Pattern Recognition of the Herbal Drug, *Magnoliae Flos* According to their Essential Oil Components

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This paper describes a pattern recognition method of *Magnoliae flos* based on a gas chromatographic/mass spectrometric (GC/MS) analysis of the essential oil components. The botanical drug is mainly comprised of the four magnolia species (*M. denudata, M. biondii, M. kobus*, and *M. liliflora*) in Korea, although some other species are also being dealt with the drug. The GC/MS separation of the volatile components, which was extracted by the simultaneous distillation and extraction (SDE), was performed on a carbowax column (supelcowax 10: 30 m × 0.25 mm × 0.25 μm) using temperature programming. Variance in the retention times for all peaks of interests was within RSD 2% for repeated analyses (n = 9). Of the 74 essential oil components identified from the magnolia species, approximately 10 major components, which is α-pinene, β-pinene, sabinene, myrcene, *d*-limonene, eucarlyptol (1,8-cineol), γ-terpinene, *p*-cymene, linalool, α-terpineol, were commonly present in the four species. For statistical analysis, the original dataset was reduced to the 13 variables by Fisher criterion and factor analysis (FA). The essential oil patterns were processed by means of the multivariate statistical analysis including hierarchical cluster analysis (HCA), principal component analysis (PCA) and discriminant analysis (DA). All samples were divided into four groups with three principal components by PCA and according to the plant origins by HCA. Thirty-three samples (23 training sets and 10 test samples to be assessed) were correctly classified into the four groups predicted by PCA. This method would provide a practical strategy for assessing the authenticity or quality of the well-known herbal drug, *Magnoliae flos*.

Key Words: *Magnoliae flos, M. biondii, M. denudata*, GC/MS, Multivariate statistical analysis

Introduction

Magnoliae flos (*M. flos*: the dried flower buds of *Magnolia denudata* or related species) is a botanical drug officially listed in the Pharmacopoeia of Asian countries. The drug name is called as Shin-Yi in Korea and Japan, Xin-Yi in China. The herbal drug has been used for managing nasal conjestion with headache, sinusitis and allergic rhinitis.[1,2] It has also a wide range of pharmacological effects including antirheumatic,[3] antiangiogenic,[4] antiallergic,[5-8] antiinflammatory,[9-10] and anitmicrobial activities.[11]

Essential oil components of mono- and sesquiterpenes, and many lignans are the pharmacologically active ingredients of mognolia drugs.[12-15] As major volatile components, bornyl acetate, eucarlyptol (1, 8-cineol), α-pinene, and eudesmol showed anti-inflammatory effects. Other components, such as camphor, cymene, linalool, limonene, myrcene, α-pinene, β-pinene, terpinene, nerolidol and citral may contribute to the antimicrobial actions of *M. flos*.

The four species, *M. biondii*, *M. denudata*, *M. kobus*, and *M. liliflora*, are the well-known herbs in Korea, but others, such as *M. sprengeri* and *M. sargentiana* in China and *M. salicifolia* in Japan, are also treated as *M. flos* or substitutes in the respective countries. Therefore, they could be misused especially when these are traded among the countries. Because the remedy and prescription should be different depending on plant species, accurate identification of the species origin is essential to assure the quality of drug in clinical applications. No reports are available for the chemical discrimination of *M. flos* as the herbal drug to date.

We established a GC/MS pattern recognition method based on the volatile components extracted using a simultaneous distillation and extraction (SDE), which is a popular method in analysis of essential oils.[16,17] The classification model for four different *Magnoliae flos* were successfully established by the multivariate statistical analysis *i.e.*, hierarchical cluster analysis (HCA), principal component analysis (PCA) and discriminant analysis (DA).

Experimental Section

Plant materials. Twenty specimens of *M. flos* (3 *M. biondii*, 8 *M. denudata*, 3 *M. kobus*, 3 *M. liliflora*, *M. denudata* var. *purpurascens*, *M. liliflora* var. *gracilis* and *M. salicifolia*) were collected from Korea and China during March to April 2008. The 20 reference specimens identified were used. The 9 drugs (U-1 ~ U-9) were purchased from oriental herbal stores in Korea, and 7 samples (U-10 ~ U-16) were obtained from Daegu Catholic University, and dried under air prior to analysis. (Table 1)

Chemicals. All standard of essential oils were provided by Seoul Perfumery Co. LTD (Seoul, Korea). The HPLC-grade diethyl ether was purchased from J. T. Baker Co. (Phillipsburg, USA) and distillated. All other solvents employed were of

Table 1. *Magnoliae flos* (Shin-Yi) samples

Label	Magnoliae flos	Source
B-1	*M. biondii*	Henan, China
B-2	*M. biondii*	Daegu, Korea
B-3	*M. biondii*	Yangsan, Korea
D-1	*M. denudata*	Yangsan, Korea
D-2	*M. denudata*	Gyungju, Korea
D-3	*M. denudata*	Youngcheon, Korea
D-4	*M. denudata*	Cheongju, Korea
D-5	*M. denudata*	Cheongju, Korea
D-6	*M. denudata*	Cheongju, Korea
D-7	*M. denudata*	Daegu, Korea
D-8	*M. denudata*	Gyungju, Korea
K-1	*M. kobus*	Seoul, Korea
K-2	*M. kobus*	Cheongju, Korea
K-3	*M. kobus*	Cheongju, Korea
L-1	*M. liliflora*	Cheongju, Korea
L-2	*M. liliflora*	Daegu, Korea
L-3	*M. liliflora*	Daegu, Korea
P	*M. denudata var. purpurascens*	Youngcheon, Korea
S	*M. liliflora var. gracilis*	Eumseong, Korea
G	*M. salicifolia*	Daejeon, Korea
U-1	herbal drug	Seoul, Korea
U-2	herbal drug	Daegu, Korea
U-3	herbal drug	Seoul, Korea
U-4	herbal drug	Seoul, Korea
U-5	herbal drug	Seoul, Korea
U-6	herbal drug	Seoul, Korea
U-7	herbal drug	Youngcheon, Korea
U-8	herbal drug	Youngcheon, Korea
U-9	herbal drug	Youngcheon, Korea
U-10	plant sample	Daegu, Korea
U-11	plant sample	Daegu, Korea
U-12	plant sample	Daegu, Korea
U-13	plant sample	Daegu, Korea
U-14	plant sample	Daegu, Korea
U-15	plant sample	Daegu, Korea
U-16	plant sample	Daegu, Korea

analytical grade quality and were redistilled before use. Anhydrous sodium sulfate was purchased from Samchun Chemical (Pyeongtak, Korea).

Sample preparation. The volatile components from *M. flos* (2 g sample plus 100 mL of distilled water) were extracted into 40 mL diethyl ether for 2 hr using a Likens-Nickerson's type SDE apparatus (Kontes, Vineland, NJ, USA). After cooling the extracts to ambient temperature (30 min), the solvent phase was collected and dried over anhydrous sodium sulfate in a refrigerator for one day to remove residual water. The extract was carefully concentrated to about 2 mL at 40 °C using a rotary evaporator at atmospheric pressure, and then finally concentrated to 1.0 mL under gentle nitrogen flow.

GC/MS analysis. GC/MS analyses were performed with an Agilent 7890 series GC system coupled to an Agilent 5975B inert MSD (Agilent, CA, USA). A supelcowax 10 column (30 m × 0.25 mm × 0.25 μm film thickness, Bellefonte, USA) was used for the GC separation. One micro-liter aliquots of each sample extracts was injected into the GC column with split (80:1). The GC oven temperature was initially set at 70 °C (hold 5 min) and ramped to 240 °C (hold 20 min) at 3 °C/min. Helium was used as a carrier gas at 1.0 mL/min. The injector temperature was set at 250 °C and the temperature of the ion source and the interface were 230 °C and 280 °C, respectively. Ionization energy was set to 70 eV and the mass range in scanning mode was m/z 35 - 400. Inherent peaks were identified using the Mass spectral search program (Wiley library 8N05ST) and/or the library, "Identification of Essential oil Components by GC/MS, 4th Edition (Allured, 2007)" combined with home-made data base.

Temperature-programmed retention indices (TPRIs), which seem to be more useful in the practice of essential oils analysis, are varied in different chromatographic operating conditions such as carrier flow-rate and temperature program. The following quasi-linear equation proposed by van den Dool and Kratz[18] was used to calibrate and build a TPRI database of natural volatile components.

Method validation. To check the reproducibility, three QC samples (D-7, U-4 and U-8) were analyzed repeatedly three times per sample on three separate days (n = 9). Variance in the retention times for all peaks of interests was within RSD 2%. The variance in percent peak area was less than RSD 30% for high-intensity peaks but was slightly higher than RSD 50% for low-intensity peaks, which might be unavoidable for analysis of crude plant materials after SDE extraction on separate days.

Statistical analysis. Thirteen peaks according to Fisher criterion were selected as components for data analysis. Multivariate statistical analysis, HCA, PCA and DA, were performed using the statistical package, SPSS (version 12.0, SPSS Inc., Chicago, USA). The HCA was performed by Ward's method using squared Euclidian distance as a measure of similarity. For PCA analysis, the eigenvalues of > 1.0 obtained by Kaiser and the cumulative proportion of eigenvalues of ≥ 80% were considered sufficiently conspicuous for interpretation. The DA was performed to develop a classification model from the model subsequently validated.

Results and Discussion

Volatile components from *Magnoliae flos*. Figure 1 shows GC/MS chromatograms of the four *M. flos* (*M. biondii*, *M. denudata*, *M. kobus* and *M. liliflora*). The chromatographic profiles were obviously different from each other's chromatogram depending on the plant origins. Although we identified total 157 inherent peaks from the 36 *M. flos* samples tested, the 83 components were included of trace (their relative intensity ≤ 0.05%) in *M. flos* samples or were detected in some few of the same species. Therefore, we selected only the 74 components that comprise more than 0.05% or have characteristics for the species. Table 2 shows the 74 components listed according to their elution order on a carbowax column

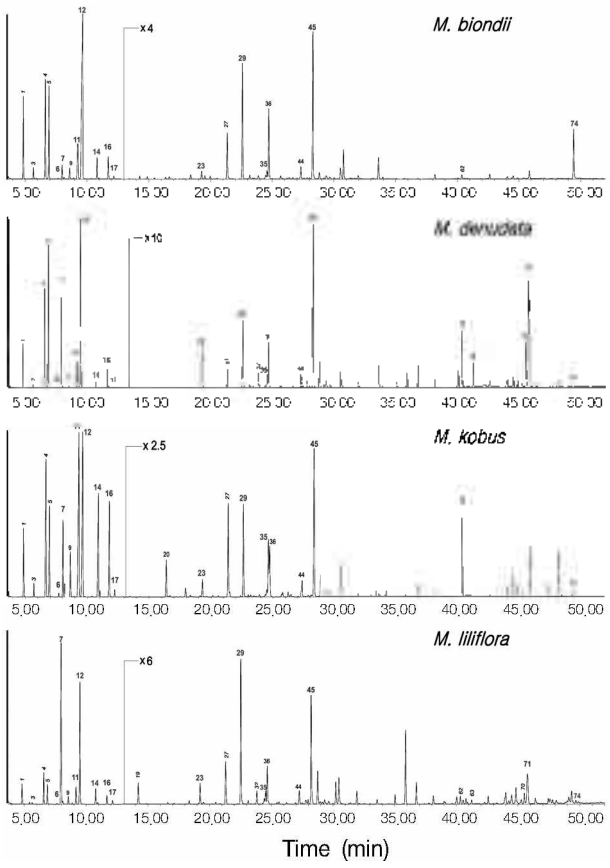

Figure 1. Representative GC/MS chromatograms of *Magnoliae flos* species.

with their relative peak areas of the total oil components. The main components comprising the mono-, sesquiterpenes and their oxides showed the content in the range of 80.4 ~ 85.4% for *M. biondii*, 79.0 ~ 91.9% for *M. denudata*, 78.9 ~ 90.0% for *M. kobus*, and 82.3 ~ 87.8% for *M. liliflora*. Our components study showed the similar result with the previous report[19] on the essential oil components from the three kinds of magnolia species (*M. biondii, M. denudata and M. sprengeri*) that the main components were found to be eucarlyptol (1, 8-cineole), sabinene, β-pinene, α-pinene, trans-caryophyllene.

In the present study, some components were present with remarkably large content from one or two species, *i.e.*, farnesol (**74**) in *M. biondii*; terpinen-4-ol (**36**) in *M. denudata*; β -caryophyllene (**35**), δ-cadinene (**52**), 1,6-germacradien-5-ol (**62**) in *M. kobus*; camphor (**27**), t-muurolol (**72**) in both *M. biondii* and *M. kobus*; terpinen-4-ol (**36**) and β-eudesmol (**71**) in both *M. denudata and M. liliflora*. It was noticeable that a few components represent the plant specificities irrelevant to their content: citronellal (**25**), geranyl acetate (**59**) and methyl isoeugenol (**67**) were detected only in *M. biondii*; α-eudesmol (**70**) and β-eudesmol (**71**) in both *M. denudata* and *M. liliflora*; 2-nonanone (**20**) in both *M. biondii* and *M. kobus*.

Principal component analysis (PCA). Of the 74 components represented in Table 2, some components showed large difference of the content within the same species because of the place of origin, harvest time, dryness condition, *etc.* Therefore, the components for statistical analysis were selected

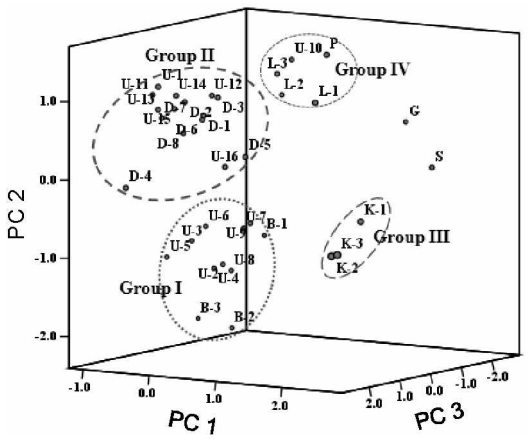

Figure 2. Score plot by principal component analysis (PCA) of 36 magnolia samples.

by Fisher method,[20] a coefficient based on the between- and within-group variations. The higher the value of Fisher coefficient is the better variable. The 13 components, which are equal to the number of the principal factors, were determined by the PCA of the 74 components. The Fisher coefficients for the selected 13 components were as follows: 139.8 (myrcene); 96.6 (*d*-limonene); 39.7 (β-eudesmol); 39.4 (δ-3-carene); 37.1 (β-caryophyllene); 24.6 (terpinolene); 19.5 (γ-terpinene); 16.7 (*p*-cymene); 11.6 (eucalyptol); 11.3 (farnesol); 9.7 (transsabinene hydrate); 3.3 (linalool); 2.6 (α-terpineol). Prior to the PCA analysis, the suitability of the data for factor analysis was checked. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.61 exceeding the recommended value (0.6)[21], that means the matrix is appropriate for PCA. The four principal components with eigenvalues exceeding one were extracted according to the Kaiser criterion, which explains up to 81.7% of the total variance. The 1st - 4th principal components were responsible for 39.8%, 19.1%, 13.5% and 9.4% of the entire information, respectively. The first factor was mainly influenced by the components with the factor loadings > 0.5 were δ-3-carene, *d*-limonene, eucalyptol, γ-terpinene, *p*-cymene, terpinolene, trans-sabinene hydrate, β-caryophyllene, α-terpineol. The second factor was closely related to myrcene, β-eudesmol, farnesol. Likewise, the third factor is related to terpinolene, β-caryophyllene, and the fourth factor is related to linalool.

The score plot of the first three principal components (Figure 2) showed the clear differentiation of the species. From the scatter points, the samples could be classified into four groups, which were marked as group I-IV according to the species: *M. biondii* (Group I); *M. denudata* (Group II); *M. kobus* (Group III) and *M. liliflora* (Group IV). *M. denudata* var. *purpurascens* (P) was clustered into group IV, while *M. salicifolia* (S) and *M. liliflora* var. *gracilis* (G) were not clustered into any of the four groups. From the score values on the principal components for each species, it can be interpreted that the contents of δ-3-carene (**6**), *d*-limonene (**11**), γ-terpinene (**14**), *p*-cymene (**16**), β-caryophyllene (**35**) on the first PC loadings are higher for *M. kobus* than the other species, while eucalyptol (**12**), trans-sabinene hydrate (**23**), α-terpineol

Table 2. Chemical composition of the essential oils from *Magnoliae flos* specimens (min-max, %)

Peak[a]	RT (min)	RI[b]	Class[c]	Components	Molecular formulae	Magnoliae flos (%)			
						M. biondii (B-1~B-3)	*M. denudata* (D-1~D-8)	*M. kobus* (K-1~K-3)	*M. liliflora* (L-1~L-3)
1	4.85	1026	1	α-Pinene	C10H16	2.87 - 5.37	1.92 - 6.61	3.42 - 4.55	2.43 - 6.32
2	5.53	1063	1	α-Fenchene	C10H16	0.02 - 0.02	0.00 - 0.03	0.01 - 0.02	0.00 - 0.05
3	5.69	1072	1	Camphene	C10H16	0.38 - 6.58	0.14 - 0.55	0.22 - 2.72	0.21 - 0.52
4	6.62	1115	1	β-Pinene	C10H16	3.80 - 9.36	5.78 - 15.42	8.64 - 9.95	3.73 - 12.56
5	6.92	1125	1	Sabinene	C10H16	5.23 - 7.89	4.45 - 16.63	3.23 - 5.87	2.65 - 11.31
6	7.73	1153	1	**δ-3-Carene**	C10H16	0.02 - 0.06	0.04 - 0.11	0.15 - 0.31	0.08 - 0.23
7	8.02	1163	1	**Myrcene**	C10H16	0.78 - 1.30	4.08 - 10.83	3.73 - 5.05	11.11 - 28.87
8	8.22	1170	1	α-Phellandrene	C10H16	0.09 - 0.20	0.10 - 0.24	0.78 - 1.56	0.25 - 0.55
9	8.62	1184	1	α-Terpinene	C10H16	0.37 - 1.26	0.57 - 1.02	1.23 - 3.66	1.07 - 1.35
10	8.92	1194	2	2,3-Dehydro-1,8-cineole	C10H16O	0.04 - 0.09	0.00 - 0.21	0.00 - 0.04	0.00 - 0.22
11	9.24	1205	1	**d-Limonene**	C10H16	3.53 - 6.60	1.98 - 4.79	15.63 - 18.81	2.83 - 6.18
12	9.56	1213	2	**Eucalyptol (1,8-cineol)**	C10H16O	16.73 - 34.93	18.54 - 29.71	7.49 - 15.44	11.86 - 23.46
13	10.41	1236	1	cis-ocimene	C10H16	0.04 - 0.06	0.00 - 0.02	0.06 - 0.10	0.02 - 0.03
14	10.84	1248	1	**γ-Terpinene**	C10H16	0.85 - 2.91	0.75 - 2.23	2.65 - 9.14	1.82 - 3.00
15	11.03	1254	1	trans-β-ocimene	C10H16	0.05 - 0.16	0.03 - 0.16	0.31 - 0.47	0.13 - 0.43
16	11.76	1273	1	**p-Cymene**	C10H14	1.02 - 2.17	0.90 - 4.69	4.72 - 7.15	1.51 - 3.23
17	12.21	1286	1	**Terpinolene**	C10H16	0.29 - 0.64	0.23 - 0.47	0.49 - 0.63	0.35 - 0.78
18	13.43	1318	5	2-Heptanol	C7H16O			0.01 - 0.02	
19	14.29	1339	4	6-Methyl-5-heptene-2-one	C8H14O	0.03 - 0.05	0.00 - 0.03	0.01 - 0.01	0.01 - 0.67
20	16.40	1392	4	2-Nonanone	C9H18O	0.05 - 0.08		0.46 - 0.88	
21	16.69	1399	1	α-Fenchone	C10H16O	0.07 - 0.30	0.02 - 0.04	0.01 - 0.14	0.01 - 0.06
22	18.43	1442	2	Linalool oxide	C10H18O2	0.04 - 0.07	0.02 - 0.22	0.00 - 0.04	0.04 - 0.12
23	19.32	1464	1	**trans-Sabinene hydrate**	C10H18O	0.47 - 0.80	0.74 - 1.64	0.43 - 0.44	0.32 - 0.91
24	19.57	1470	2	Linalool oxide	C10H18O2	0.04 - 0.05	0.01 - 0.15	0.00 - 0.02	0.03 - 0.08
25	20.01	1481	2	Citronellal	C10H18O	0.01 0.26			
26	20.59	1495	3	α-Copaene	C15H24	0.02 - 0.03	0.01 - 0.08	0.03 - 0.05	0.03 - 0.09
27	21.40	1516	2	Camphor	C10H16O	0.45 - 25.43	0.48 - 1.73	0.25 - 9.33	0.14 - 2.09
28	22.39	1541	3	β-Cubebene	C15H24	0.01 - 0.02	0.01 - 0.06	0.02 - 0.04	0.00 - 0.04
29	22.63	1547	1	**Linalool**	C10H18O	3.71 - 4.80	1.52 - 4.06	1.98 - 3.23	1.13 - 5.07
30	23.22	1562	1	**cis-p-2-menthen-1-ol**	C10H18O	0.06 - 0.13	0.10 - 0.43	0.04 - 0.05	0.10 - 0.11
31	23.45	1568	1	Pinocarvone	C10H14O		0.00 - 0.11	0.02 - 0.03	0.00 - 0.05
32	23.93	1580	1	Bornyl acetate	C12H20O2	0.06 - 1.09	0.00 - 0.65	0.06 - 1.01	0.32 - 0.50
33	24.42	1592	3	β-Elemene	C15H24	0.02 - 1.13	0.02 - 0.27	0.03 - 0.10	0.01 - 0.10
34	24.52	1595	5	exo-methyl camphenilol		0.00 - 2.42	0.05 - 0.19	0.03 - 1.07	0.00 - 0.22
35	24.68	1599	3	**β-Caryophyllene**	C15H24	0.24 - 0.49	0.14 - 1.11	1.56 - 1.74	0.27 - 0.70
36	24.77	1601	1	**Terpinen-4-ol**	C10H18O	0.66 - 1.99	1.30 - 6.62	0.36 - 1.12	1.12 - 1.75
37	24.85	1603	2	Carvacryl methyl ether	C11H16O		0.03 - 0.08		0.03 - 0.10
38	25.51	1620	1	β-cyclocitral	C10H16O		0.01 - 0.03	0.00 - 0.12	0.00 - 0.07
39	25.71	1626	1	1-Terpineol	C10H18O	0.04 - 0.09	0.00 - 0.33	0.00 - 0.08	0.00 - 0.08
40	25.77	1627	1	Myrtenal	C10H14O		0.00 - 0.06		0.00 - 0.02
41	25.90	1631	1	Sabina ketone	C9H14O		0.00 - 0.03		0.00 - 0.00
42	26.41	1644	6	3-oxocineole	C12H20O2		0.00 - 0.21		0.00 - 0.05
43	26.71	1652	1	**trans-Pinocarveol**	C10H16O		0.04 - 0.13	0.00 - 0.02	0.00 - 0.04
44	27.37	1669	3	**α-Humulene**	C15H24	0.27 - 0.47	0.37 - 1.11	0.36 - 0.68	0.42 - 0.52
45	28.37	1696	1	**α-Terpineol**	C10H18O	4.13 - 7.37	3.43 - 10.88	2.47 - 4.61	3.33 - 5.77
46	28.43	1697	1	**l-Borneol**	C10H18O	0.03 - 0.14	0.00 - 0.07	0.00 - 0.08	0.03 - 0.14
47	28.93	1711	3	Germacrene d	C15H24	0.32 - 0.57	0.38 - 2.42	0.57 - 0.68	0.63 - 1.17
48	29.24	1720	2	exo-2-hydroxycineole	C10H18O2		0.00 - 0.19		0.00 - 0.41
49	29.41	1724	3	α-Muurolene	C15H24	0.10 - 0.18	0.17 - 0.70	0.21 - 0.27	0.00 - 0.17
50	29.74	1733	3	Bicyclogermacrene	C15H24	0.00 - 0.26	0.00 - 0.44	0.00 - 0.21	0.00 - 0.18
51	30.34	1750	3	α-Farnesene	C15H24	0.00 0.02	0.00 0.55		0.35 0.76
52	30.65	1759	3	δ-Cadinene	C15H24	0.58 - 0.85	0.05 - 1.25	1.06 - 1.48	0.25 - 0.92
53	30.82	1764	1	Citronellol	C10H20O	0.39 - 1.79	0.00 - 0.09		0.00 - 0.04
54	31.06	1770	3	β-Sesquiphellandrene	C15H24		0.00 0.03		
55	31.72	1789	1	Myrtenol	C10H16O	0.00 - 0.01	0.02 - 0.11	0.00 - 0.02	0.00 - 0.03
56	32.02	1797	1	Nerol	C10H18O	0.10 - 0.21	0.16 - 0.49	0.08 - 0.09	0.18 - 0.49
57	33.23	1830	1	trans-Carveol	C10H16O		0.00 - 0.04	0.00 - 0.03	0.00 - 0.01
58	33.56	1840	1	Geraniol	C10H18O		0.00 1.04		0.00 0.19
59	34.01	1853	1	Geranyl acetate	C12H20O2	0.00 0.06			
60	39.18	2007	2	Methyl eugenol	C11H14O2		0.01 - 0.10		0.00 - 0.22
61	40.13	2036	3	trans-Nerolidol	C15H26O	0.00 - 0.02	0.16 - 0.69	0.05 - 0.10	0.11 - 0.26
62	40.44	2046	3	1,6-Germacradien-5-ol	C15H26O	0.87 - 3.86	0.37 - 1.76	2.34 - 3.38	0.27 - 0.40
63	41.33	2074	3	Elemol	C15H26O		0.17 - 0.85	0.00 - 0.02	0.00 - 0.20
64	42.68	2117	3	Spathulenol	C15H24O	0.10 - 0.15	0.06 - 0.22	0.04 - 0.16	0.12 - 0.27
65	44.02	2161	1	Thymol	C10H14O	0.04 - 0.36	0.00 - 0.09	0.08 - 0.84	0.00 - 0.20
66	44.03	2162	3	γ-Eudesmol	C15H26O		0.06 - 0.28		0.00 - 0.45
67	44.44	2175	7	Methyl isoeugenol	C11H14O2	0.07 - 0.27			
68	44.59	2180	3	α-Cadinol	C15H26O	0.36 - 0.47	0.07 - 0.44	0.17 - 1.12	0.00 - 0.41
69	45.28	2203	3	Bulnesol	C15H26O		0.04 - 0.15		0.00 - 0.06
70	45.60	2213	3	α-Eudesmol	C15H26O		0.31 - 1.48		0.31 - 0.61
71	45.84	2222	3	**β-Eudesmol**	C15H26O		0.82 - 4.44		1.24 - 1.73
72	45.93	2224	3	t-Muurolol	C15H26O	1.04 - 1.50	0.00 - 0.14	1.36 - 3.20	
73	49.20	2337	2	Isoeugenol	C10H12O2		0.00 - 0.14		0.03 - 0.29
74	49.54	2349	3	**Farnesol**	C15H26O	6.91 - 9.59	0.00 - 0.20	0.00 - 0.51	0.00 - 0.05

a) The bold-character number denote the peaks slected for statistical analysis. b) Retention index definded as shown in experim ental selection. c) Chemical class: 1. Monoterpene hydrocarbons; 2. Monoterpene oxiders; 3. Sesquiterpene hydrocarbons; 4. Ketones; 5. Alcohols; 6. Esters; 7. Ethers

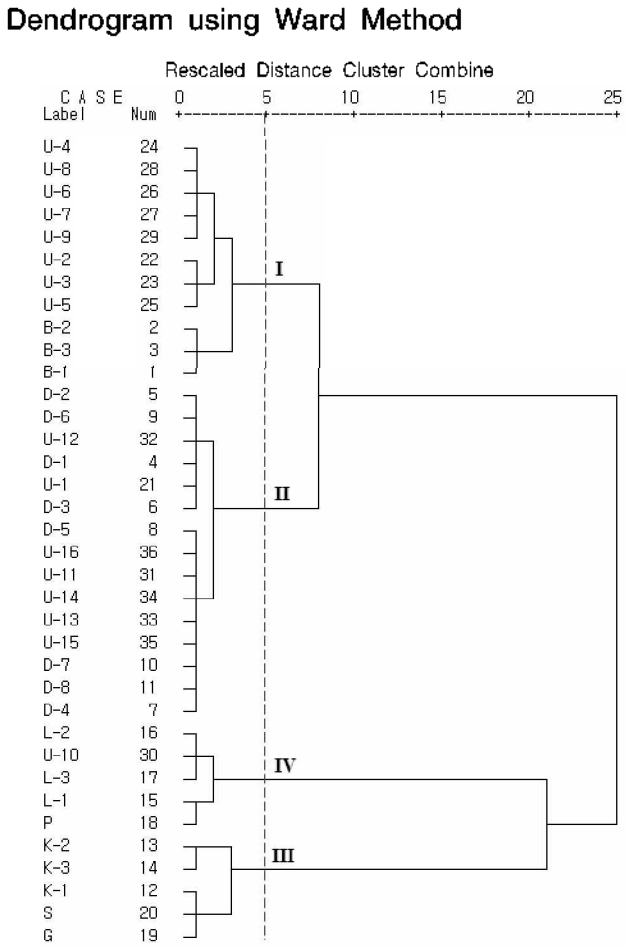

Figure 3. A Dendrogram of hierarchical cluster analysis (HCA) of 36 *Magnoliae flos* samples.

(**45**) are lower. From the second PC loadings, myrcene (**7**) showed the highest contents for *M. liliflora* among the four species, and the content of farnesol (**74**) is highest for *M. biondii*. From the third PC loadings, terpinelone (**17**) showed the higher content both for *M. salicifolia* (S) and *M. liliflora* var. *gracilis* (G) than the other species. From the fourth PC loading, linalool (**29**) showed higher content for *M. biondii* than the other species.

Hierarchical cluster analysis (HCA). The dataset for HCA was consisted of the 13 selected components and relative peak area for a total of the 36 magnolia samples. Dendrogram obtained from HCA was shown in Figure 3. The 36 samples were grouped into the predicted four clusters (I-IV): *M. biondii* (group I); *M. denudata* (group II); *M. kobus* (group III) and *M. liliflora* (group IV). The three species with each one specimen. *M. saliciflora* (S) and *M. liliflora* var. *gracillis* (G) were grouped into group III, while *M. denudata* var. *purpurascens* (P) was classified into to the group IV.

Discriminant analysis (DA). DA was performed to develop a discrimination model of the 4 groups classified by the PCA using the relative peak area of the 13 selected components as input data. The four groups of the 33 samples determined by PCA are in the predicted groups. The three specimens (only one sample per species), *M. denudata* var. *purpurascens* (P),

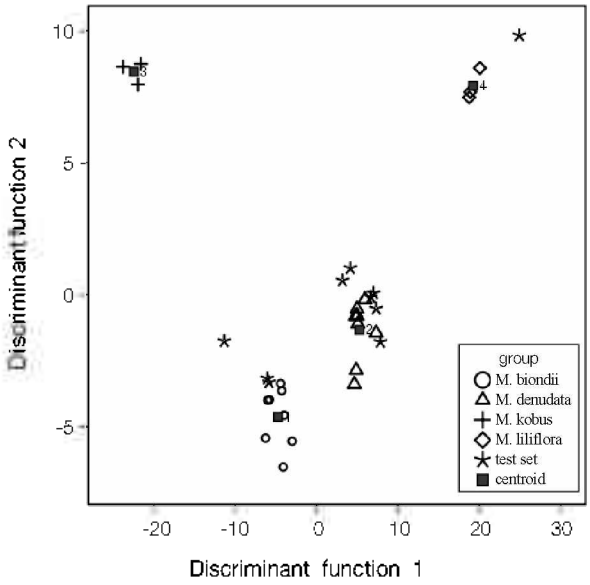

Figure 4. Discriminant analysis (DA) plots of the *Magnoliae flos* samples on the space defined by the first two discriminant functions.

M. salicifolia (S) and *M. liliflora* var. *gracilis* (G), were not included in DA. Feature selection was performed by stepwise DA using a Wilk's Lambda selection criterion. The 8 featured components, δ-3-carene (**6**), myrcene (**7**), *d*-limonen (**11**), eucalyptol (**12**), γ-terpinene (**14**), terpinolene (**17**), β-eudesmol (**71**), farnesol (**74**), were selected as the most important variables for differentiating the 4 groups of samples. All samples in the predicted groups by PCA were correctly classified (100%). To determine the predictive ability of the resulting model, 23 samples (training set consisting of 8 *M. biondii*, 9 *M. denudata*, 3 *M. kobus*, 3 *M. liliflora*) were selected at random to construct a DA model that could then be used to predict the group of remaining 10 samples (U-7 ~ U-16, test set). A 100% correct classification was also obtained when the validation procedure was used. Figure 4 shows the 33 samples on the plane defined by the two discriminant functions obtained, and test set was represented as asterisks. The 10 test samples were classified in *M. biondii* (U-7 ~ U-9), *M. denudata* (U-11 ~ U-16), and *M. liliflora* (U-10). The assignment of the 10 samples of test set permits to estimate the good possibilities of our procedure.

Conclusion

A GC/MS pattern recognition method based on the data of essential oil components successfully characterized the herbal drugs according to the four classes of plant origins. The method was able to facilitate discrimination of the fingerprint patterns from different *M. flos* samples. The 33 samples were classified into 4 groups by PCA and all group members determined by PCA were in the predicted group that 100% of all samples correctly classified by DA. This fingerprint pattern recognition would provide a practical strategy for assessing the authenticity or quality of the well-known herbal drug, *M. flos*.

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