Chemical Composition and Total Terpenoids of Aerial Parts Essential Oil from Salvia Miltiorrhiza Bge.

Yanwen Chen[a], Jinghong Hu[b,*], Fangxue Xu, Yongqing Zhang[c,*], Yingxue Song, Yu Ge

College of Pharmacy, Shandong University of Traditional Chinese Medicine, Jinan,250355 China.

[a]18765804058@126.com, [b]hujinghong97@126.com, [c]zyq622003@126.com

Abstract

The present study aimed to analyzed the chemical composition and determined the total terpenoids content of essential oil from aerial parts of S. Miltiorrhiza. The essential oil from aerial parts of S. Miltiorrhiza was obtained by steam distillation and then was analyzed through GC-MS. And the total terpenoids content were measured using a modified UV-Vis Spectrophtometry method. The result showed that terpenoids was the main components of essential oil from aerial parts of S. Miltiorrhiza, accounting for about 50.18% of the total essential oil, and β-caryophyllene (8.58%), 6,10,14-trimethyl-2-pentadecanone (7.97%), dihydro-neoproene (7.96%), germacrene D (6.37%) caryophyllene (4.22%) were the main and characteristic compositions of the essential oil. And the results showed that α-caryophyllene had a good linearity（r=0.999 0）between 0.030 9~0.1309 5 mg·mL-1, the content of terpenoids in essential oil of S. Miltiorrhiza was about 612 mg g-1. All the above suggest that terpenoids was the main components of essential oil from aerial parts of S. Miltiorrhiza, that provide a reference for the comprehensive development and utilization of S. Miltiorrhiza resources.

Keywords

***Salvia miltiorrhiza*; Aerial parts; Essential oil; Chemical Composition; total terpenoids.**

1. Introduction

As a traditional Chinese medicine, *Savlia Miltiorrhiza* Bge. is mainly distributed in Shandong, Shanxi, Shaanxi, Hebei, Henan, Anhui, Zhejiang, Hubei and Jiangsu province of China and Japan [1]. The main ingredients of *S. Miltiorrhiza* contain tanshinones, phenolic acids, flavonoids, polysaccharides, amino acids and terpenes.This medicinal herb has the efficacy of promoting blood circulation to remove blood stasis, inducing menstruation to relieve menalgia, clearing away heat, relieving vexation, cooling the blood to relieve carbuncles, and so on [2-3]. Recent pharmacological and phytochemical studies were mainly focused on the fat-soluble quinone abietanes and water-soluble phenolic acids, which have the pharmacological activity of anti-bacterial, anti-inflammatory, anticoagulant, anti-thrombosis,and cell protection, were the two kinds of major active constituents in the root of *S. Miltiorrhiza* [4-6]. Traditionally, the root and rhizome of *S. Miltiorrhiza* are the main drug parts, the aerial part is not for medicine. With the continuous improvement of people's living standards, the demand for Chinese medicine is increasing. Since Fufang Danshen Dripping Pills were listed in US market, the demand of *S. Miltiorrhiza* showed a sharp increase. According to statistics, only in Shandong,China, the annual demand of Salvia medicinal herbs reached 10,000 tons and the weight of the aerial parts take up about two thirds of the total weight of S. Miltiorrhiza [7]. In the process of search for new drug source, it was found that some of the chemical composition and pharmacological effects of the aerial part of *S. Miltiorrhiza* had some similarity with its root[8-10] which will greatly improve the development and utilization of resources if the aerial parts of *S. Miltiorrhiza* can be full used. This will not only reduce the waste of resources, but also ease the current environmental pollution situation to a certain extent.

Essential oil is one of the important active ingredients of the genus *Salvia*, with antibacterial, anti-inflammatory, anti-oxidation, anxiolytic, anti-tumor and other pharmacological activities [11-16]. The components and pharmacological activities of essential oil from *S. miltiorrhiza* were studied by many researchers [17-21], and the results showed that terpenoids and oxygen compounds were the main components of it. Li [19] analyzed the components of essential oil from aerial parts of *S. Miltiorrhiza*. by GC-MS, and found that β-caryophyllene, α-caryophyllene, germacrene D, bourbonene and other sesquiterpenes were the characteristic components in stems, leaves, flowers of *S. Miltiorrhiza.* Another studies found that terpenoids have significant pharmacological effects, such as antioxidant, anti-inflammatory, antibacterial, anti-tumor and improving the symptoms of Alzheimer's disease [22-26]. These provide a theoretical basis for the study of the anti-AD effect of the essential oil from aerial parts of *S. Miltiorrhiza.*

In this study, GC / GC-MS was used to analyze the essential oil components of aerial parts of *S. Miltiorrhizae*, and the total terpenoids were measured using a modified UV-Vis Spectrophtometry method. Through this experiment, we expected to provide a reference for the comprehensive development and utilization of *S. Miltiorrhiza* resources, and to find a new natural medicine for the treatment of AD.

2. Material and methods

2.1 Chemicals and reagents

Standards of α-caryophyllene,were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standards was prepared as stock solutions in anhydrous ethanol. Stock working solutions of the standards were stored in darkness at -20 ℃. Vanillin, glacial acetic acid, perchloric acid, anhydrous ethanol, HPLC grade petroleum ether and other chemicals and solvents are of analytical grade were purchased from the Tianjin Chemical Reagent Co., Ltd. (Tianjin, China).

2.2 Isolation of essential oil

Aerial parts of *S. Miltiorrhiza* were collected in the medicinal botancial garden of Shandong University of Traditional Chinese Medicine, Shandong, Jinan, China in September 2016. It was identified by Pro. Yongqing Zhang and a vouncher specimen was deposited at the Herbarium of Shandong University of Traditional Chinese Medicine. The air-dried aerial parts of *S. Miltiorrhiza* (10kg) were crushed into a coarse powder, and the essential oil were isolated by hydrodistillation using a Clevenger-type apparatus for 6 h according to the method recommended in the Chinese Pharmacopoeia 2010 edition. The essential oil was dried over anhydrous sodium sulfate and stored at -20 ℃ until analysis.

2.3 Identification of components of essential oil

Identification of the components of the essential oil was based on GC retention indices relative to n-alkanes and computer matching with the Wiley 275 L mass spectra library. In addition, the analysis included comparisons of the fragmentation patterns of the mass spectra to those reported in the literature.

2.4 Total Terpenoids Content of essential oil

The essential oil and α-caryophyllene were diluted with anhydrous ethanol. Total terpenoids were measured using a modified UV-Vis Spectrophtometry method, as previously described [27-28]. In brief, aliquots of test samples (1.0 mL, 0.1 mg/mL) were mixed with 1.5 mL of 5% vanillin-glacial acetic acid solution, and 2.0 mL perchloric acid was added, and then incubated at 65 ℃ constant temperature incubator for 20 min. Take out, addition of 5.0 mL glacial acetic acid, after which the absorbance was read at 560 nm. Measurements were carried out in triplicate and calculations were based on a calibration curve obtained with α-caryophyllene (0.030 9~0.1309 5 mg·mL^{-1}). The total terpene were expressed as milligrams of α-caryophyllene per gram of extract. The calibration equation for α-caryophyllene was A=*4.412 9C+0.026 4 (r = 0.999 0).*

2.5 Statistical Analysis

All data were analyzed by a one-way analysis of variance, and the differences between means were established by Duncan's multiple-range test. All data were expressed as mean±S.E.M. The values of $p<0.05$ were were considered significant.

3. Results

3.1 GC-MS analysis

The results of composition analysis of essential oil from *S. Miltiorrhiza* aerial parts and their percentages were given in **Table 1**, and the components are listed in order of their elution from the HP - 5MS column. The total ion current of essential oil from *S. Miltiorrhiza* aerial parts are given in **Fig1**.

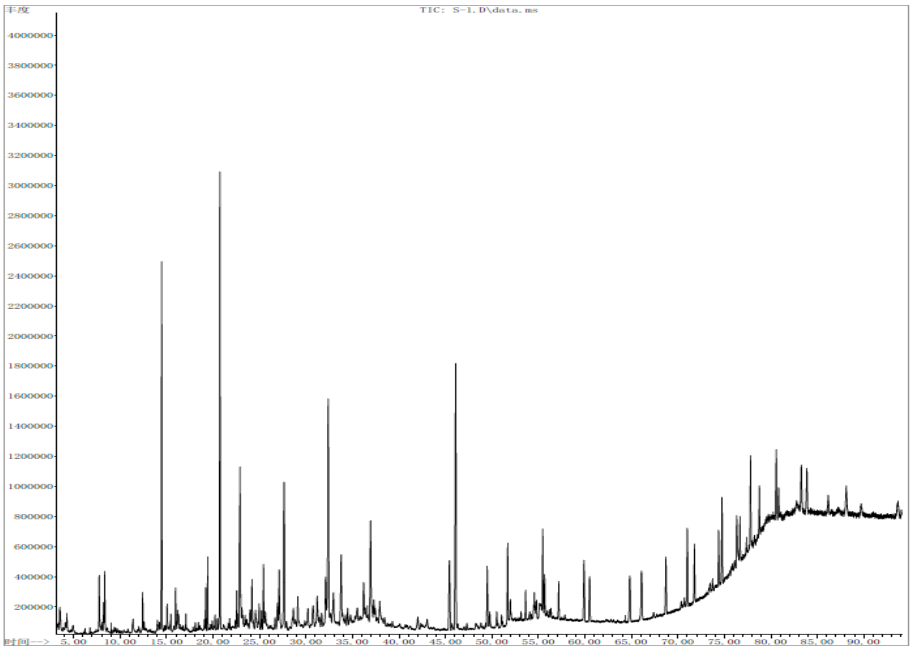

Fig. 1. The total ion current of essential oil from *Salvia Miltiorrhiza* Bge. Aerial parts by GC-MS analysis

Table 1. Chemical composition of essential oil from *Salvia Miltiorrhiza* Bge. Aerial parts

NO.	Retention time/min	Compound	Molecular formula	Molecular weight	Relative content/%
1	4.277	O-xylene	C_8H_{10}	106	0.260
2	6.749	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	$C_{10}H_{16}$	136	0.103
3	7.734	1-Octen-3-ol	$C_8H_{16}O$	128	0.826
4	8.332	camphene	$C_{10}H_{16}$	136	1.633
5	11.370	3-acetoxy-4- (1-hydroxy-1-methylethyl) -1-methyl-cyclohexene	$C_{12}H_{20}O_3$	128	0.466
6	12.422	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{10}H_{18}O$	156	0.650
7	14.467	2-methyl-undecane	$C_{11}H_{24}$	156	6.765
8	15.046	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	$C_{10}H_{18}O$	156	0.507
9	15.960	3-Cyclohexene-1-methanol, .alpha.,.alpha.4-trimethyl-	$C_{10}H_{18}O$	156	0.806
10	18.079	2-Cyclohexen-1-one,5-methyl-2-(1-methyle thyl)-	$C_{10}H_{18}O$	156	0.137
11	19.255	N-[4-(Ethyl-methyl-amino)-phenyl]-acetami	$C_{11}H_{16}N_2$	192	0.639

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12	19.406	Isoborneol	$C_{10}H_{18}O$	154	1.149
13	20.733	β-caryophyllene	$C_{15}H_{24}$	204	8.282
14	22.544	Pinene	$C_{15}H_{24}$	204	0.530
15	22.887	caryophyllene	$C_{15}H_{24}$	204	3.271
16	24.177	caryophyllene	$C_{15}H_{24}$	204	0.950
17	25.222	Octahydro-7-methyl-3-methylene-4-(1-methylethyl) -1H-cyclopenta [1,3] cyclopropane [1,2]	$C_{15}H_{24}$	204	0.349
18	25.419	α-caryophyllene	$C_{15}H_{24}$	204	1.278
19	25.629	α-caryophyllene	$C_{15}H_{24}$	204	0.849
20	26.670	γ-Vanillin	$C_{15}H_{24}$	204	0.346
21	27.102	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{13}H_{20}0$	192	1.266
22	27.637	germacrene D	$C_{15}H_{24}$	204	4.366
23	29.102	(1S-cis) -1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl) naphthalene	$C_{15}H_{12}$	192	0.730
24	31.205	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	$C_{15}H_{26}O$	212	0.982
25	32.388	dihydro-neoproene	$C_{15}H_{26}$	206	5.958
26	32.935	2,6-Lutidine-N-oxide	C_7H_9NO	123	1.103
27	33.768	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	$C_9H_{14}O$	138	2.040
28	36.190	Cis-2,3,4,4a, 5,6,7,8-octahydro-1,1,4a, 7-tetramethyl-1H-benzocyclohepten-7-ol	$C_{15}H_{26}O$	222	0.824
29	36.947	2,3,4,4a, 5,6,7,8-octahydro-1,1,4a, 7-tetramethyl-1H-benzocyclohepten-7-ol	$C_{15}H_{26}O$	222	2.724
30	45.429	Huma-1,6-dien-3-ol	$C_{15}H_{24}O$	220	1.358
31	46.089	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	7.986
32	51.689	Isophytol	$C_{20}H_{40}O$	296	1.636
33	51.981	β-Caryophyllene oxide	$C_{15}H_{24}O$	220	1.526
34	53.619	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10 ,10a-octahydrophenanthrene	$C_{20}H_{30}$	270	0.561
35	55.457	phytol	$C_{20}H_{40}O$	296	1.574
36	57.182	dodecane	$C_{22}H_{46}$	310	0.771
37	59.896	Caryophyllene oxide	$C_{15}H_{24}O$	220	1.846
38	60.512	Tricosane	$C_{23}H_{48}$	324	1.329
39	64.865	Tetracosane	$C_{24}H_{50}$	338	1.636
40	66.092	palmital	$C_{16}H_{32}O$	240	1.871
41	68.726	Pentacosane	$C_{25}H_{52}$	352	1.554
42	76.342	13-Docosenamide, (Z)-	$C_{22}H_{23}NO$	317	1.129
43	76.717	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380	0.881
44	77.818	Squalene	$C_{30}H_{50}$	410	1.823
45	80.575	Methyl,oleate	$C_{19}H_{36}O_2$	296	1.860
46	83.354	Stigmastera-3,5-diene	$C_{29}H_{48}$	396	1.382
47	86.187	Octacosane	$C_{28}H_{58}$	394	0.486
48	89.614	Ursa-9(11),12-dien-3-one	$C_{30}H_{46}O$	424	0.423

3.2 Total Terpenoids Content of essential oil

The content of total terpenoids of essential oil from *S. miltiorrhiza* aerial part was determined by the above method, the content was determined in triplicate. The content of total terpenoids were 612 $mg \cdot g^{-1}$, 625 $mg \cdot g^{-1}$ and 599 $mg \cdot g^{-1}$, respectively.

4. Discussion

In this experiment, GC-MS was used to analyze the components of the essential oil from *S. Miltiorrhizae* aerial parts, and the total ion current map and the peak mass spectrum were obtained. The components were identified by computer search (NIST05a.L libraries date of the GC-MS system), artificial interpretation and literature proofing. The results were given in the Table 1. A total of 79 species were detected from the essential oil of aerial parts of *S. Miltiorrhiza*, and 48 species were identified, accounting for 81.42% of the total essential oil. The most of which is terpenoids, accounting for about 50.18% of the essential oil. Among terpenoids, β-caryophyllene, dihydro-neoproene, germacrene D, caryophyllene and α-caryophyllene were the main constituents of sesquiterpenes, representing 8.58%, 7.96%, 6.37%, 4.22% and 2.13%, respectively. Phytol and isophytol were the main constituents of diterpenes, representing 1.64% and 1.55%, respectively. Camphene (1.63%) and isoborneol (1.15%) were the main constituents of monoterpenes. Other compounds constituted 31.24% of the essential oil with 6,10,14-trimethyl-2-pentadecanone (7.97%) of ketone compounds, 3,4-dimethyl-3-cyclohexene-1-carbaldehyde (2.04%) of aldehydes and methyl oleate (1.86%) of esters. The essential oil from *S. Miltiorrhiza* aerial parts was characterized by a high content of β-caryophyllene (8.58%) as the principal compound, followed by 6,10, 14-trimethyl-2-pentadecanone (7.97%), dihydro- neoproene (7.96%), germacrene D (6.37%) and caryophyllene (4.22%).

The components of the essential oil from *S. Miltiorrhizae* aerial parts have been analyzed by many researchers. According to the study carried out by Li et al. [19], hexadecanoic acid (17.0%), germacrene D (9.1%), phytol (8.9%), β-caryophyllene (7.1%) and methyl linolenate (5.3%) were characterized as the major constituents in the leaves of *S. Miltiorrhizae.* The components of essential oil in stem, leaf, and flower of *S. Miltiorrhizae* were identified by Chen [20] through the headspace solid-phase microextraction coupled by gas chromatography-mass spectrometry (HS-SPME /GC-MS). The result showed that β-caryophyllene (22.22%, 5.41%, 36.16%), germacrene D (9.69%, 16.76%, 8.00%) and bourbonene (4.61%, 13.05%, 13.05%) were the main compositions of the essential oil in stem, leave, flower of *S. Miltiorrhizae*, respectively. Ji [29] used the method of steam distillation extracted the essential oil from stem, leave and flower of *S. Miltiorrhizae*, and the components of essential oil were identified by GC-MS. The result showed that germacrene D (15.47%, 36.68%, 23.42%), caryophyllene (15.37%, 15.32%, 22.77%) and α-caryophyllene (5.97%, 6.06%, 10.37%) were the main compositions of the essential oil in stem, leave and flower of *S. Miltiorrhizae*, respectively. Throughout the literature there have been numerous reports on the chemical compositions of the essential oils from the aerial parts of *S. Miltiorrhizae*. The main components of essential oils have significantly different according to different researchers, and most of the reports indicated that β-caryophyllene, α-caryophyllene, caryophyllene, germacrene D, hexadecanoic acid, phytol and bourbonene were the main and/or characteristic constituents of the essential oil of the aerial parts of *S. Miltiorrhizae.* The present study showed that β-caryophyllene, dihydro-neoproene, 6,10,14-trimethyl-2-pentadecanone, germacrene D, caryophyllene and α-caryophyllene were detected in the aerial parts essential of *S. Miltiorrhizae*, among which dihydro-neoproene and 6,10,14-trimethyl-2-pentadecanone as the main compositions of aerial parts essential oil of *S. Miltiorrhizae.* were rare reported in previously researches. The extraction method, the variety of *S. Miltiorrhiza*, the growth period, the time of harvesting and the drying method may be the cause of the difference in the composition of the essential oil.

Monoterpene, sesquiterpene, diterpene and its oxide compounds were the main components of essential oil from *S. miltiorrhiza* aerial part. The components of the essential oil from *S. Miltiorrhizae* aerial parts have been analyzed by many researchers, and find the terpenoids was the main

components of it. But its total terpenoids content has not been reported. The commonly used method for determination of terpenoids is vanillin-glacial acetic acid solution and perchloric acid colorimetric method[27-28]. This method was used to determine the total terpenoids content of essential oil from *S. miltiorrhiza* aerial part, in the first time. The results showed that α-caryophyllene had a good linearity（r=0.999 0）between 0.030 9~0.1309 5 mg·mL^{-1}, the content of terpenoids in essential oil of *S. Miltiorrhiza* was 612 mg·g^{-1}, 625 mg·g^{-1} and 599 mg·g^{-1}, respectively. All the above suggest that terpenoids was the main components of essential oil from aerial parts of *S. Miltiorrhiza*, that provide a reference for the comprehensive development and utilization of *S. Miltiorrhiza* resources.

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