

Study on the Difference between Volatile Constituents of the Different Parts from *Elsholtzia ciliata* by SHS-GC-MS

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Abstract

In this study, the comparison of *Elsholtzia ciliata* volatile compounds from the stem (with leaf) and flower was acquired. The volatile compounds of these two parts from *Elsholtzia ciliata* aerial parts were respectively analyzed by a rapid and convenient static headspace injection technique coupled with gas chromatography-mass spectrometry and the relative contents of each constituent between the two different parts were determined by peak area normalization. 61 compounds were identified in the stem (with leaf) and flower, among which 39 components simultaneously exist. 47 and 53 compounds were separated and identified in the volatile compounds from the different parts of *Elsholtzia ciliata*, respectively. There were differences among some volatile constituents of the two parts, but the main constituents were all *Elsholtzia* ketone, caryophyllene, 3-octanol and Dehydroelsholtzia ketone. The most abundant components in the stem (with leaf) included *Elsholtzia* ketone (84.20%), caryophyllene (4.3%) and 3-octanol (3.11%), while *Elsholtzia* ketone (88.03%), caryophyllene (3.33%) and 3-octanol (1.53%) were the main components in the flower. 8 single constituents were identified in the stem (with leaf) volatiles including 3-heptanone and linalool, while 14 constituents only including 2-methylbutanoic acid and Perillene have been found in the flower volatiles. *Elsholtzia* ketone was the main compound, and its concentration was significantly higher than other substances (>80% of the total oils in *Elsholtzia ciliata*). The study provided an important scientific base for the further utilization of *Elsholtzia ciliata* resources and may be helpful for systematically understanding the constituents of volatile compounds of *Elsholtzia ciliata*.

Keywords

Elsholtzia ciliata, Volatile Constituents, Static Headspace Injection

1. Introduction

Elsholtzia ciliata (Thunb.) Hyland. (the Chinese name “Xiang Ru”) is an annual herb that belongs to the *Elsholtzia* species that is widely distributed in China, Korea and Europe, and as a folk medicine in China for thousands of years.

There has been an increasing interest in *Elsholtzia* species because of its long history for the treatment of cold, fever, emesis, edema, diarrhea, edema, rheumatic arthritis, nephritis and so forth. *Elsholtzia ciliata* contains many bioactive constituents, including flavonoids, terpenes and essential oil components. Modern pharmacological studies have shown the effect of antibacterial, antiviral, analgesic, antifebrile, antioxidant, antispasmodic, diuretic, antitussive, expectorant and antiasthmatic activities. Their chemical compositions are diverse, including several classes of flavonoids, phenylpropanoids, terpenes and other compounds. Several studies on the chemical constituents of *Elsholtzia ciliata* have reported the presence of abundant volatile components [1] [2].

Elsholtzia ciliata, as a kind of aromatic plants with fragrant aroma, is rich in volatile component which is one of its main effective components. The oils of some *Elsholtzia* species show therapeutical efficacy against fever, cold, vomiting, heat stroke, edema and the enhancement of immunity [3] [4], which have a significant medicinal value in clinical application.

Different methods are available to analyze the essential oils in *Elsholtzia ciliata* including steam distillation and extraction with organic solvents. Conventional analytical techniques, such as solvent extraction and steam distillation, have been used to analyze the essential oils in *Elsholtzia ciliata*. Mostly, steam distillation requires a relatively large amount of sample, and it is time-consuming. Only a few extractions with carbon dioxide methods—supercritical fluid extraction (SFE) were also applied for this purpose [5] [6]. It has been widely adopted in headspace (HS) sampling which is a fundamental technique to characterize the volatile fraction of aromatic plants.

Due to the advantage of simple, rapid, low-cost and solvent-free, HS technique offers a more useful tool than the conventional analytical techniques [7]. However, there is little paper reported that HS was applied to analyze *Elsholtzia ciliata*. Depending on the applied method, the results of our experiments have shown that static headspace (HS) is a simple and fast technique because no sample preparation or solvent is needed. Therefore, the direct coupling with a mass spectrometer could carry out the detection of *Elsholtzia ciliata* oils. In this work, HS methods coupled with GC-MS were applied for extraction and identification of volatiles of the *Elsholtzia ciliata* stem (with leaf) and flower. The comparison of the volatiles of the *Elsholtzia ciliata* stem (with leaf) and flower was performed by using HS-GC-MS.

Compared with the conventional methods, HS-GC-MS provides a simple preparation, small amounts of the sample, short times to extract the volatile constituents, and has no use for organic solvent. As the stem (with leaf) and flower volatiles of *Elsholtzia ciliata* were not yet investigated in detail, it is reported here the results obtained by HS-GC-MS analyses on the volatile compounds of the *Elsholtzia ciliata* stem (with leaf) and flower.

In this work, a direct, reliable and effective automated method has been built for the determination of the different portions of the plant based on headspace technique. Separation, identification and quantization were carried out by HS-GC-MS. Sample pretreatment or clean-up were not necessary, as samples are put directly into an HS vial, automatically processed and then injected to the GC-MS for chromatographic analysis.

The plant consisted of varies of tissues and organs. Generally, chemical compositions of the plant are probably a whole macro characteristic referring to all the plant parts. However, only a few researches on the comparison of different parts of the plant. The description of medicinal parts in the clinical application mostly used the whole plant or taken from a certain parts of the plant, such as only using roots, rhizomes, leaves, flowers or fruits-divided into the medicinal parts and non-medicinal parts [8]. The medicinal parts and non-medicinal parts have certain differences in medicinal properties and pharmacological effects. In addition, the harvesting time and location may also affect the differences, leading to the lack of unified standard for the selection of the medicinal parts. In practice, the correlation of different parts was always ignored and the non-medicinal part was often discarded as waste. To some extent, the different plant parts may act synergistically in ways we don't know. Full utilization of plant resources is an unsolved problem due to the lack of unified standard for the selection of medicinal parts.

The medicinal parts of *Elsholtzia ciliata* were the aerial parts in the Chinese Pharmacopoeia, flowering whole parts is recorded in the Traditional Chinese Medicine Dictionary, while the whole parts or aerial parts in the Chinese Materia Medica. Roots were picked-off in order to obtain the processed products. In ancient china, only the *Elsholtzia ciliata* leaves or the flowers and leaves were used together. Most experiments are carried out by using the *Elsholtzia ciliata* aerial parts. Researches on the pharmacological effect of the different parts from *Elsholtzia ciliata* have shown a certain difference [9]. In China, the *Elsholtzia ciliata* have been applied to the treatment of lots of diseases for a long time. However, up to date, the medicinal parts are still unclear. However, the volatile components of the different *Elsholtzia ciliata* parts were not investigated in detail. In our project, efforts were devoted to analysis and compare the difference of the volatiles of the different *Elsholtzia ciliata* parts by HS-GC-MS.

The whole plants contained 0.2% - 1% essential oils, the fresh stems and flowers contained 0.26% - 0.59% essential oils and the dried stems and flowers contained 0.8% - 2% essential oils. Respectively, with the main chemical classes: El-

sholtziane (85%) and Acetophenone (1%). In order to figure out whether the differences existed or not, this project aimed to quickly compare the volatiles of different parts of *Elsholtzia ciliata* with regards to the conventional direct HS-GC-MS method. In the proposed method, sampling with HS technique for GC analysis is a rapid and effective method for the analysis of volatiles in samples. Based on the chemical compounds, the project revealed the differences in chemical composition of different parts, explored the possibility of material basis of Traditional Chinese Medicine and the pharmacological effects, selected the medicinal parts, strengthened quality management and to standardize clinical application.

2. Materials and Methods

2.1. Plant Materials

The aerial parts of *Elsholtzia ciliata* were collected from Qixia Mountain, Shandong, China in the middle of September, 2012, and were authenticated by Associate Professor Sun Zhiying from the Department of Pharmacy, Shan Dong University of Traditional Chinese Medicine in China. The two parts of stem (with leaf) and flower samples were separated from the aerial parts in our laboratory, respectively. These two parts were stored in the dark at room temperature until use. The dried stems (with leaves) and flowers were respectively ground into powder (40 mesh) and kept at a desiccators prior to analysis.

2.2. HS-GC/MS Analyses

2.2.1. Extraction of Volatile Components

Dried stems (with leaves) and flowers powder (0.5 g) were precisely weighed into 10 mL HS vial and sealed hermetically with a PTFE-silicone septum and an aluminum cap and then carefully marked, respectively. An Agilent 7694 EAutomatic sampler (Agilent Technologies, USA) was used as a HS sampler. The HS sampling parameters were set as follows: Vial temperature, 120 °C; Loop temperature, 140 °C; Transfer-line temperature, 160 °C; Vial equilibration, 30 min; Loop equilibration, 0.05 min; sample injection, 1 min.

2.2.2. GC-MS Analyses

The analysis of volatile compounds of the two parts obtained by HS was performed on an Agilent 6890-5975B GC-MS system (Agilent Technologies, USA). The chromatography conditions were as follows: HP-5MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness); helium as the carrier gas, flow rate of 1.0 mL·min⁻¹ and with split ratio of 3:1; the injector temperature was at 250 °C. The temperature programmed as follows: from 40 °C (3 minutes) to 100 °C at a rate of 2 °C·min⁻¹, from 100 °C (2 minutes) to 150 °C at a rate of 1 °C·min⁻¹, and then from 150 °C (2 minutes) to 280 °C at a rate of 10 °C·min⁻¹, with a final hold of 5 minutes at 280 °C.

The mass spectrometer was fitted with EI source operated 70 eV, and mass spectra were recorded in the m/z range of 50 to 550 m/z with full-scan acquisi-

tion mode. A “Standard Tune” option was used. The ion source of the mass spectrometer was set at 230°C. Quadrupole temperature and transfer-line temperature were 150°C and 280°C, respectively.

3. Results and Discussions

The analysis of the two different parts from *Elsholtzia ciliata* was performed on the above mentioned HS-GC-MS conditions; the total ionization chromatography of the volatiles of the two different parts was shown in **Figure 1** and **Figure 2**. The separated volatile components were identified by the NIST MS library search (NIST MS Search 2.0) and comparison with literatures [10]-[19]. The relative contents of each constituent from the volatile constituents were determined by area normalization method. The identified compound with their relative amounts from *Elsholtzia ciliata* stem (with leaf) and flower was summarized in **Table 1**. The comparison of the major constituents between the two different

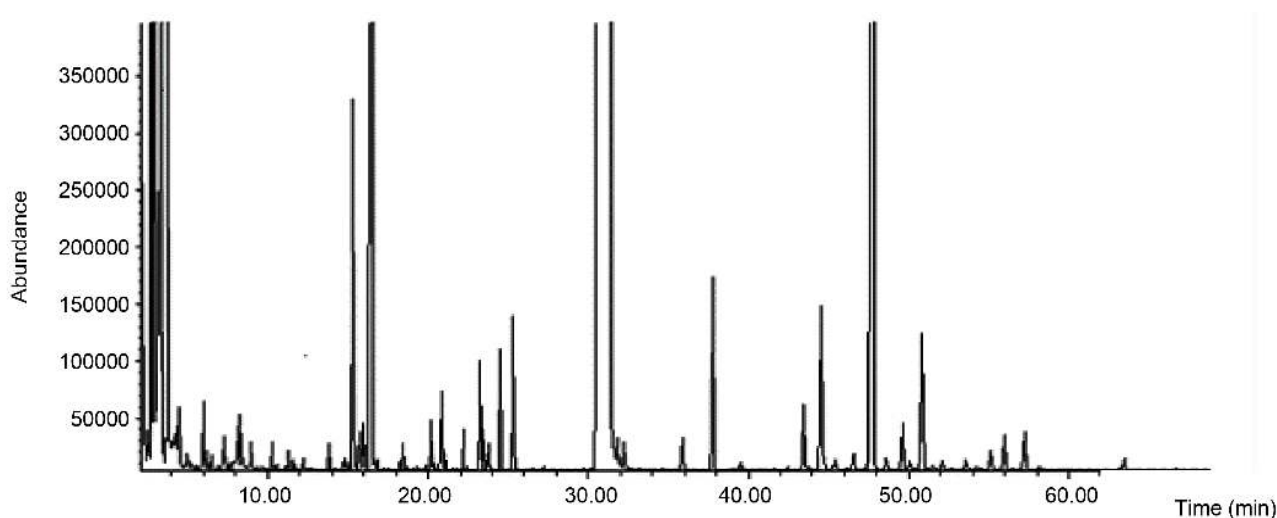


Figure 1. Total ionization chromatography of volatile compounds of *Elsholtzia ciliata* stem and leaf by HS-GC-MS.

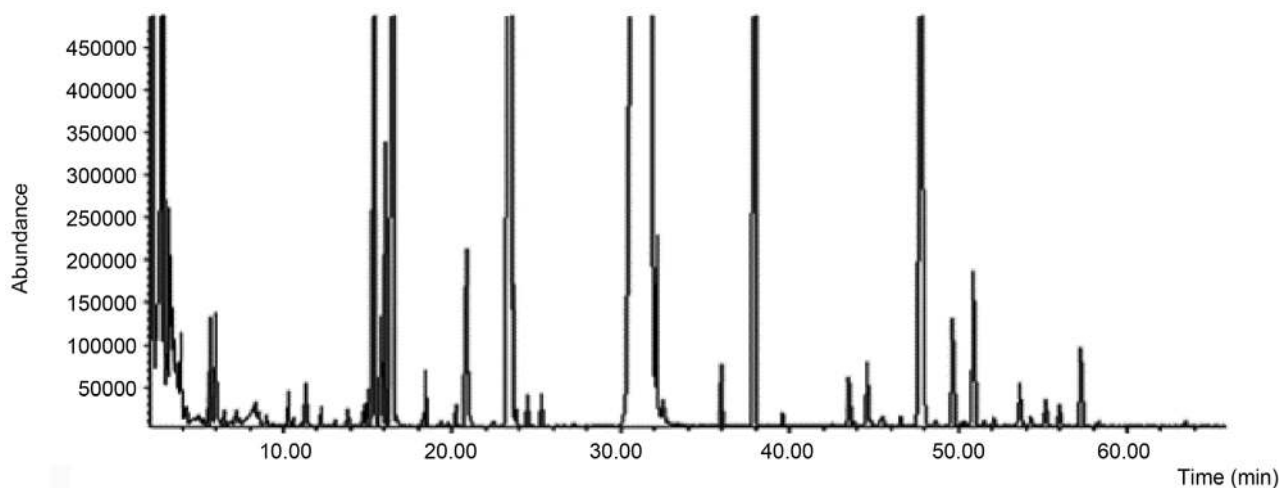


Figure 2. Total ionization chromatography of volatile compounds of *Elsholtzia ciliata* flower by HS-GC-MS.

parts was listed in **Table 2**. In total, 61 compounds (more than 90% of match scores) were identified in the two different parts. 47 compounds accounting for 96.07% of total volatiles were identified in the *Elsholtzia ciliata* stem (with leaf). 53 compounds constituting 97.94% of total volatiles were identified in the *Elsholtzia ciliata* flower (**Table 1**). 13 main compounds were found in both the two different parts, but each of them exhibited different relative contents (**Table 2**).

Seen from **Table 1**, a total of 61 compounds were identified in the two different parts, 47 compounds constituting 96.07% of the total volatiles were identified in stems (with leaves) with Elsholtzia ketone being the main constituent (84.20%) and 53 compounds accounting for 97.94% of the total volatiles were identified in flowers with Elsholtzia ketone being the main component (88.03%). 39 compounds were found both in the two different parts. 8 compounds were only detected in the stem and leaf, or shown extremely low content in the flower. In contrast, 14 compounds were only detected in the flower, or were little contained in the stem and leaf. The differential peak accounted for a very small but significant proportion, indicating that the types of the volatile components contained in the stem and leaf were different to some extent with those contained in the flower of *Elsholtzia ciliata*. The difference can serve as a basis to quickly analysis the volatile components contained in different parts of *Elsholtzia ciliata*.

Several literatures reported the volatile components of this plant [13]-[19] used different techniques of extractions including HD, SFE and HS-SPME, they showed significant differences in the composition of the volatile constituents. Saini R *et al.* [19] firstly made a comparative evaluation of the constituents using those three different extraction procedures, the proposed HS method is simple, fast and inexpensive without complicated sample pre-treatment. γ -Terpinene, Perillene, 1-Octen-3-yl-acetate, Linalool, Geranyl acetate, Caryophyllene, Humulene, Germacrene D were found as common constituents of these three extractions. 3-Octen-1-ol, Cubebene and Cadinene were only found in SFE and HD samples. In our study, the compositions of the volatile components were mainly ketones, terpenes, alcohols, aldehydes and phenols, etc. (**Figure 3**). Other key marker volatile constituents of different species such as Elsholtzia ketone, Dehydroelsholtzia ketone and Thymol were also reported in the *Elsholtzia ciliata*. Linalool, Geranyl acetate have earlier been reported as one of the major constituents of *E. blanda*, but was absent from all the other species. It was identified in low percentage in the present investigation. Perillene is one of the key marker volatile constituents of *E. fruticosa* oil, but was identified in low percentage in the *Elsholtzia ciliata* flower.

Some alcohols and ketones with a lower boiling point and earlier RT were only detected in the stem and leaf such as Linalool; some terpenes with a higher boiling point and later RT were only detected in the flower, among which a major active component, Perillene, showed a content of 0.22%. The different composition was manifested as alcohols and ketones mainly in the stem and leaf and terpenes mainly in the flower. The main constituents of the volatile components

Table 1. HS-GC-MS analytical results of volatile compounds from the two different parts of *Elsholtzia ciliata*.

No	RT/min	Compound	Formula	Relative Content/%	
				stem (with leaf)	flower
1	5.51	3-Hexanone	C ₆ H ₁₂ O	—	0.01%
2	5.67	(R, R)-2,3-Butanediol	C ₄ H ₁₀ O ₂	—	0.06%
3	5.99	Hexanal	C ₆ H ₁₂ O	0.09%	0.09%
4	6.15	2,3-Butanediol	C ₄ H ₁₀ O ₂	0.03%	—
5	7.24	Furfural	C ₅ H ₄ O ₂	0.06%	0.03%
6	7.37	3-Heptanone	C ₇ H ₁₄ O	0.03%	—
7	8.13	3-Methylpentanoic acid	C ₆ H ₁₂ O ₂	0.11%	0.06%
8	8.31	3-Hexen-1-ol	C ₆ H ₁₂ O	0.11%	0.04%
9	8.56	2-Methylbutanoic acid	C ₅ H ₁₀ O ₂	—	0.03%
10	8.81	p-Xylene	C ₈ H ₁₀	0.01%	—
11	8.97	1-Hexanol	C ₆ H ₁₄ O	0.04%	0.01%
12	10.28	2,5-Diethyltetrahydrofuran	C ₈ H ₁₆ O	0.04%	0.03%
13	10.59	Heptanal	C ₇ H ₁₄ O	—	0.01%
14	11.34	β-Pinene	C ₁₀ H ₁₆	0.02%	0.05%
15	12.23	α-Pinene	C ₁₀ H ₁₆	0.02%	0.02%
16	13.05	Camphene	C ₁₀ H ₁₆	—	0.01%
17	13.84	Benzaldehyde	C ₇ H ₆ O	0.04%	0.02%
18	14.78	α-Thujene	C ₁₀ H ₁₆	0.03%	0.02%
19	15.01	2-Acetyl-5-methylfuran	C ₇ H ₈ O ₂	—	0.04%
20	15.38	3-Octen-1-ol	C ₈ H ₁₆ O	0.61%	0.69%
21	15.78	3-Octanone	C ₈ H ₁₆ O	0.08%	0.12%
22	16.03	β-Myrcene	C ₁₀ H ₁₆	0.07%	0.24%
23	16.47	3-Octanol	C ₈ H ₁₈ O	3.11%	1.53%
24	16.68	Neodihydrocarveol	C ₁₀ H ₁₈ O	0.02%	0.01%
25	16.82	Octanal	C ₈ H ₁₆ O	0.02%	—
26	17.51	Isoterpinolene	C ₁₀ H ₁₆	—	0.01%
27	18.31	D-Limonene	C ₁₀ H ₁₈ O	0.01%	0.01%
28	18.45	Eucalyptol	C ₁₀ H ₁₈ O	0.04%	0.05%
29	20.23	Menthone	C ₁₀ H ₁₈ O	0.08%	0.02%
30	20.44	γ-Terpinene	C ₁₀ H ₁₆	0.01%	—
31	20.85	Acetophenone	C ₈ H ₈ O	0.15%	0.26%
32	23.28	Thymol	C ₁₀ H ₁₄ O	0.17%	0.58%
33	23.50	Linalool	C ₁₀ H ₁₈ O	0.08%	—
34	23.60	Perillene	C ₁₀ H ₁₄ O	—	0.22%
35	23.80	Nonanal	C ₉ H ₁₈ O	0.04%	0.02%
36	24.51	Octen-1-ol, acetate	C ₁₀ H ₁₈ O ₂	0.19%	0.03%
37	25.33	3-Octanol, acetate	C ₁₀ H ₂₀ O ₂	0.25%	0.03%
38	26.19	Camphor	C ₁₀ H ₁₆ O	—	0.01%
39	31.37	Elsholtzia ketone	C ₁₀ H ₁₄ O ₂	84.20%	88.03%

Continued

40	35.96	Eucarvone	C ₁₀ H ₁₄ O	0.06%	0.07%
41	37.80	Jasmone	C ₁₁ H ₁₆ O	—	0.01%
42	37.97	Dehydroelsholtzia ketone	C ₁₀ H ₁₂ O ₂	0.41%	1.23%
43	38.70	Thymol acetate	C ₁₂ H ₁₆ O ₂	—	0.01%
44	43.48	Geranyl acetate	C ₁₂ H ₂₀ O ₂	0.15%	0.06%
45	44.57	β -Bourbonene	C ₁₅ H ₂₄	0.45%	0.08%
46	44.85	Artemesiatriene	C ₁₀ H ₁₆	—	0.01%
47	45.46	π -Gurjunene	C ₁₅ H ₂₄	0.04%	0.02%
48	46.60	Isocaryophyllene	C ₁₅ H ₂₄	0.04%	0.02%
49	47.82	Caryophyllene	C ₁₅ H ₂₄	4.3%	3.33%
50	48.60	π -Cubebene	C ₁₅ H ₂₄	0.03%	—
51	49.63	α -Bergamotene	C ₁₅ H ₂₄	0.12%	0.17%
52	50.06	π -Muurolene	C ₁₅ H ₂₄	0.02%	—
53	50.88	Humulene	C ₁₅ H ₂₄	0.37%	0.24%
54	52.10	π -Sesquiphellandrene	C ₁₅ H ₂₄	0.02%	0.01%
55	53.62	Germacrene D	C ₁₅ H ₂₄	0.03%	0.07%
56	54.29	π -Bisabolene	C ₁₅ H ₂₄	—	0.01%
57	55.16	γ -Elemene	C ₁₅ H ₂₄	0.05%	0.04%
58	55.99	(Z, E)- α -Farnesene	C ₁₅ H ₂₄	0.09%	0.03%
59	57.26	Nerolidol	C ₁₅ H ₂₆ O	0.1%	0.12%
60	58.13	π -Cadinene	C ₁₅ H ₂₄	—	0.01%
61	63.46	Caryophyllene oxide	C ₁₅ H ₂₄ O	0.03%	0.01%

Table 2. Analytical results of the main volatile compounds from the two different parts of *Elsholtzia ciliata*.

No	RT/ min	Compound	Formula	Relative Content/%	
				Stem (with leaf)	Flower
1	15.38	3-Octen-1-ol	C ₈ H ₁₆ O	0.61%	0.69%
2	16.03	β -Myrcene	C ₁₀ H ₁₆	0.07%	0.24%
3	16.47	3-Octanol	C ₈ H ₁₈ O	3.11%	1.53%
4	20.85	Acetophenone	C ₈ H ₈ O	0.15%	0.26%
5	23.28	Thymol	C ₁₀ H ₁₄ O	0.17%	0.58%
6	31.37	Elsholtzia ketone	C ₁₀ H ₁₄ O ₂	84.20%	88.03%
7	37.97	Dehydroelsholtzia ketone	C ₁₀ H ₁₂ O ₂	0.41%	1.23%
8	43.48	Geranyl acetate	C ₁₂ H ₂₀ O ₂	0.15%	0.06%
9	44.57	β -Bourbonene	C ₁₅ H ₂₄	0.45%	0.08%
10	47.82	Caryophyllene	C ₁₅ H ₂₄	4.30%	3.33%
11	49.63	α -Bergamotene	C ₁₅ H ₂₄	0.12%	0.17%
12	50.88	Humulene	C ₁₅ H ₂₄	0.37%	0.24%
13	57.26	Nerolidol	C ₁₅ H ₂₆ O	0.10%	0.12%

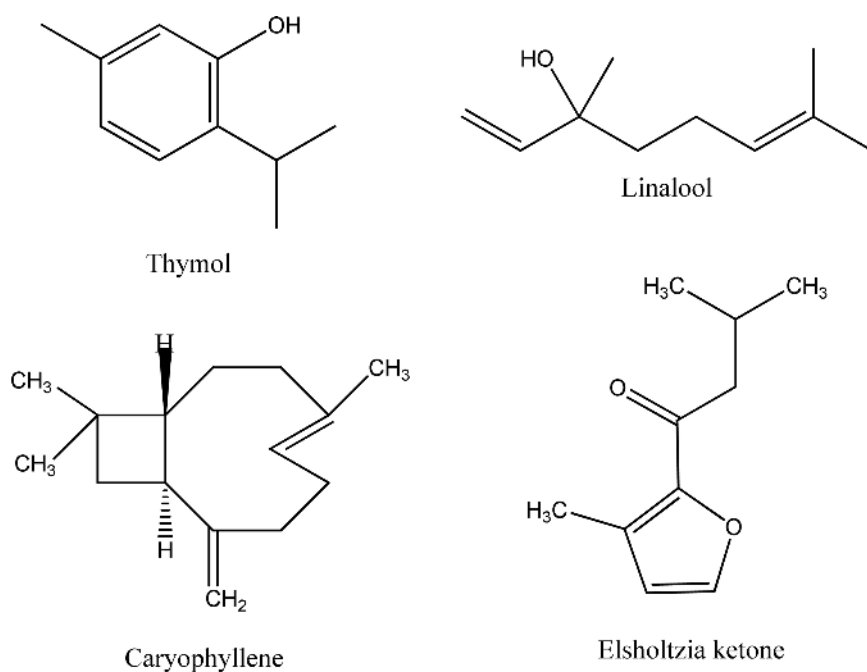


Figure 3. Typical constituents, elsholtziaketone, caryophyllene, thymol and linalool.

were similar in the two parts of *Elsholtzia ciliata*, while the flower contained a slightly higher content of volatile components than the stem and leaf. 39 compounds were acquired in two different parts, including Elsholtzia ketone, Caryophyllene, 3-Octanol, Dehydroelsholtzia ketone, 1-Octen-3-ol, Thymol, β -Bourbonene, Acetophenone and so on. Elsholtzia ketone, which is regarded as a typical compound to identify *Elsholtzia ciliata*, showed the highest content in both parts (88.03% in the flower and 84.20% in the stem with leaf).

Seen from **Table 2**, the main volatile components of more than 0.1% were almost same in both parts. In terms of the content, Caryophyllene accounted for a secondary content, 4.3% and 3.33% in the total quantity of volatile components (with certain difference in contents), respectively. The other components with a content of more than 0.1% were common among these two parts, varying in their contents in certain degrees. It could be concluded that, the main compositions of the volatile components in two parts were similar and shared a large number of components, only different in their contents. Thus we could further infer that both parts of *Elsholtzia ciliata* may have similar pharmacologic actions.

The present study shows only the comparison result between two different parts of the aerial parts of *Elsholtzia ciliata* collected at the same harvesting period and region while further work is needed to determine whether the comparison result of both parts at different harvesting periods is consistent with that obtained at the same harvesting period and region.

On the one hand, both parts share many components and their main components are similar, accounting for a large proportion; however, there are also difference in some compositions and contents. Such a difference is possibly related

with the synthetic position and distribution of chemical components inside the plant. On the other hand, the differential peaks account for a small but significant proportion, attention should be paid to the existence of the differential peaks and the content control of various main components in selection of medicinal parts.

4. Conclusions

The HS-GS-MS technique is a rapid and efficient technique for the analysis of volatiles in samples with many advantages. In this project, it was successfully applied to the rapid analysis and comparison of the volatiles of the two different parts from *Elsholtzia ciliata* harvesting at the same time and region. For this method, the sample amount taken was only 0.5 g and operated in a highly automated way. The volatiles obtained by the method, when compared with hydro-distillation reported in some literatures, are that HS-GS-MS technique is more suitable for the analysis of the low boiling compounds.

In our experiments, a large amount of common constituent was detected in the two different parts of *Elsholtzia ciliata*. For the majority of compounds, the main constituent obtained in these two different parts was both the *Elsholtzia* ketone, accounting for more than 80% in the total volatiles. Other previous reports have studied that *Elsholtzia* ketone was the main bioactive components of *Elsholtzia ciliata*. Our results have shown that these two different parts both have a certain medicinal value in a degree. Therefore, it's significant to select the *Elsholtzia ciliata* aerial parts in the clinical application.

There is a difference observed between the contents of the volatile components of the two different parts. Although the differences were small and not obvious, it can still provide basis for comparison of the two different parts. Eventually, these findings provide a reference for further exploitation of *Elsholtzia ciliata*. The experimental results suggest that the proposed method in analysis of volatile compounds of *Elsholtzia*, has less sample consumption, no organic solvent extraction, and highly automatic analysis process. It can be applied for the fast analysis and distinguishing of the volatile compounds in *Elsholtzia ciliata* stems (with leaves) and flowers.

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