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A Study for the Standardization of *Elsholtzia ciliata* (Thunb.) Hylander and *Elsholtzia splendens* Nakai ex F. Maekawa

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ABSTRACT

A Study for the Standardization of Elsholtzia ciliata (Thunb.) Hylander and Elsholtzia splendens Nakai ex F. Maekawa

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The purpose of present study is to clarify the differences between *Elsholtzia ciliata* (Thunb.) Hylander (향유) and *Elsholtzia splendens* Nakai ex F. Maekawa (꽃향유) for standardization and the proper usage as medicinal herbs. The major ingredients of both species were isolated by distillation and extraction. The qualitative and quantitative analyses of major distillates were carried out by the use of GC/MS.

There was a significant difference between the components of *Elsholtzia ciliata* and *Elsholtzia splendens* in the aspects of major components. Several common ingredients were identified as linalool,

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cumene, elsholtzia ketone, naginata ketone isomer, naginata ketone, myristicin, and sesquiterpene alcohol. Comparison between *Elsholtzia ciliata* and *Elsholtzia splendens* was done in the aspect of major compounds. Myristicin (33.7%) has been shown to be the major component in *Elsholtzia ciliata* whereas naginata ketone isomer (26.1%) was believed to be a major ingredient in *Elsholtzia splendens*. The elsholtzia ketone was also one of the major differentiating factors be- tween *Elsholtzia splendens* and *Elsholtzia ciliata*, and the quantity is 15.1% in *Elsholtzia splendens* compared to 2.87% in *Elsholtzia ciliata*. Moreover, in the *Elsholtzia splendens*, 4- vinylguaiacol and isoosmorhizole were absent, but both compounds were present in the *Elsholtzia ciliata*.

Key Word : *Elsholtzia ciliata*, *Elsholtzia splendens*, myristicin, naginata ketone isomer, elsholtzia ketone, linalool, cumene

Ⅰ. Introduction

Since Hyang-Yu (香薷) appeared for the first time in MYUNG-EUI-BYUL-ROG (名醫別錄)[4], its use has been reported subsequently in the literatures including DO-KYUNG-BON-CHO (圖經本草)[5] and BON-CHO-GANG-MOG (本草綱目)[6]. It was commonly used for headache with heat, chill symptom without sweat, abdominal pain, vomiting, diarrhea, edema and beriberi caused by cold body sick and chill absorption during summer.

Hyang-Yu could be obtained from dry part of perennial herb, either *Elsholtzia ciliata* Hylander (향유) or *Elsholtzia splendens* Nakai ex F. Maekawa (꽃향유). These herbs get fruits ripe in summer or fall, and then aerial parts of the herbs are cut and dehydrated under the sunlight or in the shade. However, it has been known that *Elsholtzia ciliata* was used for medical purpose in Korea while *Elsholtzia splendens* was used in China and known as the Chinese origin. But, according to Sato[9], Hyang-Yu is the dried parts (stems and leaves) of *Elsholtzia ciliata* in its blooming season, which was grown in northeastern part of China.

The ingredients of *Elsholtzia ciliata* include 0.5-1.0% of essential oil; it is known that the major components are elsholtzia ketone, naginata ketone, α-pinene, cinole, ρ-cumene, isovaleric acid, isobutyl-isovalerate, acetic acid, octanol, 1-octen-3-ol, linalool, camphor, geraniol, η-caproic acid, and isocaproic acid. The major ingredients of *Elsholtzia splendens* are essential oils with elsholtzidiol and sterol, phenoloid and flavonoid. Ji and coworkers[2] examined the amounts of essential oils contained in *Elsholtzia ciliata* growing in the central area of the Korean penninsula, and found carvacrol elsholtzia ketone, naginata ketone, β-carprophyllene, and sesquiterpenes, etc.. Park and coworkers[1] reported several flavonoids in *Elsholtzia ciliata;* apigenin-7-glu-coside, luteolin-7-glucoside, and apigenine-4-r-utinoside. Zheng and Shangzhen[7] found 1-octen-3-ol, linalool, ethylbenzoate, α-carvone, dihydroactinidiolide, limonene, terpenene, 2-m-ethoxy-1,3,5-trime-

thylbenzene, aromadendrene, and found flavonoid-type-material like 5-hydr-oxy-6-methylflavanone-7-O-D-galactopyranoside, 5-hydroxy-6,7-dimethoxyflavone, 5-hydroxy-7,8-demethoxyflavone,5-hydroxy-7,4'-dim-ethoxyflavanonol,5,7-dimethoxy-4'-dimethoxyflavone. Zhu[8] reported elsholtzia ketone, carvacrol, and thymol in the *Elsholtzia ciliata*.

The medical effects of *Elsholtzia ciliata* were reported that essential oil of *Elsholtzia ciliata* enhances breathing, blood circulation, relieving sweating and heat. Also the external use of the essential oil is good for blood circulation and for relieving convulsive pains for muscle. The essential oil of *Elsholtzia splendens* helps to enhance filtering capability of the kidney blood vessels, and is good for activating urination and for removing phelgm, influenza virus, and a significant antibacterial effect against *Staphylococcus aureus, β-hemolytic streptococcus, Corynebacterium diphtheriae, Salmonella typhi,* and *Shigella flexineri.* Yun[3] reported an experiment with the extracts of *Elsholtzia ciliata* and *Elsholtzia splendens* collected in the wild forest in Korea that *Elsholtzia splendens* has revealed better effect against anti-inflammatory, analgesic, and anti-pyretic effects compared that with *Elsholtzia ciliata.*

Herein we wish to report the standardization method for the comparison of *Elsholtzia ciliata* with *Elsholtzia splendens* in terms of their ingredients for the proper usage of Hyang-Yu. Because *Elsholtzia ciliata* is used in Korea as the origin of Hyang-Yu, but *Elsholtzia splendens* is regarded as the original Hyang-Yu in China.

II. Experimental

1. Material and Reagents

The *Elsholtzia ciliata* was harvested at Eaebang-ri, Sudong-meon, Namyangzu-shi in Keongki-do on late September, 1995. The other species, *Elsholtzia splendens*, was also collected at wild forest around Chukryong mountain on early October, 1995. These samples, collected at different places, were dryed in the shade followed by sieving between 40 and 100 mesh for the experiment. All the reagents used were HPLC or reagent grade from J.T.Baker (Phillipsburg, U.S.A.) including diethyl ether, n-hexane, methylene chloride, methanol, chloroform, ethanol, ethylacetate, isopropanol and acetone. Distilled water used was doubly distilled and passed through ion exchange resin for removal of ions.

2. Instruments

The GC/MS with Varian 3400 Gas Chromatography (GC) [DB-5 column (J&W, USA, 30m x 0.25mm x 0.25μm)] and Magnum Ion Trap Mass Spectrometer (MS) (Finnigan, San Jose, U.S.A.) was used. The semi-preparative scale High Pressure Liquid Chromatography (HPLC) (Waters Model 510, U.S.A.) with 254㎚ UV detector or refractive index detector was used. Bruker IFS120HR Fourier Transform / Infrared spectrometer (FT/IR) (Germany) was used. The sample was treated with the grinder by Scientific Apparatus (Thomas Co., Philadelphia, U.S.A.).

3. Identification of major components in *Elsholtzia ciliata* and *Elsholtzia splendens*

After grinding *Elsholtzia ciliata* and *Elsholtzia splendens* below 50 mesh, a 10.0g grinded herb was added to the 250㎖ water, boiled for an hour and distilled to 120-140㎖ as distillate for about 2 hours by simple distillation apparatus. The distillate was extracted three times with 50㎖ portions of diethyl ether in 500㎖ separatory funnel. After major components were transported to diethyl ether layer more than 99.5%, anhydrous sodium sulfate (10-20g) was added to the etherial solution and organic layer was collected and concentrated under vacuum below 35℃. The concentrated organic layer was diluted with 1 ㎖ of methanol to make analytical sample solution for qualitative and quantitative analyses of *Elsholtzia ciliata* and *Elsholtzia splendens*. The 0.5㎕ of sample solution was injected to Finnigan GC/MS for seperation and the experimental conditions for GC/MS were listed at Table I. The compounds were identified by the comparison of the IR, UV and MS spectrum of the literature values.

4. Separation of major components of *Elsholtzia splendens*

The concentrated solution of organic layer of *Elsholtzia splendens* was purified by semi-preparative normal phase HPLC with 254㎚ UV detector or IR detector and the ㎛(micro)-porasil column (25㎝ × 1㎝ i.d., Waters Co., U.S.A.) was used. The mixture of n-hexane : methylene chloride (1:1) was used as a mobile phase and the flow rate was 2.5㎖/min. The two major components from *Elsholtzia splendens* were collected followed by sequential concentration and structural determination was done by the spectrometer.

Table I. Seperation condition (GC/MS) for the determination of active ingredients in *Elsholtzia ciliata* and *Elsholtzia splendens*

GC : Varian 3400		
column : DB-5(30m×0.25㎜×0.25㎛)		
oven :	initial temperature	: 100℃ for 3min
	final temperature	: 250℃ for 5min
	ramp	: 10℃/min
injector temperature		: 250℃
manifold temperature		: 220℃
carrier gas : He (3.5㎖/min)		
MS : Finnigan Magnum Ion Trap Mass Spectrometer		
mass scan range :45 - 450 amu		
solvent delay : 180 sec		
ionization mode : EI at 70eV		
emission current : 10㎂		

Ⅲ. Results and Discussion

1. Analysis of major components in *Elsholtzia ciliata* and *Elsholtzia splendens*

The extracts of *Elsholtzia ciliata* and *Elsholtzia splendens* were obtained after boiling the grinded herbs of *Elsholtzia ciliata* and *Elsholtzia splendens* with 250㎖ water for an hour, distillation to 120-140㎖ as distillate, extraction with 50㎖ portions of diethyl ether (three times) and concentration *in vacuo*. The analytical samples of *Elsholtzia ciliata* and

Elsholtzia splendens were prepared by the dilution of the concentrated organic extracts with 1㎖ of methanol for further qualitative and quantitative analyses, respectively. The 0.5 ㎕ of sample solution was injected to Finnigan GC/MS for the analysis of each herb. The quantitative analytical TIC chromatogram for *Elsholtzia ciliata* and *Elsholtzia splendens* by GC/MS is shown at Figure 1. Based on the chromatogram of quantitative analysis of *Elsholtzia ciliata*, a 3.26% of linalool was appeared at the retention time of 4.27 min, a 2.87% of cumene at 4.97 min, a 2.87% of elsholtzia ketone at 7.90 min, a 1.24% of 4-vinylguaiacol at 9.77 min, a 1.13% of naginata ketone isomer at 9.95 min, a 2.71% of naginata ketone at 10.88 min, a 1.45% of isoosmorhizole at 11.63 min, a 33.7% of myristicin at 13.72 min, a 0.93% of sesquiterpene alcohol at 14.48 min, a 2.92% of $C_{10}H_{16}$ isomer at 7.48 min, a 1.57% of $C_{10}H_{14}O_2$ isomer at 12.42 min and a 6.68% of $C_{12}H_{16}O_3$ isomer at 13.85 min. On the other hand, the quantitative chromatogram of *Elsholtzia splendens* showed a 0.86% of linalool at the retention time of 4.27, a 0.54% of cumene at 4.97 min, a 15.1% of elsholtzia ketone at 7.90 min, a 26.1% of naginata ketone isomer at 9.95 min, a 1.10% of naginata ketone at 10.88 min, a 2.29% of myristicin at 13.72 min, a 1.80% of sesquiterpene alcohol at 14.48 min, and a 1.20% of $C_{12}H_{16}O_3$ isomer at 13.85 min.

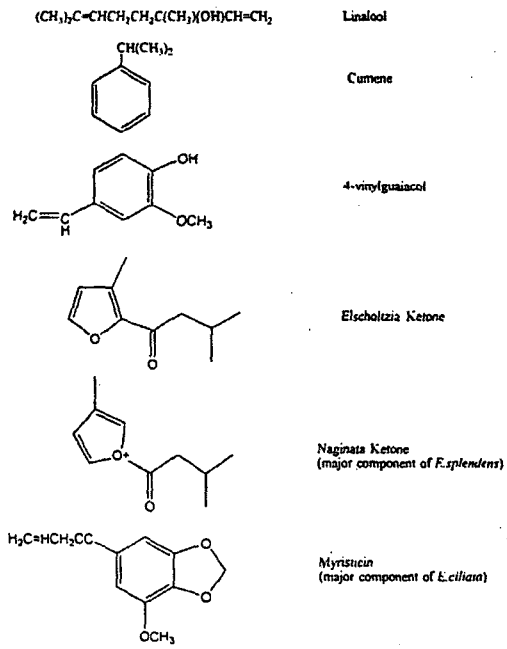
But in the *Elsholtzia splendens*, both 4-vinylguaiacol and isoosmorhizole were absent compared to the chromatogram of *Elsholtzia ciliata*. The result is summerized at Table II.

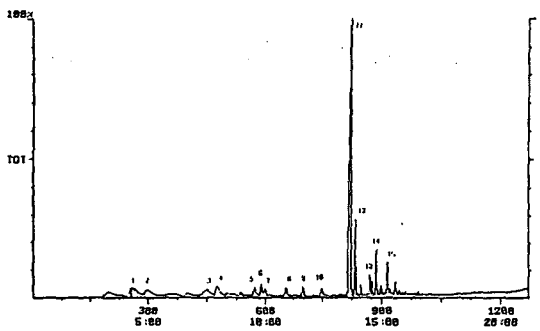
Table II. Comparison of active ingredients between *Elsholtzia ciliata* and *Elsholtzia splendens* by GC/MS (Unit : total area % ratio)

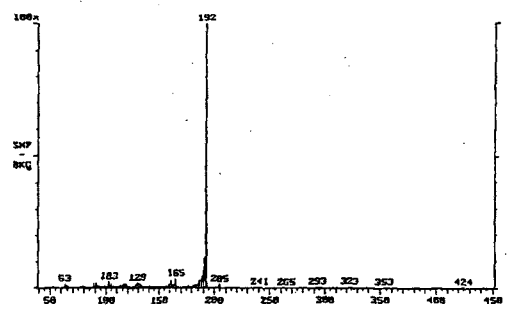
	retention time(min)	compound name	molecular formular	MW	*E. ciliata*	*E. splendens*
1	4.27	linalool	$C_{10}H_{18}O$	154	3.26	0.86
2	4.97	cumene	C_9H_{12}	120	2.87	0.54
3	7.48	unknown	$C_{10}H_{16}$	136	2.92	-
4	7.90	elsholtzia ketone	$C_{10}H_{14}O_2$	166	2.87	**15.1**
5	9.48	unknown	-	-	1.41	-
6	9.77	4-vinylguaiacol	$C_9H_{10}O_2$	150	1.24	-
7	9.95	**naginata ketone isomer**	$C_{10}H_{12}O_2$	164	1.13	**26.1**
8	10.31	unknown	-	-	-	**7.67**
9	10.88	naginata ketone	$C_{10}H_{12}O_2$	164	2.71	1.10
10	11.63	isoosmorhizole	$C_{11}H_{14}O_2$	178	1.45	-
11	11.88	sesquiterpene류	-	-	-	0.75
12	12.10	sesquiterpene류	-	-	-	1.16
13	12.42	unknown	$C_{10}H_{14}O_2$	166	1.57	0.04
14	13.72	**myristicin**	$C_{11}H_{12}O_3$	192	**33.7**	2.29
15	13.85	unknown	$C_{12}H_{16}O_3$	208	**6.68**	1.20
16	14.48	sesquiterpene alcohol	$C_{15}H_{24}O$	220	0.93	1.80
17	14.73	#15 isomer	$C_{12}H_{16}O_3$	208	1.06	1.91
18	15.20	#15 isomer	$C_{12}H_{16}O_3$	208	**4.80**	-

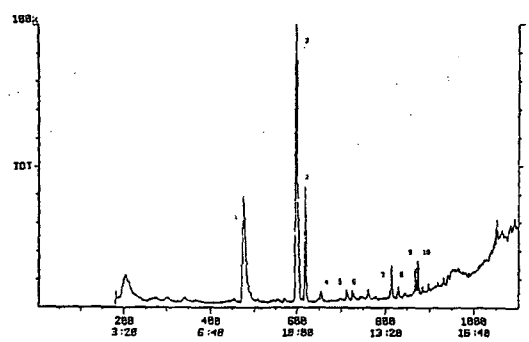
Also the chemical structures of major compounds for both species are shown in Figure 2. The mass spectrum of myristicin and naginata ketone isomer are shown at Figures 3 and 4, respectively.

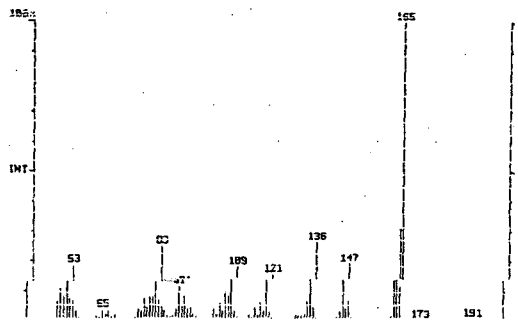
The chromatogram indicated that more hydrophobic component or heavier ingredients were contained in *Elsholtzia ciliata*. And the chromatogram of *Elsholtzia splendens* showed the characteristics of polar and smaller molecules. In *Elsholtzia ciliata*, myristicin (33.7%) is a major component which can differentiate with *Elsholtzia splendens*, where the naginata ketone isomer (26.1%) and elsholtzia ketone (15.1%) are the major ingredients.

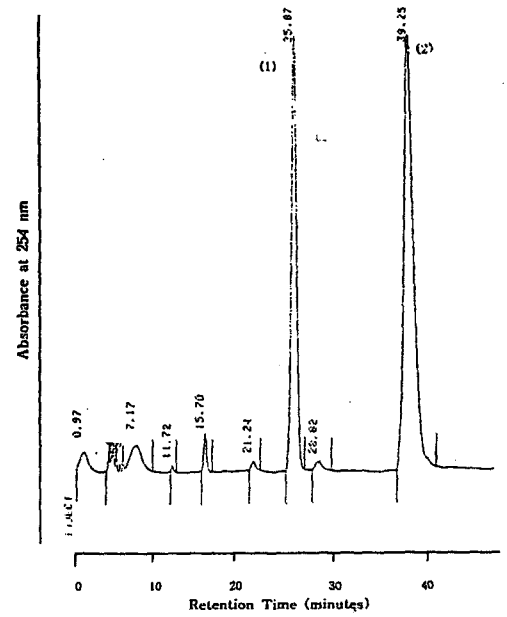


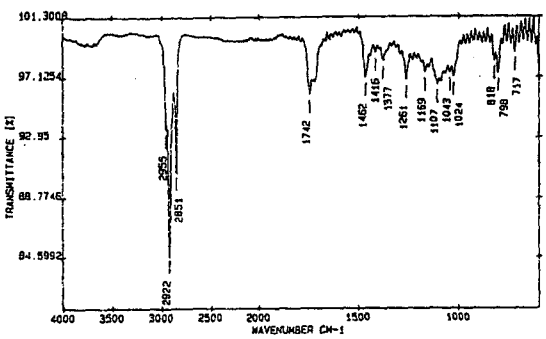


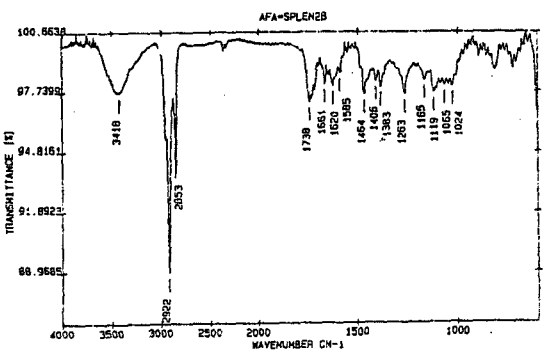












IV. Conclusion

There was a significant difference between the components of *Elsholtzia ciliata* and *Elsholtzia splendens* in the aspect of major compound.

Myristicin (33.7%) has been shown as a major component in *Elsholtzia ciliata* whereas naginata ketone isomer (26.1%) and elsholtzia ketone (15.1%) were believed as major ingredients in *Elsholtzia splendens*. The common compounds in two species were identified as linalool, cumene, elsholtzia ketone, naginata ketone isomer, naginata ketone, myristicin and sesquiterpene alcohol. But in the *Elsholtzia splendens*, both 4-vinylguaiacol and isoosmorhizole were absent compared to the *Elsholtzia ciliata*. According to the qualitative and quantitative analysis of two species, their major components and structual formular are found to be different each other.

For the quality control of Hyang-yu (香薷), the accurate differenciation between *Elsholtzia ciliata* (Thunb.) Hylander (향유) and *Elsholtzia splendens* Nakai ex F. Maekawa (꽃향유) is in need and it was clearly demonstrated in this article.

But further clinical investigation will be necessary for the proper medicinal usage of Hyang-yu (香薷) in the future.

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