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# Comparison of volatile constituents in two types of mugwort leaves (produced in Qichun and Nanyang) using the headspace GC-MS

## 顶空进样 GC-MS 比较蕲春与南阳产艾叶的挥发性成分

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**Abstract**

**Objective:** To compare the volatile constituents in mugwort leaves produced in Qichun, Hubei Province and Nanyang, Henan Province.

**Methods:** The volatile constituents were extracted using headspace heating and analyzed using gas chromatography-mass spectrometry (GC-MS). Then a qualitative analysis was made according to the standard database provided by the National Institute of Standards and Technology (NIST) and the relative contents of each constituent were calculated using the peak area normalization method.

**Results:** A total of 59 compounds were identified from the mugwort leaves from Qichun and 51 compounds were identified from the mugwort leaves from Nanyang. These mainly include monoterpenoids, sesquiterpenoids, $C_xH_yO_z$ and other compounds involving the aldehyde, ketone, alkane and benzene. The mugwort leaves from Qichun and Nanyang share 32 common volatile constituents. The chromatographic peak area of identified compounds accounting for 96.38% of GC-MS total chromatographic peak areain Qichun mugwort leaves, versus 95.54% of that in Nanyang mugwort leaves.

**Conclusion:** The headspace heating extraction combined with GC-MS technology can evidently display similarities and differences of volatile constituents in mugwort leaves produced in different areas and thus provide scientific basis for the quality and screening of mugwort leaves.

**Keywords:** Artemisia Argyi; Qichun; Nanyang; Volatile Organic Compounds; Gas Chromatography-mass Spectrometry; Headspace Sampler

**【摘要】目的:** 观察比较湖北蕲春与河南南阳两个产地艾叶的挥发性成分的种类。**方法:** 采用顶空加热提取艾叶挥发性成分并进行气相色谱-质谱(gas chromatography-mass spectrometry, GC-MS)分析, 结合美国国家标准与技术研究院(National Institute of Standards and Technology, NIST)提供的标准数据库进行定性分析, 以峰面积归一化法计算各组分的相对含量。**结果:** 从湖北蕲春产艾叶中鉴定出 59 种化合物, 从河南南阳产艾叶中鉴定出 51 种化合物, 主要为单萜类、倍半萜及其含氧衍生物, 及其他的醛、酮、烷及苯系化合物。两地艾叶药材挥发性成分中有 32 种共有成分, 具有一定差异; 所鉴定化合物的色谱流出峰面积占湖北蕲春艾叶 GC-MS 色谱总流出峰面积的 96.38%, 占河南南阳艾叶的 95.54%。**结论:** 顶空加热提取结合 GC-MS 技术可以便捷地揭示不同产地艾叶的挥发性成分异同, 为艾材的质量评价和筛选提供一定科学依据。

**【关键词】**艾叶; 蕲春; 南阳; 挥发性有机成分; 气相色谱-质谱法; 顶空进样
**【中图分类号】**R245.8　　**【文献标志码】**A

Mugwort leaves are dry leaves of herbaceous perennial plant *Artemisia Argyi*. This plant is widely distributed in northeastern, northern, eastern, southwestern and northwestern parts of China as well as neighboring Asian countries. Mugwort leaves are gathered in summer when the plant is in flower[1]. It has been used as a moxibustion material in traditional Chinese medicine for over 1 000 years.

In ancient times, our ancestors realized mugwort leaves that are produced in different areas and gathered in different times have different qualities. In addition, they may also be associated with clinical effects of

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moxibustion. According to the *Tu Jing Ben Cao* (*Illustrated Classic of Materia Medica*) by Su Song in the Song Dynasty (960-1279), mugwort leaves from Siming (now Ningbo, Zhejiang Province) and Fudao (now Tangyin County, Anyang, Henan Province) have the best quality[2] and are considered as Dao-di herbs. In the Ming dynasty (1368-1644), according to the *Ben Cao Pin Hui Jing Yao* (*Essentials of Materia Medica Distinctions*) and *Ben Cao Gang Mu* (*Compendium of Materia Medica*), mugwort leaves from Qichun County, Hubei Province have the best quality[3-4]. Evidently, ancient people realized that the quality of mugwort leaves is associated with the origins of production and believed the clinical effect of moxibustion is related to the quality of moxibustion materials.

Over the recent years, numerous studies have reported the effect of production origins, gather time, processing and storage on chemical components of mugwort leaves[5-9]. The quality of mugwort leaves varies greatly in production origin, gather time, processing and storage, particularly the production origin and years of storage. Currently, there are two high-quality brand names of mugwort leaves in China: *Qi* mugwort (from Qichun, Hubei Province) and *Bei* mugwort (from Nanyang or Tangyin, Henan Province). These two areas have large production of mugwort leaves and processed products. Qichun, Hubei Province (the hometown of Li Shi-zhen) has long been an origin of Dao-di herbs. Nanyang, Henan Province (the hometown of Zhang Zhong-jing) has now become the largest processing base of mugwort leaves.

The headspace sampling and heating extraction technology is used to heat the samples to enable the vapor phase (thermodynamic equilibrium) to be simultaneously in a closed system with samples being analyzed. Then the volatile constituents are specifically gathered for analysis. Compared with the liquid-liquid extraction and solid phase extraction, this extraction method can avoid loss of volatile components in removing solvent and at the same time reducing the extraction noise and has higher sensitivity and faster analysis. This method can protect the analytical column from being overloaded or contaminated by humidity, high-boiling compounds or non-volatile matter. In addition, this method causes minimal potential hazard to laboratory personnel and the environment and thus meets the requirement for green analytical chemistry[5]. Also, because of its convenience and reproducibility, this method is now widely used in analytical test of volatile constituents. Along with the advance in gas chromatography, the headspace analytical method has been greatly upgraded and developed. Today, modern headspace analysis can be combined with GC-MS technology, allowing for fast, sensitive, accurate and quantitative analysis of samples[5]. To provide scientific foundation for the quality assessment and screening of mugwort leaves, this study conducted comparative study on volatile components of mugwort leaves produced in Nanyang and Qichun and gathered in May using the headspace sampling and heating combined with GC-MS.

## 1 Materials and Instruments

Mugwort leaves in Qichun, Hubei Province. Time of gathering: 6th of June, 2014. Place of gathering: Song Yong base, Hongmenlou Village, Qichun Township, Hubei Province. Gatherer: Guo Shuang-xi.

Mugwort leaves in Nanyang, Henan Province. Time of gathering: 5th of June, 2014. Place of gathering: Wancheng district, Nanyang City, Henan Province. Gatherer: Huang Xian-zhang.

Trace 1300 Series TSQ800 GC-MS (Thermo Company, USA), TG-5MS GC capillary column (30 m × 0.25 mm × 0.25 μm, Thermo Company, USA), Xcalibur software containing NIST database (Mass Spectral Library & Search Software-Version 2014, Thermo Company, USA), 1/10 000 electronic analytical balance (Mettler Company, Switzerland) and Core TQ-2000Y Grinder (Yongkang Tianqi Shengshi Industry and Trade Co., Ltd., China).

## 2 Method

Ground the dry samples of mugwort leaves from two places into fine powder (approximately 10% of the total mass), weighted 1 g of moxa floss, placed into a 20 mL sample bottle and then covered the bottle with PETF tubing.

The Headspace GC-MS conditions: the 100 μm polydimethylsiloxane (PDMS) solid-phase micro-extraction (SPME) was used for 40-minute extraction at a temperature of 80 ℃. The inlet port temperature was 250 ℃, thermal desorption for 3 min. The GC-MS heating procedure of the column temperature was as follows: the temperature was first programmed at 40 ℃ (hold for 2.5 min), raised to 200 ℃ at a rate of 5 ℃/min and then to 240 ℃ at a rate of 10 ℃/min (hold for 5 min). The helium (He) was used as the carrier gas, coupled with un-split stream sampling with a flow rate of 100 mL/min. The transfer line temperature was set at 230 ℃ with an electron ionization (EL) of 70 eV. The ion source temperature was set at 250 ℃. The mass scan ranged from 35-400 Dalton (Da). The emission current was 100 μA and the test voltage was 1.4 kV.

## 3 GC-MS Analysis on Volatile Constituents

Automatic retrieval on mass spectral data of each constituent from the Xcalibur Library Browser showed the similarity was more than 800 and further checked and confirmed with the reference spectra[6-10]. A total of

59 compounds were identified in mugwort leaves from Qichun, Hubei Province and 51 compounds in mugwort leaves from Nanyang, Henan Province. The total ion chromatography was shown in Figure 1.

The contents of each GC-MS determined volatile constituent in mugwort leaves were calculated using the area normalization method. In addition, the differences between components in two types of mugwort leaves were compared and analyzed (Table 1).

It can be seen from Figure 1 that GC-MS separated and manifested the volatile constituents of mugwort leaves from Qichun and Nanyang. As shown in table 1, a total of 59 volatile constituents were identified from mugwort leaves from Qichun and its chromatographic peak area accounting for 96.38% of GC-MS total chromatographic peak area; 51 volatile constituents were identified from mugwort leaves from Nanyang and its chromatographic peak area accounting for 95.54% of GC-MS total chromatographic peak area. The mugwort leaves from two places were different in volatile constituents and relative contents. These components mainly contain monoterpenoids (such as terpinene, phellandrene and camphene), sesquiterpenoids [such as caryophyllene, humulene, S-(E, E) dihydro]-1-methyl-5-methylene-8-(1-methylethyl)-1, 6-cyclo-decadieneand Copaene), Ketones (such as camphor, thujone, 6,10,14-trimethyl-2-pentadecanone); alcohols (such as terpineol and agarospirol), a small amount of esters (methyl hexadecanoate), phenol (eugenol), aldehydes and alkanes (such as heptadecane and octadecane), etc.

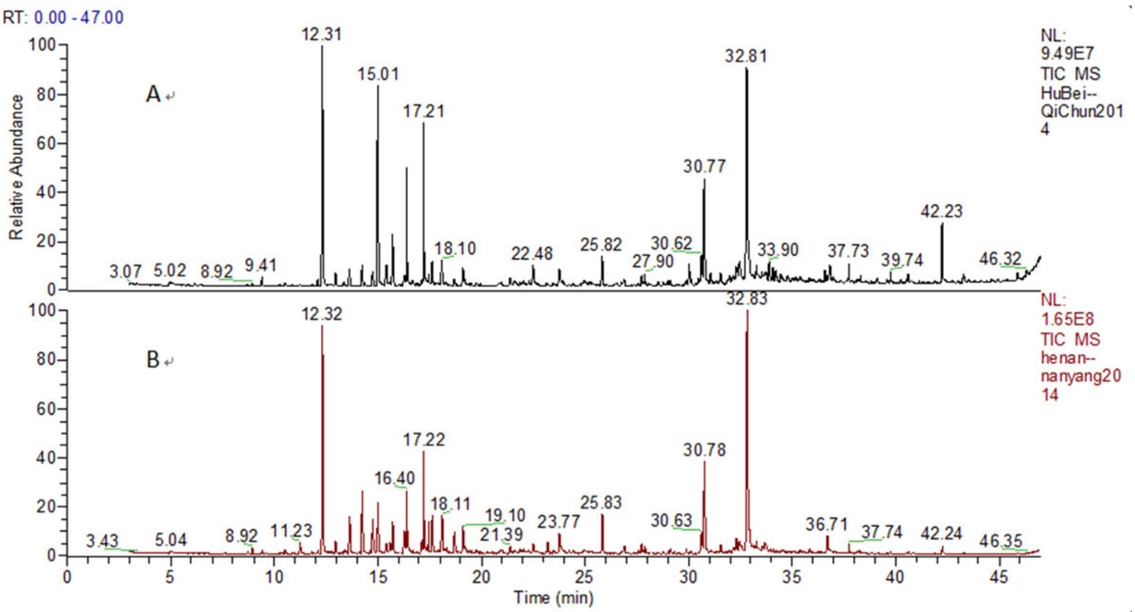


**Figure 1. Total ion chromatography of headspace sampling GC-MS analysis on mugwort leaves from Qichun (A) and Nanyang (B)**

**Table 1. GC-MS identification results of volatile constituents in mugwort leaves from Qichun and Nanyang**

| Serial Number | Retention time (minute) | Compounds | Molecular formula | Relative mass fraction (%) | |
|---|---|---|---|---|---|
| | | | | Qichun | Nanyang |
| 1 | 17.21 | Borneol | $C_{10}H_{18}O$ | 27.58 | 20.82 |
| 2 | 15.01 | Thujone | $C_{10}H_{16}O$ | 18.40 | 2.68 |
| 3 | 12.31 | Eucalyptol | $C_{10}H_{18}O$ | 9.53 | 10.98 |
| 4 | 16.39 | Bornanone | $C_{10}H_{16}O$ | 8.58 | 5.52 |
| 5 | 32.81 | 1-Naphthalenol,decahydro-1,4a-dimethy-l-7(1-methylethylidene),[1R-(1à, 4aá,8aà)] | $C_{15}H_{26}O$ | 5.68 | 8.29 |
| 6 | 42.23 | 1,6,10,14-Hexadecatetraen,3-ol,3,7,11,15-tetramethy,-(E,E) | $C_{20}H_{34}O$ | 4.41 | 0.51 |
| 7 | 14.24 | 1,5-Heptadien4-ol,3,3,6-trimethyl | $C_{10}H_{18}O$ | 3.59 | 18.41 |
| 8 | 15.70 | Bicyclo[3.1.1]hept-3-en-2-one,4,6,6trimethyl,-(1S) | $C_{10}H_{14}O$ | 3.20 | 2.59 |
| 9 | 30.77 | Caryophyllene oxide | $C_{15}H_{24}O$ | 2.26 | 2.34 |
| 10 | 22.48 | 1-Hexen-3-yne,2,5,5-trimethyl | $C_9H_{14}$ | 1.13 | 1.08 |
| 11 | 36.82 | Hexadecane, 2,6,10,14-tetramethyl | $C_{20}H_{42}$ | 0.48 | 0.05 |

**Table 1. GC-MS identification results of volatile constituents in mugwort leaves from Qichun and Nanyang: continued**

| Serial Number | Retention time (minute) | Compounds | Molecular formula | Relative mass fraction (%) | |
|---|---|---|---|---|---|
| | | | | Qichun | Nanyang |
| 12 | 39.75 | Hexadecanoic acid, methyl ester | $C_{17}H_{34}O_2$ | 0.33 | 0.04 |
| 13 | 17.62 | 3-Cyclohexen1-ol,4-methyl-1-(1-methylethyl),® | $C_{10}H_{18}O$ | 0.31 | 2.96 |
| 14 | 18.10 | Terpineol | $C_{10}H_{18}O$ | 0.30 | 2.36 |
| 15 | 37.73 | 2-Pentadecanone,6,10,14-trimethyl | $C_{18}H_{36}O$ | 0.21 | 0.25 |
| 16 | 23.77 | Eugenol | $C_{10}H_{12}O_2$ | 0.04 | 1.32 |
| 17 | 19.09 | 2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-, cis | $C_{10}H_{16}O$ | 0.20 | 1.66 |
| 18 | 14.76 | Cyclohexanol, 1-methyl-4(1-methylethenyl), cis | $C_{10}H_{18}O$ | 0.08 | 0.88 |
| 19 | 26.89 | Humulene | $C_{15}H_{24}$ | 0.08 | 0.07 |
| 20 | 13.63 | Cyclohexanol, 1-Methy-4(1-methylethenyl), cis | $C_{10}H_{18}O$ | 0.08 | 1.37 |
| 21 | 13.34 | Terpinene | $C_{10}H_{16}$ | 0.12 | 0.07 |
| 22 | 15.60 | 2-Cyclohexen-1-ol,1-methyl-4(1-methylethyl-), trans | $C_{10}H_{18}O$ | 0.08 | 0.07 |
| 23 | 20.54 | Bicyclo[3.1.1]hept-2-en-6-ol,2,7,7-trimethyl,acetate, [1S-(1à,5à,6á)] | $C_{12}H_{18}O_2$ | 0.06 | 0.08 |
| 24 | 21.38 | Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl,-acetate, (1S-endo) | $C_{12}H_{20}O_2$ | 0.06 | 0.06 |
| 25 | 27.73 | 1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl)-,[S-(E,E)] | $C_{15}H_{24}$ | 0.08 | 0.19 |
| 26 | 27.90 | Naphthalene,decahydro-4-amethyl-1-methylene-7-(1-methylethenyl), 4aR-(4aà,7à,8aá)] | $C_{15}H_{24}$ | 0.05 | 0.06 |
| 27 | 29.74 | Vitamin A aldehyde | $C_{20}H_{28}O$ | 0.53 | 0.16 |
| 28 | 25.82 | Caryophyllene | $C_{15}H_{24}$ | 0.04 | 0.83 |
| 29 | 31.53 | 12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl,-[1R-(1R*,3E, 7 E,11R*)] | $C_{15}H_{24}O$ | 0.06 | 0.03 |
| 30 | 33.94 | Heptadecane | $C_{17}H_{36}$ | 0.06 | 0.06 |
| 31 | 24.99 | Bicyclo[3.1.1] hept-2-en-6-one,2,7,7-trimethyl | $C_{10}H_{14}O$ | 0.05 | 0.05 |
| 32 | 38.21 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 0.21 | 0.03 |
| 33 | 33.91 | Nonadecane | $C_{19}H_{40}$ | 1.24 | - |
| 34 | 36.57 | Octadecane | $C_{18}H_{38}$ | 0.94 | |
| 35 | 40.62 | Dibutyl phthalate | $C_{16}H_{22}O_4$ | 1.94 | - |
| 36 | 38.31 | 1,2-Benzenedicarboxylic-acid,bis(2-methylpropyl),ester | $C_{16}H_{22}O_4$ | 1.56 | - |
| 37 | 9.41 | Camphene | $C_{10}H_{16}$ | 0.12 | |
| 38 | 34.21 | Benzoic acid, 2-ethylhexyl ester | $C_{15}H_{22}O_2$ | 0.17 | - |
| 39 | 45.88 | Trans Geranylgeraniol | $C_{20}H_{34}O$ | 0.09 | - |
| 40 | 30.02 | 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-,® | $C_{15}H_{26}O$ | 0.43 | - |
| 41 | 34.07 | Tetradecane, 3-methyl | $C_{15}H_{32}$ | 0.48 | - |
| 42 | 34.21 | Benzoic acid, 2-ethylhexyl-ester | $C_{15}H_{22}O_2$ | 0.34 | - |
| 43 | 8.52 | À-Phellandrene | $C_{10}H_{16}$ | 0.10 | - |
| 44 | 8.70、10.28 | Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl) | $C_{10}H_{16}$ | 0.10 | - |
| 45 | 8.92 | (1R)-2,6,6-Trimethylbicyclo[3.1.1]-hept-2-ene | $C_{10}H_{16}$ | 0.10 | - |
| 46 | 5.84 | Phenol | $C_6H_6O$ | 0.15 | - |
| 47 | 10.51 | 1-Octen-3ol | $C_8H_{16}O$ | 0.06 | - |
| 48 | 11.08 | Allene | $C_3H_4$ | 0.10 | - |
| 49 | 11.81 | Cyclohexene, 1-methyl-4-(1-methylethylidene) | $C_{10}H_{16}$ | 0.09 | - |
| 50 | 12.09 | Benzene, 1-Methyl-2(1-methylethyl) | $C_{10}H_{14}$ | 0.09 | - |
| 51 | 14.63 | 2,7-Dimethyl-2,6-Octadien-4-ol | $C_{10}H_{18}O$ | 0.07 | - |

**Table 1. GC-MS identification results of volatile constituents in mugwort leaves from Qichun and Nanyang: continued**

| Serial Number | Retention time (minute) | Compounds | Molecular formula | Relative mass fraction (%) | |
|---|---|---|---|---|---|
| | | | | Qichun | Nanyang |
| 52 | 16.27 | Bicyclo[3.1.1]heptan-3-ol,6,6-dimethyl-2-methylene,[1S-(1à,3à,5à) | $C_{10}H_{16}O$ | 0.03 | - |
| 53 | 28.07 | Pentadecane | $C_{15}H_{32}$ | 0.04 | - |
| 54 | 28.54 | Naphthalene,2,3,4,4a,5,6,8a-octahydro-7-Methyl-4-methylene1(1-methylethyl-)(1à,4aá,8aà) | $C_{15}H_{24}$ | 0.07 | - |
| 55 | 28.76 | Butanoic acid, 3-methyl,1,7,7-trimethylbicyclo [2.2.1] hept-2-yl-ester, exo | $C_{15}H_{26}O_2$ | 0.03 | - |
| 56 | 29.12 | 2(4H)-Benzofuranone,5,6,7,7-atetrahydro-4, 4,7-atrimethyl | $C_{11}H_{16}O_2$ | 0.05 | - |
| 57 | 30.12 | 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl,® | $C_{15}H_{26}O$ | 0.05 | - |
| 58 | 30.49 | 1-Hcycloprop[e]azulen-7-ol,Decahydro1,1,7-trimethyl-4-methylene,[1ar(1aà,4aà,7á,7aá,7bà)] | $C_{15}H_{24}O$, | 0.03 | - |
| 59 | 30.62 | Spathuleneol | $C_{15}H_{24}O$ | 0.04 | - |
| 60 | 34.10 | Dodecane, 2,6,10-trimethyl | $C_{15}H_{32}$ | - | 0.05 |
| 61 | 38.32 | Phthalic acid, hept-4-ylisobutyl ester | $C_{19}H_{28}O_4$ | - | 0.02 |
| 62 | 40.64 | Dibutyl phthalate | $C_{16}H_{22}O_4$ | - | 0.18 |
| 63 | 18.70 | 2-Cyclohexen-1-ol,3-methyl-6-(1-methylethyl)-, trans | $C_{10}H_{18}O$ | - | 1.43 |
| 64 | 19.23 | 2-Oxabicyclo[2.2.2]octane-6-ol,1,3,3-trimethyl | $C_{10}H_{18}O_2$ | - | 0.05 |
| 65 | 19.40 | Cis-p-mentha-1(7),8-dien-2-ol | $C_{10}H_{16}O$ | - | 0.06 |
| 66 | 19.51 | Carveol | $C_{10}H_{16}O$ | - | 0.05 |
| 67 | 14.11 | P-Menth-8-en-ol | $C_{10}H_{18}O$ | - | 0.05 |
| 68 | 14.63 | 1,5-Heptadien-4-ol,3,3,6-trimethyl | $C_{10}H_{18}O$, | - | 0.07 |
| 69 | 17.44 | 2-Octen-4-ol,2-methyl- | $C_{10}H_{18}O$ | - | 6.38 |
| 70 | 25.28 | Coniferyl alcohol | $C_{10}H_{12}O_3$ | - | 0.26 |
| 71 | 27.57 | 2-Isopropenyl-4-a,8-dimethyl-1,2,3,4,4a,5,6,7-otcahydronaphthalene | $C_{15}H_{24}$ | - | 0.07 |
| 72 | 28.15 | Cubenol | $C_{15}H_{26}O$ | - | 0.05 |
| 73 | 28.22 | Elemene | $C_{15}H_{24}$ | - | 0.06 |
| 74 | 29.13 | 2-(4H) Benzofuranone,5,6,7,7-atetrahydro-4,4,7-atrimethyl,® | $C_{11}H_{16}O_2$ | - | 0.05 |
| 75 | 30.63 | 1H-Cycloprop[e]azulen-7-ol,decahydro1,1,7-trimethyl-4-methylene | $C_{15}H_{24}O$ | - | 0.23 |
| 76 | 24.43 | Copaene | $C_{15}H_{24}$ | - | 0.04 |
| 77 | 32.30 | Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol,4,4-dimethyl | $C_{15}H_{24}O$ | - | 0.54 |
| 78 | 33.14 | Agarospirol | $C_{15}H_{26}O$ | - | 0.03 |

As shown in Table 1, borneol has the highest content in mugwort leaves from both places. The mugwort leaves from Qichun and Nanyang share 32 common constituents such as eucalyptol, caryophyllene, thujone, bornanone, eugenol, humulene, terpinene, and caryophyllene oxide. Eucalyptol acts to stop coughing. Borneol acts to unblock orifices, refresh the brain, resolve swelling and alleviate pain. Bornanone acts to kill bacteria and resolve inflammation[11]. However, bornanone and thujone are known to be toxic to the liver, kidney and nervous system; and bornanone is believed to be toxic to reproduction[12]. Toxicological studies with mice have proven that the median lethal dose (LD 50) of α-thujone was 45 mg/(kg·bw)[13]. Volatile constituents in mugwort leaves are essential parts associated with their efficacy and toxicity. Literature studies have suggested that there is a dose-response between volatile constituents and their efficacy/toxicity[14]. As a result, comparison of differences in volatile constituents and relative contents using headspace sampling can provide reference for their clinical application. According to the pharmacopoeia standard, the content of eucalyptol ($C_{10}H_8O$) should be no less than 0.05%. Considered from the analytical results, the eucalyptol in mugwort leaves from Qichun is slightly lower than that from Nanyang but there is no significant difference. In terms of toxic components, the contents of thujone and bornanone were 18.40% and 8.51% respectively in mugwort leaves from Qichun, versus 2.68% and 5.42% respectively in mugwort leaves from Nanyang. Evidently, the mugwort leaves from Nanyang have relatively lower contents of toxic components. This is probably related to the geographic and climate features.

Volatile constituents in mugwort leaves are essential parts associated with their efficacy and toxicity. Extraction of volatile constituents using headspace sampling and heating can analyze the specific constituents and thus provide scientific basis and reference for the quality assessment and screening of mugwort leaves. However, there are no unified and standardized criteria for the quality of mugwort leaves. In addition, the volatile constituents change with the years of storage. It's generally believed that old mugwort leaves (＞3 years) are better for clinical use, probably because their toxic components may diminish or disappear over time. This requires further systematic and follow-up research. Only by then can we provide more accurate scientific data for quality assessment of mugwort leaves[15-19].

On another matter, the dosage of mugwort leaves needs our attention, especially on the development of oral preparation. In this regard, we can refer to the thujone limit in food and drink regulated in the European Union, USA and Canada. This also requires more systematic and in-depth study in the future.

**Conflict of Interest**

The authors declared that there was no potential conflict of interest in this article.

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