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Identification and quantification of 13 components in *Angelica sinensis* (Danggui) by gas chromatography–mass spectrometry coupled with pressurized liquid extraction

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Abstract

Angelica sinensis (Danggui in Chinese), a well-known traditional Chinese medicine, is also used as a health food product for women's care in Europe and America. Therefore, the demand for Danggui is enormous throughout the world. Due to the shortage of *Angelica sinensis*, *Angelica acutiloba* and *Angelica gigas* are commonly used as the substitutes of Danggui in the market of southeast Asia. However, the three common *Angelica* roots showed variation in their genetic and chemical composition. Up to date, it is thought that ferulic acid, ligustilide and other phthalides such as butylidenephthalide are the biologically active components of Danggui. In this paper, the contents of 13 compounds including ferulic acid, *Z*-ligustilide, *E*-ligustilide, *Z*-butylidenephthalide, *E*-butylidenephthalide, 3-butylphthalide, 3-butylidene-4-hydroxyphthalide, senkyunolide A, 6,7-epoxyligustilide, senkyunolide F, senkyunolide H, senkyunolide I, and 6,7-dihydroxyligustilide were determined or estimated by using gas chromatography–mass spectrometry (GC–MS) coupled with pressurized liquid extraction (PLE). The results showed that GC–MS coupled with PLE offered a simple, rapid and high sensitive method to analysis of components in *Angelica* root. And the contents of investigated compounds in *Angelica sinensis*, *Angelica acutiloba* and *Angelica gigas*, which are used as Danggui in China, Japan and Korea, respectively, were highly variant. It is thought that interaction of multiple chemical compounds contributes to the therapeutic effects of Chinese medicines. However, the overall clinical efficacy of these different Danggui has not been determined. Therefore, comparison of chemical components and pharmacological activities of different *Angelica* root is helpful to elucidate the mechanism of therapeutic effects of Danggui.

Keywords: *Angelica sinensis*; *Angelica acutiloba*; *Angelica gigas*; Gas chromatography–mass spectrometry; Pressurized liquid extraction

1. Introduction

Angelica sinensis (Danggui in Chinese), one of the most important traditional Chinese medicines, is used for tonifying blood and treating female irregular menstruation and amenorrhoea. It is also used for treatment of anemia, hypertension, chronic bronchitis, asthma, rheumatism and cardiovascular diseases [1–3]. It is recorded that 70 formulae in China and 56 formulae in Japan contain Danggui [1,4]. Besides the common usage in Asia, Danggui is also used as a health food product for women's care in Europe and America. Therefore, the demand for Danggui is enormous throughout the world [5]. The Chinese pharmacopoeia (2000) recorded that Danggui is derived from root of *Angelica sinensis* (Oliv.) Diels

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(Umbelliferae) [1]. However, *Angelica acutiloba* (Sieb. et Zucc.) Kitag. and *Angelica gigas* Nakai, which are mainly found in Japan and Korea, respectively, are commonly used as the substitutes of Danggui in the market of southeast Asia due to the shortage of *Angelica sinensis* [6–9]. However, the three common *Angelica* roots showed variation in their genetic and chemical composition [10]. These problems, therefore, compromise the values of traditional Chinese medicine or even jeopardize the safety of the consumers.

Among over 70 compounds isolated and identified from Danggui [11], ferulic acid, ligustilide and other phthalides are thought to be the biologically active components [12–16]. Unfortunately, only ferulic acid and ligustilide were quantitated and compared among different species and/or geographical sources of Danggui [10]. In addition, high performance liquid chromatography (HPLC) [10] and gas chromatography (GC) [17] are limited for quantitative determination of chemical components in Danggui because of the absence of chemical standards. Gas chromatography–mass spectrometry (GC–MS) offers a powerful tool for identification of chemical components in essential oil [18,19]. In present study, a method of GC–MS coupled with pressurized liquid extraction (PLE) was developed for simultaneous determination of 13 active components including ferulic acid, *Z*-ligustilide, *E*-ligustilide, *Z*-butylidenephthalide, *E*-butylidenephthalide in Dangui. The amount of 13 components in different species and/or geographical sources of *Angelica* root were also compared.

2. Materials and methods

2.1. Materials and chemicals

The roots of *Angelica sinensis* were obtained from Minxian of Gansu Province, Lijiang of Yunnan Province collected by us. The roots of *Angelica acutiloba* were collected from Japan by Dr. Hui Y. Li of National Research Institute for Traditional Sino-Japanese Medicines, Toyama Medical and Pharmaceutical University. The roots of *Angelica gigas* were collected from Korea by Dr. Xiu H. Ji of National Products Chemistry Laboratory, Department of Applied Biological and Environmental Chemistry, Seoul National University. All the plant materials were collected in September or October after they had been cultivated for 2 years. The botanical origins of all the materials in forms of whole plants were identified morphologically by us during the field collection. The voucher specimens of *Angelica* root were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macau, China.

Ferulic acid, *Z*-butylidenephthalide and *E*-butylidenephthalide were purchased from Sigma (St. Louis, MO, USA). *Z*-ligustilide was purchased from Chroma-Dex (St. Santa Ana, CA, USA). Methanol for GC was purchased from Merck (Darmstadt, Germany).

2.2. Pressurized liquid extraction

Pressurized liquid extractions were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. In brief, raw materials of *Angelica* root were dried at 40 °C for 6 h and were ground into powder of 0.09–0.13 mm. Powder of Danggui (0.3 g) was mixed with diatomaceous earth (2 g) and placed into 11 ml stainless steel extraction cell, respectively. The use of a dispersion agent, such as diatomaceous earth, is recommended in order to reduce the solvent volume used for the extraction [20]. The extraction cell was extracted under the extraction conditions. Then, extract was transferred to a 25 ml volumetric flask which was brought up to its volume with extraction solvent and filtered through a 0.45 μm Econofilter (Agilent Technologies) prior to injection into the GC–MS system.

2.3. GC–MS analysis

GC–MS was performed with an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies, Palo Alto, CA). Compounds were separated on a 30 m × 0.25 mm i.d. capillary column coated with 0.25 μm film 5% phenyl methyl siloxane. The column temperature was at 50 °C for injection, then programmed at 4 °C min^{-1} to 180 °C, then at 20 °C min^{-1} to 300 °C. Split injection (2 μl) was conducted with a split ratio of 1:10 and helium was used as carrier gas of 1.0 ml min^{-1} flow-rate. The spectrometers were operated in electron-impact (EI) mode, the scan range was 50–550 amu, the ionization energy was 70 eV and the scan rate was 0.34 s per scan. The inlet, ionization source temperatures were 320 and 300 °C, respectively.

3. Results and discussions

3.1. Optimization of PLE procedure

PLE procedure was optimized. And the parameters include the type of solvent, particle size, temperature, static extraction time, pressure and flush volume were studied by using univariate approach. *Z*-ligustilide, *E*-ligustilide, *Z*-butylidenephthalide, *E*-butylidenephthalide and ferulic acid were used as the markers for evaluation of extraction efficiency. Influences of solvent, particle size, temperature, static extraction time, pressure and flush volume on the PLE was shown in Figs. 1 and 2, respectively. The recovery efficiency for the PLE procedure was determined by performing consecutive pressurized liquid extractions on the same sample under the optimized PLE conditions, until no investigated compounds were detected by the analysis. The recovery was calculated based on the total amount of individual investigated components. Taking into account the results of optimization and recovery experiment, the conditions of the

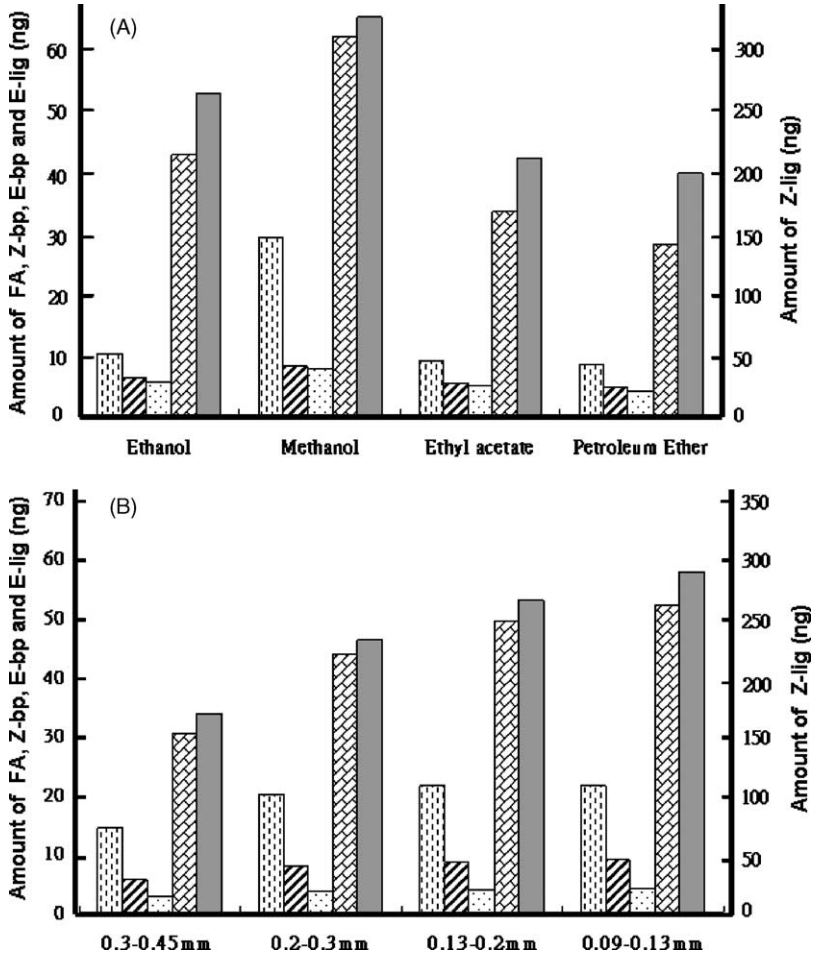

Fig. 1. Effects of solvent (A) and particle size (B) on pressurized liquid extraction of *Z*-ligustilide (*Z*-lig, ■), *E*-ligustilide (*E*-lig, ▨), *Z*-butylidenephthalide (*Z*-bp, ▨), *E*-butylidenephthalide (*E*-bp, ⬚) and ferulic acid (FA, ⬚) in *Angelica sinensis*. Condition: particle size, 0.125–0.2 mm (A), or solvent, methanol (B); temperature, 100 °C; static extraction time, 5 min; pressure, 1500 psi; flush volume, 60%; extraction cycle, 1 and extraction times, 1. The mean values of three determinations are presented. The variation is less than 3% of the mean.

PLE method proposed were: solvent, methanol; temperature, 100 °C; particle size, 0.09–0.13; static extraction time, 10 min; pressure, 1200 psi; static cycle, 2 and 60% of the flush volume.

3.2. Identification of components in Danggui

Chromatograms of PLE extracts from *Angelica* root were shown in Fig. 3. All the main components were separated completely, and 13 of them were identified according to the mass spectrum of each component. By comparing the mass spectra of the sample with literature data [18,21–26], peaks 1–13 were identified as ferulic acid, 3-butylphthalide, *Z*-butylidenephthalide, 3-butylidene-4-hydroxyphthalide, *E*-butylidenephthalide, senkyunolide A, *Z*-ligustilide, *E*-ligustilide, 6,7-epoxyligustilide, senkyunolide F, senkyunolide H, senkyunolide I, and 6,7-dihydroxyligustilide, respectively. The structures are shown in Fig. 4. The results are summarized in Table 1.

3.3. Quantitation of components in Danggui

The selected ion monitoring (SIM) method was used for the quantification of investigated compounds. A fragment ion *m*/*z* 150 was used for ferulic acid, *m*/*z* 161 for *Z*-ligustilide and *E*-ligustilide, and *m*/*z* 149 for *Z*-butylidenephthalide and *E*-butylidenephthalide. The mass spectra of *Z*-ligustilide and *E*-ligustilide are very similar (data not shown). Therefore, the content of *E*-ligustilide was estimated using the calibration curve of *Z*-ligustilide.

The calibration curves, which obtained from the ions peak area, for ferulic acid, *Z*-butylidenephthalide, *E*-butylidenephthalide, *Z*-ligustilide were linear over the range 6.35–381, 1.9–475, 1.24–62 and 5.4–540 ng absolute on column, respectively. The square coefficients of correlation (r^2) were between 0.9992 and 0.9997.

The short-term (12 h) repeatability as well as the long term (24 h) repeatability of ferulic acid, *Z*-butylidenephthalide, *E*-butylidenephthalide and *Z*-ligustilide were calculated for 10 times. The peak area of selected ions was relatively stable ex-

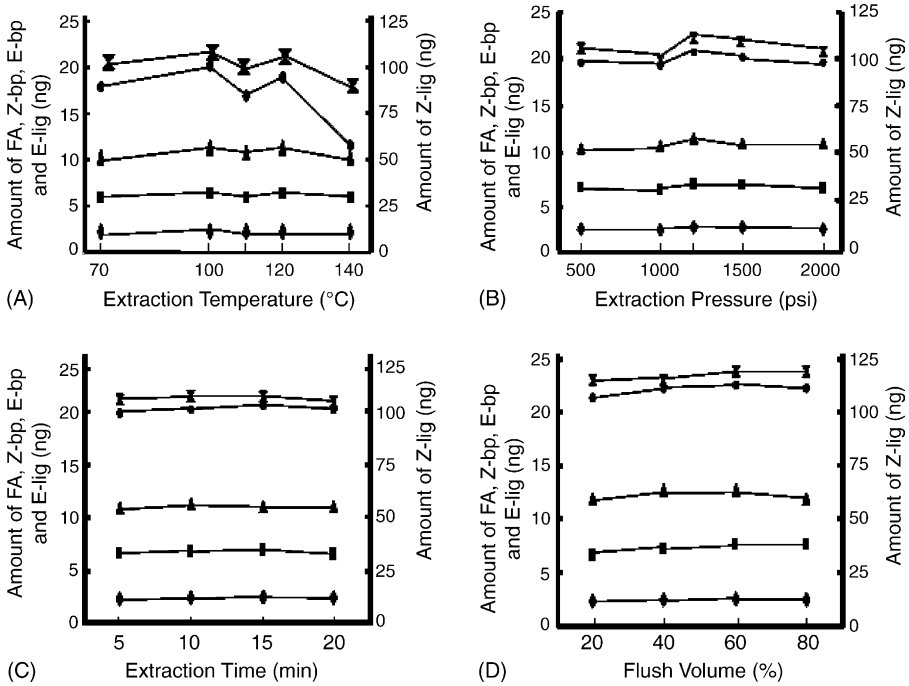

Fig. 2. Influence of selected factors including temperature (A), pressure (B), static extraction time (C) and flush volume (D) on the PLE extraction of *Z*-ligustilide (*Z*-lig, ⧗), *E*-ligustilide (*E*-lig, ●), *Z*-butylidenephthalide (*Z*-bp, ■), *E*-butylidenephthalide (*E*-bp, ▲) and ferulic acid (FA, ♦) in *Angelica sinensis*. Condition: to determine one of the parameters including temperature, pressure, static extraction time and flush volume, the others were set at the system default value (temperature, 100 °C; pressure, 1500 psi; static extraction time, 5 min; flush volume, 60% and extraction cycle, 1). Solvent, methanol; particle size, 0.09–0.13 mm.

cept *Z*-ligustilide showed a higher variation in the long-term repeatability. The R.S.D. of short (long) term repeatability for ferulic acid, *Z*-butylidenephthalide, *E*-butylidenephthalide and *Z*-ligustilide was 1.93% (4.55%), 0.94% (5.32%), 1.48% (5.21%) and 3.65% (7.41%), respectively. Thus, the quantitation of components such as ligustilide in *Angelica* root must be preformed within 12 h after the sample extraction.

In order to validate the presented method, a known amount of ferulic acid, *Z*-butylidenephthalide, *E*-butylidenephthalide and *Z*-ligustilide was added into the *Angelica* root sample and extracted at optimized conditions mentioned above. The extracted material was subjected to GC–MS, and the content of the analytes was calibrated. The recovery of the tested compounds was between 100.8% and 102.9% with relative standard deviation (R.S.D.) of 1.94–2.49%, where $n = 5$.

The contents of ferulic acid, *Z*-butylidenephthalide, *E*-butylidenephthalide and *Z*-ligustilide of different *Angelica* root were determined by using the calibrated GC–MS. GC or HPLC cannot identify the compounds of the peaks without standard. However, it is easy for using GC–MS. The content of identified components based on mass spectra in *Angelica* root was estimated by using *Z*-ligustilide which is one of the

Table 1
Mass data of 13 compounds identified from Danggui

Peak no.	Compound	Rt (min)	Mass data[a]
1	Ferulic acid	18.64	150(100), 135(74), 118(3), 107(29), 89(4), 77(25), 63(4), 51(6)
2	3-Butylphthalide	28.96	190(M^+,4), 144(3), 133(100), 134(11), 105(24), 77(9), 51(3)
3	*Z*-Butylidenephthalide	29.43	188(M^+,21), 160(13), 159(100), 146(32), 131(21), 103(17), 77(13)
4	3-Butylidene-4-hydroxyphthalide	30.64	204(M^+,34), 175(100), 162(39), 147(21), 91(16), 73(23), 57(31)
5	*E*-Butylidenephthalide	30.74	188(M^+,20), 159(100), 146(32), 131(22), 104(12), 103(20), 77(13)
6	Senkyunolide A	30.90	192(M^+,23), 163(3), 135(5), 107(100), 79(22)
7	*Z*-Ligustilide	31.29	190(M^+,66), 161(100), 148(78), 134(15), 106(32), 105(44), 77(21), 55(33)
8	*E*-Ligustilide	32.84	190(M^+,64), 161(100), 148(72), 134(15), 106(33), 105(45), 77(23), 55(36)
9	6,7-Epoxyligustilide	34.52	206(M^+,100), 177(66), 164(30), 150(20), 149(29), 135(34), 77(30), 55(61)
10	Senkyunolide F	34.58	206(33), 177(100), 150(61), 149(64), 135(37), 107(29), 104(29), 77(34), 71(31), 55(30)
11	Senkyunolide H	35.19	224(M^+,36), 181(16), 180(100), 165(22), 151(42), 138(15), 123(9), 95(14), 55(23)
12	Senkyunolide I	35.64	224(M^+,30), 181(17), 180(100), 165(18), 151(41), 138(13), 123(10), 95(13), 55(21)
13	6,7-Dihydroxyligustilide	36.07	224(M^+,35), 180(100), 165(26), 151(51), 95(28), 55(53)

[a] *m*/*z*, relative intensity is shown in parenthesis, and the ion of relative intensity 100 was used for the quantification.

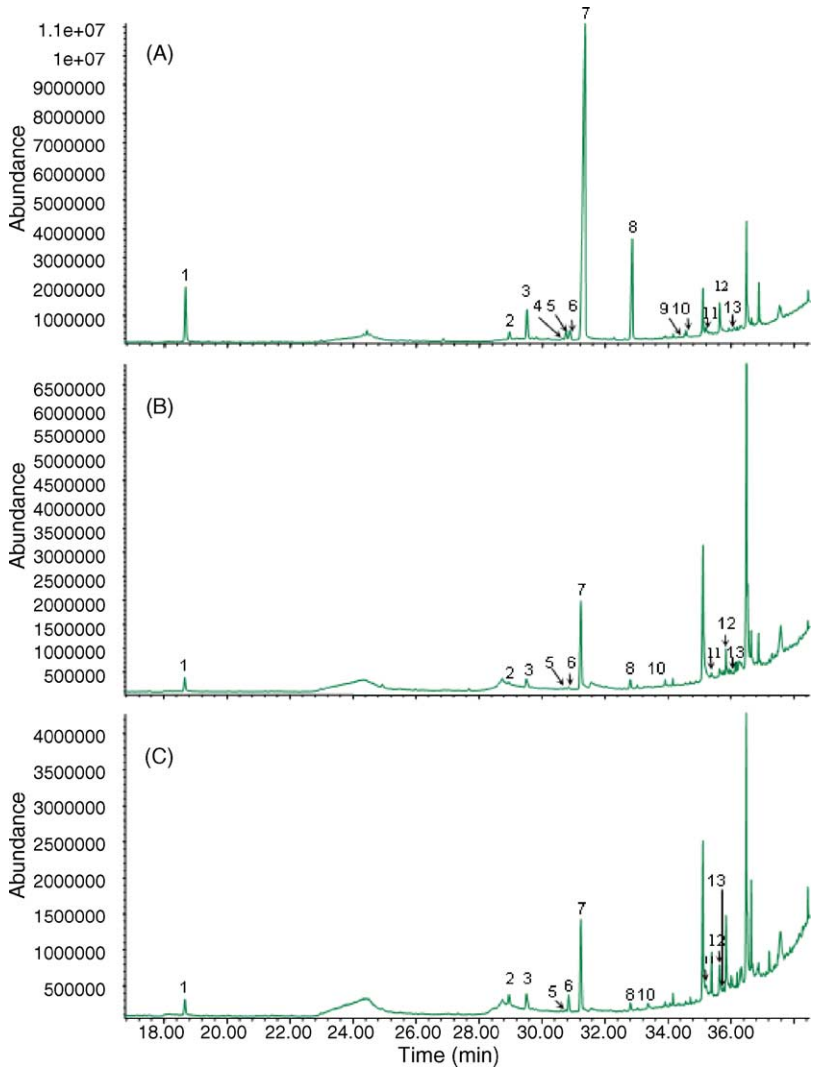

Fig. 3. GC–MS total ion chromatograms of PLE extract from *Angelica sinensis*, *Angelica acutiloba* and *Angelica gigas*. (1) Ferulic acid; (2) *Z*-ligustilide; (3) *E*-ligustilide; (4) *Z*-butylidenephthalide; (5) *E*-butylidenephthalide; (6) 3-butylphthalide; (7) 3-butylidene-4-hydroxyphthalide; (8) senkyunolide A; (9) 6,7-epoxyligustilide; (10) senkyunolide F; (11) senkyunolide H; (12) senkyunolide I; (13) 6,7-dihydroxyligustilide.

Table 2
Contents of 13 compounds in different *Angelica* root (%)

	Angelica sinensis				*Angelica acutiloba*		*Angelica gigas*
	Gansu 1	Gansu 2	Gansu 3	Yunnan	Hokkado	Toyama	Korea
Ferulic acid	0.37 (6.06)	0.40 (6.91)	0.42 (7.05)	0.34 (8.82)	0.13 (7.44)	0.14 (8.38)	0.12 (7.09)
3-Butylphthalide	0.26 (4.23)	0.19 (3.27)	0.19 (3.20)	0.47 (12.29)	0.15 (8.56)	0.14 (8.63)	0.20 (11.21)
Z-Butylidenephthalide	0.12 (2.01)	0.15 (2.59)	0.12 (2.10)	0.08 (2.21)	0.06 (3.30)	0.05 (2.90)	0.05 (2.97)
3-Butylidene-4-hydroxyphthalide	0.14 (2.25)	0.13 (2.25)	0.14 (2.32)	0.14 (3.79)	0.12 (6.94)	–	–
E-Butylidenephthalide	0.05 (0.86)	0.05 (0.89)	0.05 (0.80)	0.04 (1.05)	0.02 (1.34)	0.02 (1.36)	0.02 (1.32)
Senkyunolide A	0.29 (4.68)	0.18 (3.09)	0.18 (3.03)	0.32 (8.37)	0.14 (7.95)	0.14 (8.32)	0.21 (12.19)
Z-Ligustilide	3.14 (50.93)	3.10 (53.19)	3.13 (53.09)	1.43 (37.68)	0.31 (17.57)	0.44 (27.37)	0.34 (19.22)
E-Ligustilide	0.68 (11.02)	0.59 (10.04)	0.47 (8.02)	0.30 (7.84)	0.14 (7.72)	0.15 (9.12)	0.14 (7.78)
6,7-Epoxyligustilide	0.13 (2.06)	0.13 (2.30)	0.13 (2.19)	0.12 (3.24)	0.12 (6.83)	–	–
Senkyunolide F	0.14 (2.32)	0.14 (2.45)	0.14 (2.36)	0.13 (3.32)	0.13 (7.11)	0.13 (7.71)	0.13 (7.15)
Senkyunolide H	0.16 (2.61)	0.18 (3.07)	0.17 (2.86)	0.13 (3.37)	0.13 (7.11)	0.13 (7.77)	0.13 (7.55)
Senkyunolide I	0.35 (5.62)	0.45 (7.63)	0.38 (6.51)	0.15 (4.03)	0.16 (9.18)	0.15 (9.12)	0.21 (11.78)
6,7-Dihydroxy-dihydroligustilide	0.33 (5.36)	0.14 (2.33)	0.38 (6.46)	0.15 (4.00)	0.16 (8.95)	0.15 (9.31)	0.21 (11.73)
Total (%)	6.17	5.83	5.90	3.80	1.79	1.62	1.75

Samples were collected from China (*Angelica sinensis*), Japan (*Angelica acutiloba*) and Korea (*Angelica gigas*). The percentage in 13 compounds is shown in parenthesis.

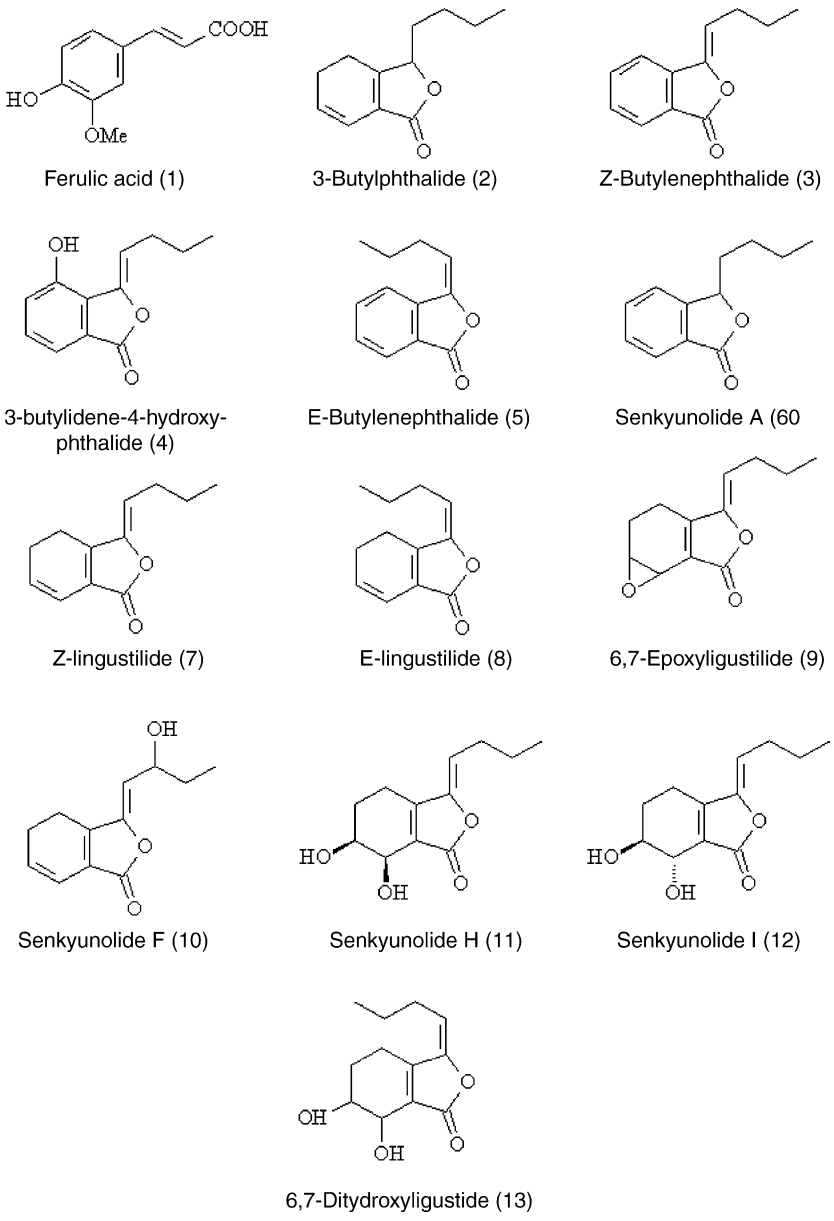

Fig. 4. The structure of 13 identified compounds in *Angelica* root.

major phthalides. Table 2 shows the summary results on the contents of investigated compounds. The results give some more valued information on the quality of samples, though some errors exist. The results showed that the contents of investigated components in *Angelica sinensis* were higher than those in *Angelica acutiloba* and *Angelica gigas*. In general, the curative effect of traditional Chinese medicine is an integrative result of a number of bioactive compounds. Up to date, ferulic acid, ligustilide and other phthalides are thought to be the biologically active components [12–16]. Thus, the contents of these components are correlated with the therapeutic effects of *Angelica* root. Therefore, the overall clinical efficacy of these different *Angelica* root should be determined and compared to distinguish their clinical use.

4. Conclusion

Danggui is a well-known Chinese traditional medicine. Many studies showed that ferulic acid, ligustilide and other phthalides such as butylidenephthalide are the biologically active components. In this paper, the contents of 13 compounds including ferulic acid, ligustilides, butylidenephthalides and other phthalides were determined or estimated by using GC–MS coupled with PLE. The results showed that the contents of investigated compounds in *Angelica sinensis*, *Angelica acutiloba* and *Angelica gigas*, which are used as Danggui in China, Japan and Korea, respectively, were highly variant. But the overall clinical efficacy of these different Danggui has not been determined. It is thought that in-

teraction of multiple chemical compounds contributes to the therapeutic effects of Chinese medicines. Therefore, comparison of chemical components and pharmacological activities of different *Angelica* root is helpful to elucidate the mechanism of therapeutic effects of Danggui.

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