt 2	CFLG2	GW: Mt. Taebaek, Taebaek-si / NMR: H. J. Kim11052104

CB: Chungcheongbuk-do, GB: Gyeongsangbuk-do, GG: Gyeonggi-do, GW: Gangwon-do, JN: Jeollanam-do, JJ: Jeju-do.

raised to 300℃, 5℃/min. Components of the extracts were identified with the aid of the database (Wiley7N).

Cluster analysis

The phenogram was prepared using 7 species comprising 18 populations in *Chrysosplenium* in Korea. Every analyzed components (≥ 80% quality) were treated as OTU (Operational Taxonomic Unit) and each OTU marked as value of "1", when founded, or "0", when not founded. After the above procedure, data matrix was prepared. The phenogram was prepared using average linkage cluster analysis according to average distance. UPGMA (unweighted pair group method with arithmetic average) of PAUP 4.02b (Swoffrd, 1998) was used for statistical analysis.

Results and Discussion

Components composition

On the basis of results from this study of 7 species comprising 18 populations in *Chrysosplenium* in Korea using GC-MS analysis, 57 components (≥ 80% quality) were identified as Table 2. Percentage composition of extracts identified from *Chrysosplenium* in Korea was listed in Table 3.

There was some difference among 7 taxa in components composition; 21 Kinds of components in *C. sphaerospermum*, 15 in *C. valdepilosum*, 19 in *C. flaviflorum*, 22 in *C. psuedofauriei*, 14 in *C. ramosum*, 24 in *C. japonicum*, 21 in *C. flagelliferum* were identified. Number of components specie in each taxa was 6 (compound 8, 12, 15, 18, 21, 57) in *C. sphaerospermum*, 1 (compound 33) in *C. valdepilosum*, 6 (compound 5, 10, 19, 36, 41, 43) in *C. flaviflorum*, 5 (compound 1, 6, 22, 40, 47) in *C. psuedofauriei*, 1 (compound 30) in *C. ramosum*, 5 (compound 7, 11, 38, 48, 49) in *C. japonicum* and 3 (compound 4, 34, 42) in *C. flagelliferum*. Neophytadiene (0.93~11.08%), Palmitic acid (0.63~3.34%), Phytol (0.97~9.45%) were detected in all the taxa experimented. There was a definite difference in components types and GC-MS profile among the taxa.

In addition, it was showed that *C. japonicum* population from Mt. Palgong contained 20 kinds of components, the most, and *C. sphaerospermum* population from Isl. Naro 6 kinds, the least. In the meanwhile, it was judged that geographical variation of *C. sphaerospermum*. *C. flaviflorum* occurred to some extent in chemical components composition of them. The chemical composition of *C. sphaerospermum* populations from Isl. Jeju and Isl. Naro was generally similar to that from the inland areas, but, compound 2, 8, 12, 18, 21, 23, 45, 56 identified from the interior population weren't identified in *C. sphaerospermum* populations from island areas and compound 3, 29, 53, 57 were specific in populations from Isl. Jeju and Isl. Naro. Collected populations of *C. sphaerospermum* were usually distributed at the margins of moist valleys. Light condition of their habitats was good because the flowering season, early spring, of them didn't overlap with the actual growing period of other plants. Above chemical composition difference might be an indication of local differnetiation between inland populations and island populations rather than different environmental conditions of their habitats. In *C. flaviflorum*, compound 13, 14, 20, 25, 26, 39 were identified in populations from Mt. Myeongji, Mt. Cheongyang and Mt. Samdobong but compound 36, 37, 53 were specific in population from Mt. Samdobong. Populations from Mt. Myeongji and Mt. Cheongyang were usually distributed at an altitude of about 100~200 m and populations from Mt. Samdobong were restricted to highland over 1,000 m. Water condition was judged to be similar in that all the populations of *C. flaviflorum* were distributed at the margins of moist valleys. Light condition of their habitats was also similar because their flowering season, early spring, didn't overlap with the actual growing period of other plants. So, it was estimated that these components differences might be caused by those of altitude of their habitats.

From this study, differences of components among taxa and populations were identified. These differences were assumed to be caused by those of geographical or inhabiting environments. So, it was suggested that additional studies on environmental characteristics, morphological variations and genetic diversity according to their natural habitats should be conducted.

Cluster analysis

For conducting cluster analysis, data matrix was made on the basis of components composition of 7 species and 18 populations in *Chrysosplenium* cluster analysis was carried out using UPGMA of data matrix drawn and phenogram was accomplished (Fig. 1.).

Table 2. The list of identified componunds

No.	Compound name	No.	Compound name
1	8-Aminoquinoline	31	Rotenalone
2	4,7,7-Trimethylbicyclo[3.3.0]octan-2-one	32	4,4,6a,8a,11,12,14b-Heptamethyl-1,2,3,4,4a,5,6,6a,7,8,8a,9,10,11,12,12a,13,14,14a,14b-eicosahydropicen-3-ol
3	5-Oxymethylfurfurole	33	Bicyclo[2.2.1]heptane, 2-chloro-2,7,7-trimethyl-, exo-
4	4-Vinylguaiacol	34	3-Eicosene, (E)-
5	Chloroquinone	35	11,14,17-Eicosatrienoic acid, methyl ester
6	Leucoglucosan	36	9-Octadecenamide, (Z)-
7	4-Methyl-2,6-di-tert-butylphenol	37	(E)-5,10-secocholest-1(10)-en-3,5-dione
8	Butylated Hydroxytoluene	38	2-Methyl-7-nonadecene
9	2-Ethylquinoline	39	1-Octadecene
10	3-Deoxy-d-mannoic lactone	40	1-Nonadecene
11	4-Propylfuro[3,2-c]pyridine	41	13-Tertadecen-1-ol acetate
12	Megastigmatrienone	42	1-Docosene
13	Tetradecanoic acid	43	1-Heptadecene
14	Neophytadiene	44	Campesterol
15	1,4-Eicosadiene	45	23S-Methylcholesterol
16	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	46	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
17	Methyl palmitate	47	Docosanoic acid, methyl ester
18	Pentadecanoic acid, 14-methyl-, methyl ester	48	α-Glyceryl linoleate
19	(E)-3-Tetradecen-5-yne	49	β-Monolinolein
20	Palmitic acid	50	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-
21	Hexadecanoic acid, trimethylsilyl ester	51	Stigmasterol, 22,23-dihydro-
22	8,11-Octadecadienoic acid, methyl ester	52	1,5-Dimethyl-6-(1,5-dimethylhexyl)-15,16-epoxy-18-oxatetracyclo[9.6.1.0(2,10).0(5,9)]octdecane-13-one
23	9,12,15-Octadecatrienoic acid, methyl ester	53	(23S)-ethylcholest-5-en-3β-ol
24	9,12-Octadecadienoic acid, methyl ester	54	γ-Sitosterol
25	Linolenic acid methyl ester	55	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
26	Phytol	56	4,9,13,17-Tetramethyl-4,8,12,16-octadecatetraenal
27	Linoleic acid	57	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene
28	Ethyl linoleolate		
29	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-		
30	4-Hexadecen-6-yne, (E)-		

Chrysosplenium in Korea comprises 5 series; *Alternifolia*, *Flagellifera, Oppositifolia*, *Sinica, Pilosa*. Ser. *Alternifolia* and Ser. *Flagellifera* have alternate leaves, and Ser. *Oppositifolia*, Ser. *Sinica* and Ser. *Pilosa* have opposite leaves (Hara, 1957; Chung and Kim, 1988). From this study, *Chrysosplenium* in Korea was divided into two groups on the basis of components analysis using GC-MS. That was agreed to system of morphological classification and view of Bohm and Collins (1979) that the arrangements of leaves were divided into alternate and opposite according to the containing degree of O-methylated flavones.

C. japonicum in Ser. *Alternifolia* and *C. flagelliferum* in Ser.

Table 3. Percentage composition of extracts identified from *Chrysosplenium* in Korea

Taxa	Compound																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
CSPH1	-	-	-	-	-	-	-	0.48	-	-	-	0.39	-	4.52	0.72	0.74	-	0.66	-	0.95	0.75
CSPH2	-	0.46	-	-	-	-	-	-	-	-	-	-	0.82	3.66	0.61	0.30	-	-	-	1.69	0.47
CSPH3	-	-	-	-	-	-	-	-	-	-	-	-	-	4.86	-	0.31	-	-	-	0.89	-
CSPH4	-	-	5.98	-	-	-	-	-	-	-	-	-	7.73	1.61	0.33	-	-	-	-	1.05	-
CSPH5	-	-	1.79	-	-	-	-	-	-	-	-	-	-	3.17	0.44	-	-	-	-	0.63	-
CVAL1	-	-	-	-	-	-	-	-	-	-	-	-	14.42	11.08	-	-	-	-	-	0.92	-
CVAL2	-	-	2.15	-	-	-	-	-	-	-	-	-	9.29	7.31	-	-	-	-	-	0.78	-
CVAL3	-	-	-	-	-	-	-	-	-	-	-	-	1.04	8.12	-	-	0.34	-	-	1.01	-
CFLV1	-	-	-	-	-	-	-	-	-	1.13	-	-	8.13	3.25	-	-	-	-	0.60	1.47	-
CFLV2	-	-	1.77	-	1.65	-	-	-	-	-	-	-	9.13	2.48	-	-	-	-	-	1.81	-
CFLV3	-	-	1.72	-	1.14	-	-	-	-	-	-	-	11.37	8.96	-	-	-	-	-	0.80	-
CPSE1	-	-	-	-	-	7.75	-	-	1.50	-	-	-	0.50	2.65	-	-	0.56	-	-	1.01	-
CPSE2	0.20	-	-	-	-	3.05	-	-	-	-	-	-	0.20	1.04	-	-	0.30	-	-	0.68	-
CRAM1	-	-	-	-	-	-	-	-	1.21	-	-	-	-	2.97	-	-	0.53	-	-	1.94	-
CJAP1	-	-	-	-	-	-	-	-	-	-	-	-	1.54	0.93	-	-	0.40	-	-	3.34	-
CJAP2	-	-	-	-	-	-	0.48	-	-	-	0.49	-	1.03	1.83	-	0.55	0.62	-	-	2.57	-
CFLG1	-	-	-	0.27	-	-	-	-	-	-	-	-	0.95	3.67	-	-	0.38	-	-	2.70	-
CFLG2	-	0.24	-	0.62	-	-	-	-	-	-	-	-	0.65	2.26	-	-	0.30	-	-	0.81	-

Table 3. Continued

Taxa	Compound																				
	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
CSPH1	-	0.28	-	-	7.69	0.75	-	-	-	-	2.57	-	-	-	-	-	-	-	-	-	-
CSPH2	-	0.93	-	-	2.36	0.65	-	-	-	-	10.08	-	-	-	-	-	-	-	-	-	-
CSPH3	-	-	-	-	1.33	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CSPH4	-	-	-	-	1.75	0.41	-	1.40	-	-	0.87	-	-	-	-	-	-	-	-	-	-
CSPH5	-	-	-	-	0.97	0.23	-	-	-	-	3.29	-	-	-	-	-	-	-	-	-	-
CVAL1	-	-	-	0.58	4.85	-	1.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CVAL2	-	-	-	0.31	4.02	-	1.09	-	-	-	8.47	-	-	-	-	-	-	-	-	-	-
CVAL3	-	-	-	0.87	8.82	-	1.94	-	-	-	0.74	0.67	-	-	-	-	-	-	-	-	-
CFLV1	-	-	-	0.33	2.03	0.34	-	2.21	-	-	-	-	-	-	-	-	-	0.33	-	1.82	-
CFLV2	-	-	-	0.37	3.16	0.27	-	2.89	-	-	-	-	-	-	-	-	-	0.54	-	-	-
CFLV3	-	-	-	0.85	8.24	-	-	-	-	-	-	-	-	-	0.72	2.20	-	0.57	-	-	-
CPSE1	0.42	-	-	1.61	3.09	0.32	2.31	-	-	-	21.02	-	-	-	-	-	-	-	-	-	-
CPSE2	-	-	0.45	1.43	2.87	0.20	-	1.20	-	21.35	33.68	-	-	-	-	-	-	-	0.93	-	-
CRAM1	-	-	2.06	2.77	9.45	-	-	-	0.39	5.54	30.30	-	-	-	-	-	-	-	-	-	-
CJAP1	-	2.42	1.20	-	1.41	1.95	0.61	8.38	-	-	2.13	-	-	-	-	-	2.42	-	-	-	-
CJAP2	-	1.39	-	-	2.40	0.78	0.81	3.05	-	-	4.56	-	-	0.19	-	-	-	5.68	-	-	-
CFLG1	-	-	0.51	1.27	3.70	0.86	-	4.79	-	-	-	-	-	0.19	-	-	-	-	-	-	0.63
CFLG2	-	-	0.54	1.51	1.98	0.34	-	1.96	-	-	24.85	-	0.43	-	-	2.11	-	-	-	-	-

Table 3. Continued

Taxa	Compound															Total (%)	Compound no.
	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57		
CSPH1	-	-	-	-	-	-	-	-	-	-	-	13.06	-	5.68	-	39.24	14
CSPH2	-	-	7.28	-	-	-	-	-	-	-	-	21.93	-	-	-	51.24	13
CSPH3	-	-	-	-	-	-	-	-	-	-	11.51	-	-	-	-	19.20	6
CSPH4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	21.13	9
CSPH5	-	-	-	-	-	-	-	-	-	-	-	4.86	-	-	1.42	16.80	9
CVAL1	-	1.87	1.37	-	-	-	-	-	-	-	-	9.02	3.68	-	-	49.26	10
CVAL2	-	3.03	-	-	-	-	-	-	-	-	-	6.63	-	-	-	43.08	10
CVAL3	-	3.51	-	-	-	-	-	-	-	9.43	-	-	3.44	-	-	39.93	12
CFLV1	-	-	-	-	-	-	-	1.57	3.26	-	-	-	-	-	-	26.47	13
CFLV2	2.43	-	-	-	-	-	-	-	5.26	-	-	-	-	-	-	31.76	12
CFLV3	-	-	-	-	-	-	-	-	-	-	9.01	-	-	-	-	45.58	11
CPSE1	-	2.70	-	-	1.17	-	-	1.85	-	-	-	12.76	-	5.81	-	67.03	17
CPSE2	-	0.36	-	-	-	-	-	1.11	-	-	-	4.20	-	0.88	-	74.13	18
CRAM1	-	0.70	-	2.55	-	-	-	2.41	6.95	-	-	-	-	-	-	69.77	14
CJAP1	-	-	-	-	-	-	2.73	7.21	-	6.51	-	-	2.70	-	-	45.88	16
CJAP2	-	-	-	2.57	-	1.86	-	3.16	-	-	-	7.03	0.80	-	-	41.85	20
CFLG1	-	-	-	-	-	-	-	4.51	4.80	-	-	11.84	1.31	-	-	42.38	16
CFLG2	-	-	-	2.68	-	-	-	3.50	-	-	-	10.60	3.29	-	-	58.67	18

Flagellifera were recombined to Group 1. *C. japonicum* and *C. flagelliferum* with altermate leaves can be classified according to the presence and absence of rossetted leaves and the number of bracteal leaves (Kim, 2007). The recent molecular phylogenetic study (Han *et al.* 2011) supported that *C. japonicum* and *C. flagelliferum* are allied species because of their close relationship.

Group 2 with opposite leaves was redivided into two subgroups. One subgroup comprised *C. psuedofauriei* in Ser. *Sinica* and *C. ramosum* in Ser. *Oppositifolia*. The number of common components in *C. psuedofauriei*, *C. ramosum* was 11 and similarity was found in chemical composition. But, compound 1, 6, 13, 22, 27, 28, 29, 40, 47, 54, 56 found in *C. psuedofauriei* weren't found in *C. ramosum* and compound 30, 46, 51 found in *C. ramosum* weren't found in *C. psuedofauriei*. These two taxa have a same important characteristic such as oppsite leaves, but have definite differences in features (spreading or erect) and colors of sepals, features of carpels (spreading or erect) and shapes of capsules. According to the recent boundary diagram using ITS analysis by Han *et al.* (2011), *C. psuedofauriei* was firstly branched from outgroup (bootstrap, 98%), and then *C. ramosum* formed close relationship with Ser. *Pilosa* (bootstrap, 95%). The study of Han *et al.* (2011) showed somewhat different results from this study. So, it was estimated that additional close studies should be needed to determine the accurate systematic relationships. The other subgroup gradationally comprised *C. flaviflorum*, *C. valdepilosum*, *C. sphaerospermum* in Ser. *Pilosa*. Type of leaf, flower, fruit, seed and frequency of hair were key characters in the morphological taxonomy in Ser. *Pilosa* (Hara, 1957; Chung and Kim, 1988). According to features of seed surface ridges, *C. flaviflorum* didn't have ridges, *C. valdepilosum* had them slightly and *C. sphaerospermum* had them definitely (Han *et al*., 2010). According to the colors of bracteal leaves, *C. valdepilosum* was bright yellow, *C. sphaerospermum* was greenish or greenish yellow (Kim, 2007). Han *et al*. (2010) reported that *C. flaviflorum* was definitely identified from other taxa in that it had subhypogaeus stolon. According to the phylogenetic study using nrDNA of ITS sequence (Han *et al*., 2011), taxa of Ser. *Pilosa* formed close relationships.

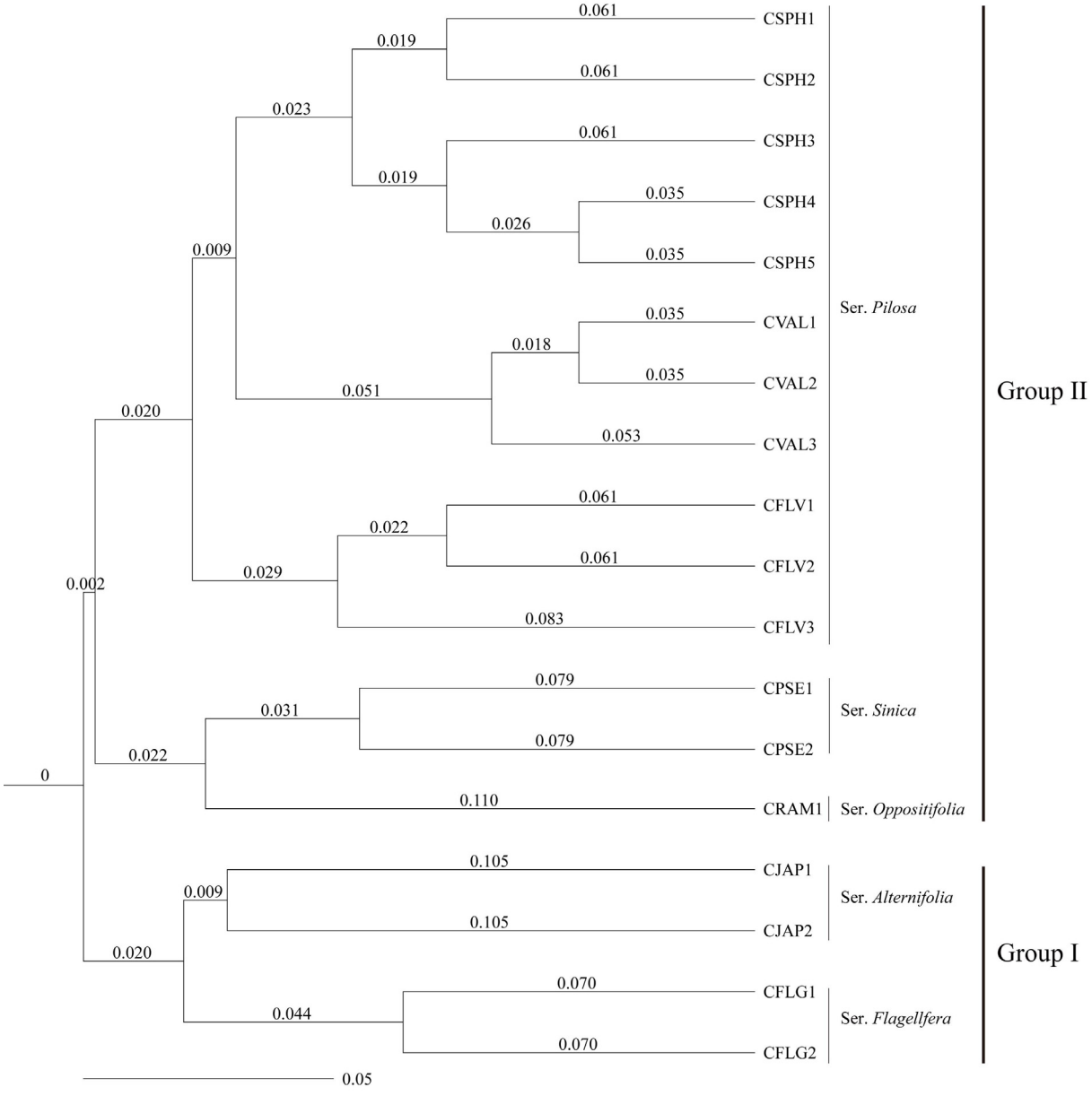

Fig. 1. UPGMA tree of the genus *Chrysosplenium*.

According to the results of this study, Ser. *Pilosa* was divided into 3 taxa; compound 3, 13, 14, 20, 26 were found commonly, but compound 5, 10, 19, 36, 41, 43 were restricted to *C. flaviflorum*, compound 33 was restricted to *C. valdepilosum* and compound 8, 12, 15, 18, 21, 57 were restricted to *C. sphaerospermum*. So, these findings could be an evidence to support those of existing morphological and molecular studies.

The results of this study were generally agreed to taxonomy according to morphological characteristics and systematics using nrDNA of ITS sequence. In conclusion, chemical composition using GC-MS analysis of 7 species and 18 populations of *Chrysosplenium* in Korea can be useful characters in understanding the relationship among interspecific and intraspecific complex. More comprehensive taxonomic studies introducing various chemotypes should be conducted and, consequently, these studies can make it possible to identify *Chrysosplenium* more accurately.

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Literature Cited

Bohm, B.A. and F.W. Collins. 1979. Flavonoid of some species of *Chrysosplenium*. Biochem. Syst. Ecol. 7:195-201.

Chung, T.H. 1965. Illustrated Encyclopedia of Fauna & Flora of Korea. Tracheophyta. Genus *Chrysosplenium*. Ministry of Education, Seoul, Korea. 5:497-483.

Chung, T.H., B.S. To, D.B. Lee and H.J. Lee. 1937. Genus *Chrysosplenium. In* Nomina Plantarum Koreanum. Chosen Nat. Hist. Inst., Seoul, Korea. pp. 80-81.

Chung, Y.H. and Y.D. Kim, 1988. Monographic Study of Endemic Plants in Korea Ⅹ. Taxonomy and Interspecific Relationship of the Genus *Chrysosplenium*. Korean J. Environ. Biol. 6(2):33-63.

Cudej, J. and P. Czapski. 2009. Components of the petroleum ether and chloroform extracts of *Chrysosplenium alternifolium.* Chem. Nat. Comp. 45(5):717-719.

Forbes, F.B. and W.B. Hemsley. 1887. An enumeration of all the plants known from China proper, Formosa, Heinan, Corea, the Luchu Archipelago and Island of Hong Kong. J. Linn. Sco. Bot. 23:271.

Franchet, A.R. 1890. Monographie du genere *Chrysosplenium* Tournfort. Nouv. Arch. Mus. Hist. Nat. Paris Ⅲ. 2:87-114.

Han, J.W., S.G. Yang, H.J. Kim, C.G. Jang and S.H. Kang. 2010. Morphological chaeacteristics and distribution of Korean endemic *Chrysosplenium flaviflorum* Ohwi. Korean J. Plant Res. 23(5):480-485 (in Korean).

Han, J.W., S.G. Yang, H.J. Kim, C.G. Jang, J.M. Park and S.H. Kang. 2011. Phylogenetic study of Korean *Chrysosplenium* based on nrDNA ITS sequences. Korean J. Plant Res. 24(4):358-369 (in Korean).

Hara, H. 1957. Synopsis of Genus *Chrysosplenium* L. Jour. Fac. Sci. Univ. Tokyo, Bot. 7:1-90.

Iwatsuki, K., D. Boufford, H. Ohba. 2001. *Chrysosplenium* L. *In* Flora of Japan, Vol. Ⅱb. Kodansha LTD., Tokyo, Japan. pp. 58-70.

Kim, Y.D. 2007. Saxifragaceae. *In* Flora of Korea Editorial Committee (eds.), The Genera of Vascular Plants of Korea, Academy Publishing Co., Seoul, Korea.

Lee, T.B. 1976. Vascular plants and their uses in Korea. Bull. Kwanak Arb. 1:1-137.

Lee, W.T. 1996a. Standard Illustration of Korean Plants. Academy Press, Seoul, Korea.

Lee, Y.N. 1996b. Flora of Korea. Kyohak Publishing Co, Seoul, Korea.

Linneus, C. 1753. Species Plantarum. p. 398.

Maximowicz, C.J. 1877 Diagnosis Plantarum Novarum Asiaticarum Scripsit. Bull. Acad. Sci. St. Petersb. 23:340-350.

Nakai, T. 1909. Flora Koreana. J. Coll. Sci. Univ. Tokyo, Japan. 26:220.

Palibin, J. 1898. Conspectus florae Koreae. Pars Prima. Acta. Horti. Petropol. p. 90.

Pan, J. 2001. *Chrysosplenium* L. *In* Wu, Z.Y. and P.H. Raven (eds.), Flora of China, Science Press and Missouri Botanical Garden Press, Beijing and St. Louis. 8:346-358.

Park, M.K. 1949. Genus *Chrysosplenium*. *In* An enumeration of Korean plants. Ministry of Education, Seoul, Korea. pp. 83-85.

Park, M.K. 1974. Genus *Chrysosplenium*. *In* Keys to the Herbaceous plants in Korea (Dicotyledoneae). Chungeumsa, Seoul, Korea. pp. 208-210.

Sponberg, G.L. 1972. The genera of Saxifragaceae in the South- eastern United States. Jour. Arnold Arb. 53:409-494.

Yang, Y.S., G.F. Shi, R.H. LU. 2004. Study on the volatile constituents of Tibetan medicine *Chrysosplenium nudicaule*. Chin. NPRD. 16:38-40.

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