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Analysis on Volatile Components in *Mentha haplocalyx* of Special Herbs in Qinghai Province

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The qualitative and quantitative analysis is performed on the volatile components in *Mentha haplocalyx* of special herbs in Qinghai Province by SPME-GC-MS. The relative percentage content of each component was calculated with peak area normalization method. There were 42 components separated from *Mentha haplocalyx*. The main constituents were ketones, alkenes, aromatic and ester compounds. The component of highest concentration was 2-methyl-5-(1-methylethenyl)cyclohexanone which reached 39.54 %.

Keywords: *Mentha haplocalyx*, Volatile component, SPME, Gas chromatography/mass spectrometry, Qinghai province.

INTRODUCTION

The *Mentha haplocalyx* is classified in Labiatae, *Mentha* and is a perennial herb, which is also called Southern *Mentha haplocalyx*, Su *Mentha haplocalyx*, Ren primrose and Fish herb, *etc*. and is mainly located in agricultural area in the eastern part of the Qinghai Province[1] and fringe wetland at an elevation of 1800-2500 m. The wild bergamot's whole herb could be used in medicine. Its nature and flavor is cool and pungent and its smell is fragrant, which enters into the lung and especially does good to the liver. Moreover, it can relieve wind-heat and superficies by cooling, keeps out the cold and swelling, does good for swallowing, aches and dizziness, regulates the flow of vital energy and removes obstruction. It is able to cure anemopyretic cold, early rheumatism, headache, cough, red eye, dyspeptic retention, painfully swelling pharynx and larynx, dental ulcer, urticaria, measles, thoracic and abdominal oppression, as well as prevents early pregnancy and some other diseases like cardiovascular and gastrointestinal diseases. Meanwhile, it also can be used as antiseptic, analeptic and local anesthetic[2].

Nowdays, a lot of studies have found that *Mentha* plants contain many different compounds, such as *Mentha haplocalyx* isoflavone glycoside, menthol, different ruifu spirit, luteolin-7-g lucoside, rosmarinci acid, caffeic acid, 3-pentanol, 3-octanol, α-terpineol, linalool, cineole, *etc.*[3-9]. Especially, the contained volatile substances have the effect of antibacterial, antioxidation[10] antiimflammatory, anticytotoxic and anticancer[11]. However, the research on the volatile components of medical plants wild *Mentha haplocalyx* in Qinghai Province is still blank. On this basis, this experiment adopts the solid phase micro extraction[12-14] on volatile components in *Mentha haplocalyx* extraction, using gas chromatography-mass spectrometry (GC/MS) detection and computer aided system for its separation identification of volatile component. The application of the chromatographic peak area normalization method for determining the percentage of each ingredients, combined with the matching degree of retention index and identification compounds, aiming at unique volatile components in medicinal plants and wild *Mentha haplocalyx* in qinghai province and provides the theory basis for the rational development and utilization of measures.

EXPERIMENTAL

Healthy wild *Mentha haplocalyx* plants was collected in Yang Jiazhai village Xining city Qinghai Province in July 2010, identified as labiatae plants of the genus *Mentha haplocalyx* by professor Wu Xue-ming from College of Biology and Geography sciences, Qinghai Normal University. Repeat 3 times.

GC-MS-QP 2010 type gas chromatography-mass spectrometry instrument (Shimadzu company); SL202N drug electronic balance (Ming Qiao precision scientific instruments co., LTD. In Shanghai); Manually Solid phase microextraction device, spme fiber is 100 µm PDMS; MLLI-Q-ultrapure water instrument (Millipore corporation in the United States).

Solid phase microextraction conditions: Clean the fresh plant of wild *Mentha haplocalyx*, grinding into powder with liquid nitrogen, weighing 0.2 g powder accurately into 10 mL solid-phase microextraction bottle for sampling, adding 2 mL ultrapure water, inserting 100 µm PDMS/DVB fiber ends manual sampler, magnetic stirring speed is 800 rpm, headspace extraction for 0.5 h under 30 °C and then take out, inserting chromatograph injection port (250 °C) quickly, desorption for 4 min.

Gas chromatographic conditions: RTX-5MS type elastic quartz capillary chromatographic column (0.25 mm × 30 m × 0.25 µm); The carrier gas is high purity helium (99.999 %); Carrier gas flow rate in the column is 1.30 mL min^{-1}; temperature of the injection port is 230 °C. Temperature process: starting from 60 °C, keeping for 2 min; Raising the temperature to 80 °C by 4 °C/min, keeping 1 min; Raising the temperature 120 °C by 10 °C/min, keeping for 1 min; then raising the temperature to 180 °C by 5 °C/min, keeping for 1 min; At last raising the temperature to 120 °C; sampling without shunting.

Mass spectrometry conditions: Electron bombardment ion source EI source; Ion source temperature 230 °C; Interface temperature 280 °C; Electronic energy 70 ev; Multiplier voltage 0.9 kV; Solvent delay 5 min; Scan range 35-600 *m/z*.

RESULTS AND DISCUSSION

The analysis of volatile components in wild *Mentha haplocalyx* depending on the above optimization conditions. The results of total ion flow diagram is shown in Fig. 1. Quantitative and qualitative analysis the chromatographic peaks corresponding to the mass spectrogram after artificial parsing and NIST27 and NIST147 spectrum library retrieval combining with the retention index, calculating relative percentage content of each component by area normalization method, the analysis results of content of relative volatile components from wild *Mentha haplocalyx* are given in Table-1. The results show content of 42 volatile components of wild *Mentha haplocalyx* plants is obviously different, the higher ones are 2-methyl-5-(1-methylethenyl)cyclohexanone, D-limonene, (2R-*cis*)-5-methyl-2-(1-methylethyl)-cyclohexanone, the datas are 39.54, 24.59 and 10.64 %, the lower ones are camphene, 1-methyl-4-(1-methylethylidene)-cyclohexene and [1S-(1-α, 2-β, 4-β)]-1-ethenyl-1-methyl-2, 4-*bis*(1-methylethenyl)-cyclohexane, the datas are 0.06, 0.04 and 0.04 % .

In preliminary experiments, we select two different polarity solid-phase microextraction fiber head PDMS/DVB (100 microns) and CAR/PDMS (75 microns) to extract volatile components in wild *Mentha haplocalyx*. Results show that the extraction sensitivity of PDMS/DVB is relatively higher than CAR/PDMS, the peak is good, so PDMS/DVB fiber head experiment is better.

Volatile components in the wild *Mentha haplocalyx* were discussed by SPME-GC-MS/MS technology. Forty two compounds were measured and identified. Relatively high mass fraction of components are 2-methyl-5-(1-methylethenyl)-cyclohexanone (39.54 %), D-limonene (24.59 %), (2 R-*cis*)-5-methyl-2-(1-methylethyl)-cyclohexanone (10.64 %), caryophyllene (2.95 %), *trans*-2-methyl-5-(1-methylethenyl)-cyclohexanone (2.73 %), (S)-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (2.53 %), (2 R-*cis*)-5-methyl-2-(1-methylethyl)-cyclohexanone (1.62 %), eucalyptol (1.33%), α-caryophyllene (1.05 %), (Z)-3,7-dimethyl-1, 3,6-octatriene (1.05 %) and so on. The *Mentha haplocalyx* volatile composition and content is different from previous studies[15-18]. The reasons of these differences may be caused by different geographical environment. At the same time, volatile components of Qinghai-tibet Plateau wild *Mentha haplocalyx* is clearer than the mainland's, may be due to sufficient sunlight, no pollution, *etc.* on the plateau region. In addition, present study also found that the method is fast, simple and economical, safe, easy operation, good reproducibility and quantity of analysis sample is small.

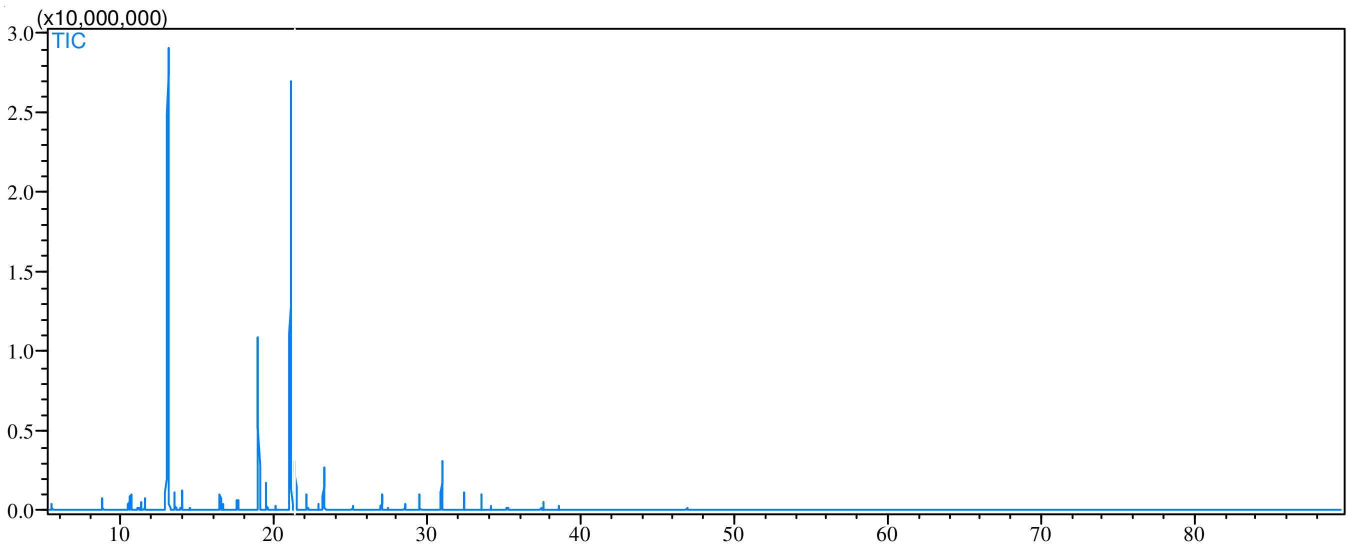

Fig.1. Total ion current diagram of chemical components of volatile oils in *Mentha haplocalyx* in Qinghai province

TABLE-1
VOLATILE COMPONENTS AND THEIR RELATIVE PERCENTAGE CONTENTS OF *Mentha haplocalyx* IN QINGHAI PROVINCE

No.	tR/min	Chemical name	m.f.	Relative molecular weight	Relative percentage content (%)
1	8.706	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	$C_{10}H_{16}$	136	0.08
2	8.833	1,3,3-Trimethyl tricyclo[2.2.1.0(2,6)]heptane	$C_{10}H_{16}$	136	0.63
3	9.442	Camphene	$C_{10}H_{16}$	136	0.06
4	10.534	α-Phellandrene	$C_{10}H_{16}$	136	0.44
5	10.651	β-Pinene	$C_{10}H_{16}$	136	0.78
6	10.864	(S)-(+)-2-Pyrrolidinemethanol	$C_5H_{11}NO$	101	0.07
7	11.148	Aminothiazole	$C_3H_4N_2S$	100	0.15
8	11.342	β-Myrcene	$C_{10}H_{16}$	136	0.48
9	11.57	Megestrol acetate	$C_{24}H_{32}O_4$	384	0.56
10	12.472	1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene	$C_{10}H_{16}$	136	0.07
11	12.967	(S)-1-Methyl-4-(1-methylethenyl)cyclohexene	$C_{10}H_{16}$	136	0.14
12	13.066	D-Limonene	$C_{10}H_{16}$	136	24.59
13	13.138	Eucalyptol	$C_{10}H_{18}O$	154	1.33
14	13.518	(E)-3,7-Dimethyl-1,3,6-octatriene	$C_{10}H_{16}$	136	0.88
15	13.996	(Z)-3,7-Dimethyl-1,3,6-octatriene	$C_{10}H_{16}$	136	1.05
16	14.468	1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene	$C_{10}H_{16}$	136	0.12
17	15.865	1-Methyl-4-(1-methylethylidene)cyclohexene	$C_{10}H_{16}$	136	0.04
18	16.458	3,7-Dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	154	0.81
19	16.652	Nonanal	$C_9H_{18}O$	142	0.36
20	17.611	3-Octanol acetate	$C_{10}H_{20}O_2$	172	0.56
21	18.982	(2S-*cis*)-5-Methyl-2-(1-Methylethyl)cyclohexanone	$C_{10}H_{18}O$	154	10.64
22	19.458	(2R-*cis*)-5-Methyl-2-(1-methylethyl)cyclohexanone	$C_{10}H_{18}O$	154	1.62
23	20.113	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	$C_{10}H_{18}O$	154	0.22
24	20.81	α,α,4-Trimethyl-3-cyclohexene-1-methanol	$C_{10}H_{18}O$	154	0.07
25	21.123	2-Methyl-5-(1-methylethenyl)cyclohexanone	$C_{10}H_{16}O$	152	39.54
26	21.396	*trans*-2-Methyl-5-(1-methylethenyl)cyclohexanone	$C_{10}H_{16}O$	152	2.73
27	22.114	*cis*-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol	$C_{10}H_{16}O$	152	0.8
28	22.86	*cis*-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol	$C_{10}H_{16}O$	152	0.24
29	23.22	(S)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-one	$C_{10}H_{14}O$	150	2.53
30	25.123	Bornyl acetate	$C_{12}H_{20}O_2$	196	0.22
31	27.031	2-Methyl-5-(1-methylethenyl)cyclohexanol	$C_{10}H_{18}O$	154	0.85
32	27.451	(1R-*cis*)-2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol acetate	$C_{12}H_{18}O_2$	194	0.15
33	28.553	*cis*-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol acetate	$C_{12}H_{18}O_2$	194	0.34
34	29.476	[1S-(1α,3aα,3bβ, 6aβ, 6bα)]-Decahydro-3a-methyl-6-methylene-1-(1-methylethyl)cyclobuta[1,2:3,4]dicyclopentene	$C_{15}H_{24}$	204	0.89
35	29.792	[1S-(1α,2β,4β)]-1-Ethenyl-1-methyl-2,4-*bis*(1-methylethenyl)cyclohexane	$C_{15}H_{24}$	204	0.04
36	30.954	Caryophyllene	$C_{15}H_{24}$	204	2.95
37	32.375	α-Caryophyllene	$C_{15}H_{24}$	204	1.05
38	33.516	[3aS-(3aα,3bα,4β,7α,7aS@)]-Octahydro-7-methyl-3-methylene-4-(1-methylethyl)-1*H*-cyclopenta[1,3]cyclopropa[1,2]benzene	$C_{15}H_{24}$	204	0.9
39	34.163	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-cyclohexane	$C_{15}H_{24}$	204	0.2
40	35.235	[1S-(1α,4aβ,8aβ)]-1,2,4a,5,8,8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	$C_{15}H_{24}$	204	0.15
41	37.576	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.43
42	46.924	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	0.24

Volatile components in wild *Mentha haplocalyx* has various biological activities and medicinal value. Some of them are the main effective components in Chinese and Tibetan medicine. For example, 3-hexene-1-alcohol (0.56 %) is natural spices with rich aroma[19]; myrcene (0.40 %) has the effect of expectorant, antitussive[20]; Green grass ene has smooth wheezing, antitussive, expectorant, treatment of tracheitis[21]; limonene (21.24 %) not only has a good the effect of antitussive, expectorant, bacteriostatic[22], but also has significant inhibitory effect on human bladder cancer EJ cells[23]; α-clove ene (0.18 %) have certain asthma effect; carvacrol has strong antimicrobial effect, especially for fungi; mondial phenol has a diuretic effect, *etc*. In addition, volatile composition of *Mentha haplocalyx* in Qinghai province also contains some special chemical composition, such as jasmine, jasmine ketone, *etc*.

This study first analyzes the volatile chemical components in Qinghai wild *Mentha haplocalyx*, it is of great importance for its further in-depth discussion to theory and reference value. However, the studies on the relationship between chemical composition and efficacy will be discussed in the subsequent experiments, aiming at better reasonable development and use of this valuable medicinal plant resources in Qinghai-Tibetan plateau.

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