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Comparative Studies on Medicinal Constituents of Korean and Chinese Angelicae Dahuricae Radix

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ABSTRACT

The medicinal constituents contained in Korean and Chinese Angelicae dahuricae Radix were compared by confirming their qualities. From the extracts of Angelicae dahuricae Radix, oxypeucedanin, imperatorin, and alloisoimperatorin etc. furanocoumarin derivatives were identified by GC/MS analysis. Through GC/FID analysis, the furanocoumarin derivatives content of Baizi cultivated in Korea was more than that cultivated in China, except for one cultivated at Ankuk province, and so confirmed to possess, on the whole, good quality medicinal constituents by content , as compared with the ones cultivated in China.

Key Words **:** Baizi, furanocoumarin derivatives, oxypeucedanin, imperatorin, alloisoimperatorin, GC/MS analysis

INTRODUCTION

Baizi, Radix Angelicae dahuricae, is the dry root of *Angelica dahurica* (Fischer) Bentham et Hooker collected between summer and fall when the leaves have turned yellow. It is often used as an antipyretic and analgesic for cold, headache, and toothache in traditional Chinese medicine(Tang and Eisenbrandt, 1992).

The roots of *A. dahurica* are known to contain a number of coumarin and furanocoumarin derivatives(Hata et al., 1963a; Yoshida et al., 1971; Saiki et al., 1971; Shlyunko et al., 1977; Fujiwara et al., 1980; Kozawa et al., 1981; Lu and Cai, 1982). In addition to the coumarin and furanocoumarin derivatives, sitosterol(Zhang et al., 1980), stigmasterol(Yen et al., 1969), and some lactones such as β-angelica lactone(Ding and Zhang, 1981), 2-hydroxy-3,4-dimethyl-2-buten-4-olide(Baba et al., 1985), γ-nonalactone, γ-decalactone(Tani et al., 1984) were isolated and identified. Among these compounds, furanocoumarin derivatives, shown on Fig. 1, like byakangelicin, imperatorin, and oxypeucedanin etc. were reported as the main medicinal constituents of Angelicae dahuricae Radix (Hata et al., 1963b; Saiki et al., 1971; Kozawa et al., 1981).

As the medicinal effects of the furanocoumarin derivatives in Angelicae dahuricae Radix, a significant inhibition of hepatic drug-metabolizing enzyme(DME) activity was known(Shin et al., 1988). Also, experiments to determine the effects of coumarins on the actions of adrenaline, ACTH, and insulin in fat cells isolated from rats showed that the furanocoumarins

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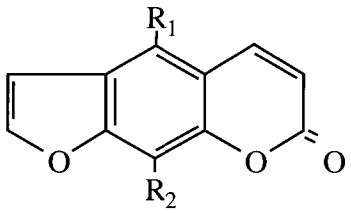
oxypeucedanin, bergapten, xanthotoxin, imperatorin, and phellopterin activated adrenaline-induced lipolysis. Oxypeucedanin hydrate, imperatorin, and phellopterin also activated ACTH-induced lipolysis, whereas the furanocoumarins byakangelicin, neobyakangelicol, and isopimpinellin strongly inhibited insulin-stimulated lipogenesis. Therefore, the roots of *A. dahurica* activates lipolytic hormons and selectively inhibits antilipolytic hormons(Kimura et al., 1982).

Recently, the cultivation of medicinal herbs in Korea is gradually diminishing. The cause of this phenomenon is not a decrease in the demand of medicinal herbs but the lower price of imported herbal medicine materials. Hence there has been a better understanding of the establishment of a higher quality in domestic medicinal herbs.

This study was conducted to ensure sustainable cultivation of domestic medicinal herbs and to supply the basic data on their standard quality by comparison to medicinal constituents of Korean and Chinese herbal medicine materials.



	R_1	R_2
byakangelicin	$-OCH_3$	$-O-CH_2-CH(OH)-C(OH)(CH_3)_2$
byakangelicol	$-OCH_3$	$-O-CH_2-CH(O)-C(CH_3)_2$
oxypeucedanin	$-O-CH_2-CH(O)-C(CH_3)_2$	$-H$
oxypeucedanin hydrate	$-O-CH_2-CH(OH)-C(OH)(CH_3)_2$	$-H$
imperatorin	$-H$	$-O-CH_2-CH=C(CH_3)_2$
isoimperatorin	$-O-CH_2-CH-C(CH_3)_2$	$-H$
bergapten	$-OCH_3$	$-H$

Fig. 1. The furanocoumarin derivatives contained in Angelicae dahuricae Radix.

MATERIALS AND METHODS

Plant material

Domestic herbal materials were collected randomly from the Kyungdong market in Seoul. In the case of the Chinese ones, low and high quality material were collected, respectively, from Yeunkil, Ankuk, and Killim province in China, considering the content differences of the main constituents in accordance with localities.

Preparation of extracts

The air-dried roots of *A. dahurica* were milled. The 10 g of each sample was extracted by refluxing at 70 ℃ for 1 hour with 100 ml methanol and filtered by Whatman No. 2 filter paper. And then, the residue was also extracted under refluxing condition at 70 ℃ for 1 hour with 100 ml methanol and filtered. After the extraction was repeated for 3 times, the methanol was removed from the combined filtrates by concentrating in vacuo at 40℃(Shin et al., 1990). Each 1 g of extracts was dissolved with 10 ml of methanol . This solution was used as the sample analyzed by GC/FID.

Preparation of acid hydrolyzates

The 2 g of extracts from Angelicae dahuricae Radix cultivated in Korea were extracted with 20 ml of 7 % methanolic sulfuric acid by refluxing at 70 ℃ for 6 hours and filtered by Whatman No. 2 filter paper. This solution was partitioned for 3 times with 50 ml of diethyl ether. Each ether solution was dried over anhydrous sodium sulfate, filtered and concentrated in vacuo at 40℃. The concentrate was dissolved with 1 ml of methanol. The solution was used as sample analyzed by HPTLC.

The HPTLC analysis of acid hydrolyzates was applied on a HPTLC plate(Silica gel 60 F_{254}) and developed with the lower phase of chloroform : methanol : water(65:35:10). The spots were detected by heating to 105 ℃ for 10 min. after spraying with 30 % sulfuric acid.

Preparation of TMS derivatives

The above 100㎕ solution of acid hydrolyzates was evaporated to dryness under a N2 stream. After adding 100㎕ BSA to this concentrate, the reaction mixture was heated at 70 ℃ for 3 hours and evaporated to dryness.

GC/FID and GC/MS analysis

TMS derivatives were analyzed by GC/FID and GC/MS. The mass spectrum of the peaks was separated through GC and compounds identified by comparing their mass spectrum with libraries of the NIST.

The conditions of GC/FID and GC/MS were as follows: SPB-1 fused silica capillary column, 0.25 mm i.d. × 30 m, Supelco; temperature programmed from 200 ℃ (3 min) to 300 ℃ at 4 ℃/min.

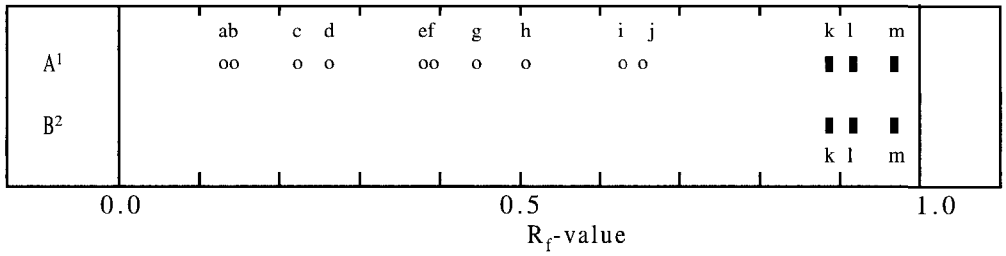


Fig. 2. TLC patterns of the extracts and the acid hydrolyzates of Angelicae dahuricae Radix.
A^1; The extracts, B^2; The acid hydrolyzates of extracts

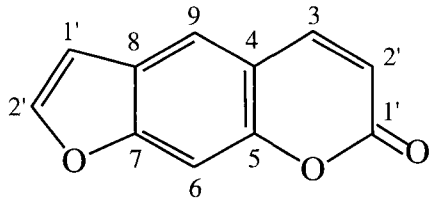


Fig. 3. The chemical structure of furanocoumarin.

RESULTS AND DISCUSSION

TLC patterns of the extracts and acid hydrolyzates

Fig. 2 shows TLC patterns of the extracts and the acid hydrolyzates of Angelicae dahuricae Radix. The 14 spots(a-m) were detected from the extracts of Angelicae dahuricae Radix. On the other hand, the 3 spots(k-m) were only confirmed on the HPTLC plate from the acid hydrolyzates of Angelicae dahuricae Radix. The main constituents of Angelicae dahuricae Radix have been reported as furanocoumarin derivatives, byakangelicin, byakangelicol etc. (Hata et al., 1963a, b; Saiki et al., 1971; Kozawa et al., 1981). The other compounds, sterols(Zhang et al., 1980; Yen et al., 1969) and lactones(Ding and Zhang, 1981; Tani et al., 1984) were separated and identified from the roots of *A. dahurica*. The constituents contained in k, l, and m were deduced to be coumarins, furanocoumarins, sterols, and lactones since there was no glycosides within the acid hydrolyzates of Angelicae dahuricae Radix.

Identification of constituents by GC/MS analysis

The TMS derivatives of the acid hydrolyzates were analyzed by GC/MS. The mass fragmentation patterns, appearing on the mass spectra of the peak No. 5, 6, 7, and 8(Fig. 4 and Table 1), were similar to those of furanocoumarin derivatives.

The peak No. 5 exhibited mass fragmentation patterns of m/z 202(100%), 63, 89, 145, and 270. Among the constituents of Angelicae dahuricae Radix, the mass spectra of imperatorin, isoimperatorin, and alloisoimperatorin have an [M]+ion at m/z 270 suggesting the molecular formular $C_{16}H_{14}O_4$. The chemical structure of imperatorin has the $-OCH_2CH=C(CH_3)_2$ moiety at No. 6 position of furanocoumarin(Fig. 3). And isoimperatorin has the $-OCH_2CH=C(CH_3)_2$ unit at No. 9 position of the furanocoumarin structure. In this case the chemical structure of alloisoimperatorin has the $-CH_2CH=C(CH_3)_2$ moiety at No. 6 position and -OH unit at No. 9 position of furanocoumarin, respectively. Therefore, the mass fragmentation pattern of imperatorin is analogous to that of isoimperatorin. In the case of alloisoimperatorin, it was assumed that the specified m/z 252(M^+-H_2O) or m/z 254(M^+-OH+H^+) moiety was in existence in its mass spectrum. From the mass fragmentation pattern of peak No. 5, the compound of peak No. 5 was identified as imperatorin or isoimperatorin judging from it not being the m/z 252(M^+-H_2O) unit but being the base peak of m/z 202(M^+-$CH_2CH=C(CH_3)_2$) unit.

The peak No. 6 showed mass fragmentation patterns of m/z 286(100%), 129, 145, 157, 173, 187, 215, and 270. Thus, the compound of peak No. 6 was identified as oxypeucedanin of molecular weight 286 and molecular formula $C_{16}H_{14}O_5$.

The peak No. 7 was identified as alloisoimperatorin of molecular weight 270 and molecular formula $C_{16}H_{14}O_4$ since the mass spectrum of peak No. 7 had the fragment of m/z 254(M^+-OH+H^+).

The peak No. 8 indicated the mass fragmentation patterns of m/z 286(100%), 63, 89, 118,145, 174, 202, and 270 etc. This compound was identified as the oxypeucedanin isomer of molecular weight 286 and molecular formula $C_{16}H_{14}O_5$.

The other furanocoumarin derivatives of Angelicae dahuricae Radix, including byakangelicin etc., were not detected from this GC/MS analysis. In the case of

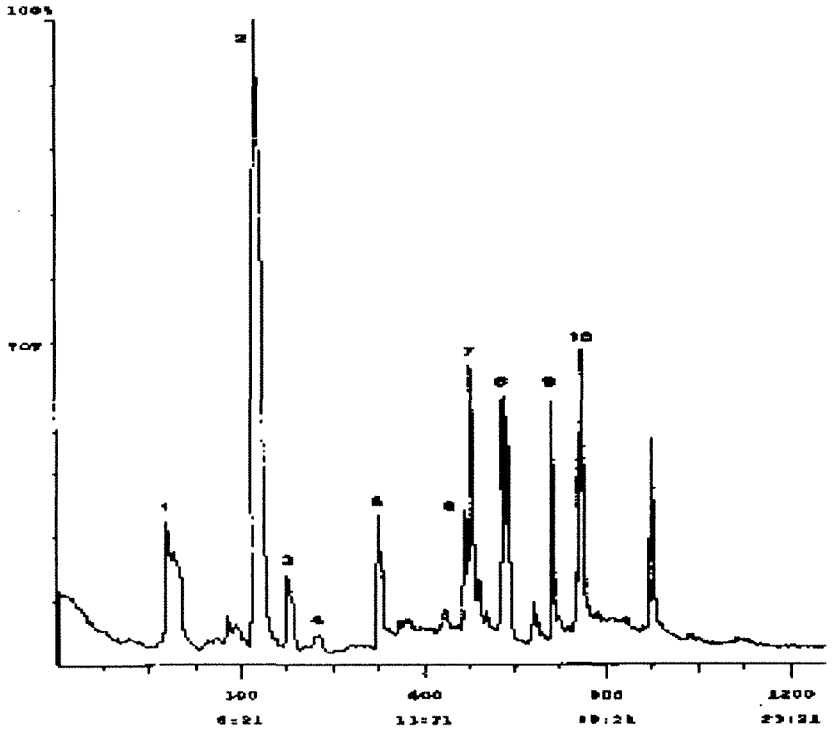


Fig. 4. Total ion chromatogram of Angelicae dahuricae Radix extracts.

Table 1. Mass fragmentation patterns of Angelicae dahuricae Radix extracts.

Peak No.	m/z (%)	Identification	GC peak No.
1	55(100%), 73, 87, 157, 256		1
2	67(100%), 55, 79, 95, 280		2
3	129(100%), 51, 65, 77, 91, 115, 143, 157		3
4	232(100%), 51, 77, 133, 161, 189, 217		4
5	202(100%), 63, 89, 145, 174, 270	Isoimperatorin or Imperatorin	5
6	286(100%), 129, 145, 157, 173, 187, 215, 270	Oxypeucedanin	6
7	270(100%), 115, 171, 186, 199, 254	Alloisoimperatorin	7
8	286(100%), 63, 89, 118, 145, 174, 202, 270	Oxypeucedanin isomer	8
9	316(100%), 175, 217, 231, 245, 299		9
10	232(100%), 189, 217, 245, 281, 304, 316		10

byakangelicin, it could be considered not to be confirmed by the experimental procedures because of two hydroxyl functional groups attached to $-CCH_2CHOHCOH(CH_3)_2$ moiety at No. 6 position of byakangelicin(Fig. 3).

Comparison of medicinal constituents by GC/FID analysis

The TMS derivatives of the acid hydrolyzates were analyzed by GC/FID. The GC Chromatogram shown in Fig. 5, was obtained as one similar to GC/MS by the same analysis conditions.

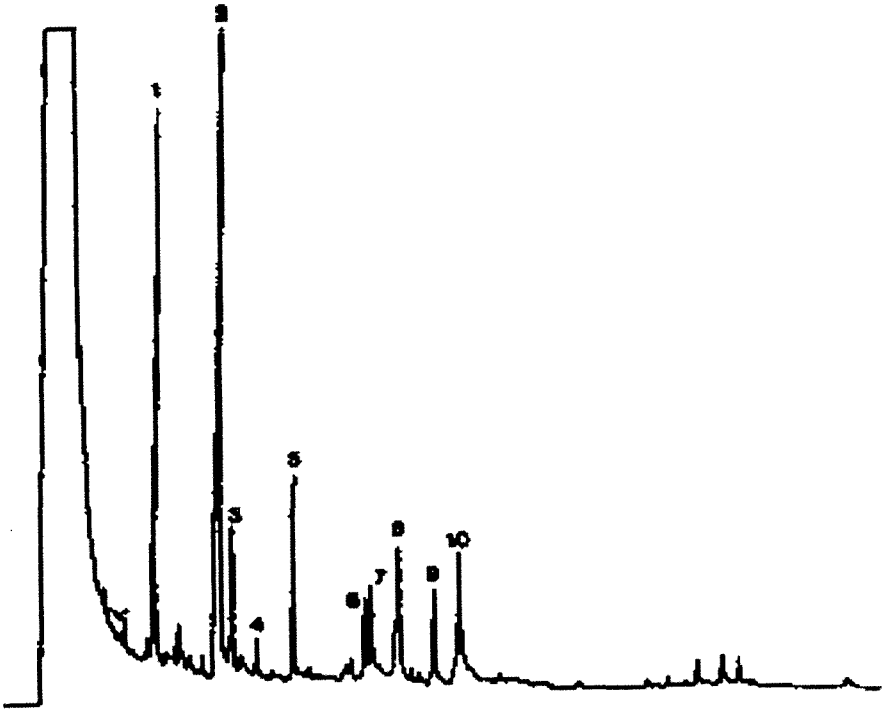


Fig. 5. GC chromatogram of Angelicae dahuricae Radix extracts.

Table 2. Composition of constituents contained in Korean and Chinese Angelicae dahuricae Radix

GC peak No.	Rt(min)	Composition ratio of peak area (%)				
		Cultivated in Korea	Cultivated in China, high quality(Yeungil)	Cultivated in China, low quality(Yeungil)	Cultivated in Chinain (Ankuk)	Cultivated in China (Killim)
1	6.3	12.6	12.6	13.9	14.5	9.9
2	8.9	37.9	33.4	38.8	44.7	15.3
3	9.5	4.3	1.9	2.1	6.2	18.8
4	10.5	1.6	6.3	3.1	6.4	1.4
5	11.9	4.8	2.4	5.3	6.2	8.2
6	14.8	2.7	1.9	1.4	1.9	1.4
7	15.1	3.5	2.4	1.7	2.9	6.1
8	16.2	5.7	3.8	3.2	5.1	2.6
9	17.7	3.3	3.8	2.5	1.9	0.6
10	18.7	6.9	2.5	2.2	2.1	1.7
The others		16.7	29.0	15.8	8.1	34.0
Total		100	100	100	100	100

Table 3. Yield of the extracts from Korean and Chinese Angelicae dahuricae Radix

	Cultivated in Korea	Cultivated in China, high quality(Yeungil)	Cultivated in China, low quality(Yeungil)	Cultivated in China, (Ankuk)	Cultivated in China, (Killim)
Quantity of extract	3.73g	3.79g	3.58g	6.54g	1.47g
Yield(%)	37.3%	37.9%	35.8%	65.4%	14.7%

Besides the sample of Killim cultivated, the composition ratio of an unidentified peak No. 2 was highest at 33.4 to 44.7 % in all the rest samples. Comparing Baizi cultivated in Korea with ones cultivated in China through the peak area of peaks No. 5, 6, 7, and 8 shown in Table 2, the furanocoumarin derivatives content of the one cultivated in Korea was more than the ones cultivated in China except for the one cultivated at Killim province. But the content of the one cultivated in Korea was more than 2.5 times the ones cultivated at Killim province, considering the yield of extracts indicated in Table 3. From the estimated yield of extracts among the samples analyzed, the one cultivated at Ankuk province showed the highest content.

Based on the results so far obtained, Angelicae dahuricae Radix cultivated in Korea was confirmed to possess good quality medicinal constituents by content, as compared to the ones cultivated in China, besides Ankuk province.

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