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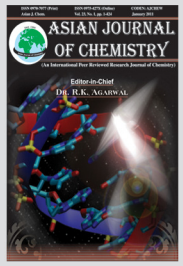

Extraction and GC-MS Analysis of the Volatile Constituents of *A Tractylodes macrocephala*

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In present studies *A tractylodes macrocephala* of three different growing areas of China is the research object. The extraction of volatile constituents from *A tractylodes macrocephala* of China has been made by steam distillation and solid-phase micro-extraction (SPME). The chemical constituents of volatile oil separated were identified by gas chromatography-mass spectrometry analysis, the relative percentage content of volatile constituents were determined by area normalization method. Gas chromatographic conditions: OV-1701 capillary-tube chromatographic column (30 m × 0.25 mm, 025 μm), temperature-increasing procedure: keep the initial temperature 50 ºC for 2 min and then increase the temperature to 130 ºC at the rate of 10 ºC min^{-1} and increase the temperature to 190 ºC at the rate of 5 ºC min^{-1} again and continuous increase the temperature to 240 ºC at the rate of 12 ºC min^{-1} and keep it for 4 min. The temperature at the sample-feeding entrance is 200 ºC, the temperature at the vaporizing room is 260 ºC, the loaded gas is high-purified (He) and the flowing rate is 0.8 mL min^{-1}, proportion of cleft sample-feeding: 30:1, capacity of sample-feeding: 0.1 mL. Mass-spectrometry conditions: the ion source of electron impact (EI), the ion energy is 70 eV, the temperature of the ion source is 200 ºC, the voltage of the checking device is 350 V, the scanning range is m/z: 33-450 AMU, the scanning speed is 0.5 s, the retrieving data-base is Willey and NIST standard mass-spectrometry data-base. Experimental results show that the type of volatile components is basic similar by steam distillation and SPME method, content of atractylon of *A tractylodes macrocephala* are high prevalent in three different growing areas. The yield of volatile oil was the highest in Lin'an of Zhejiang province, China, reached 1.92 %, the relative content of atractylon in total volatile oil was the highest, reached 46 %. This experiment result enhances our understanding of volatile constituents in *A tractylodes macrocephala* and content, the result provides further reference for the development and utilization of *A tractylodes macrocephala* of China.

Key Words: *A tractylodes macrocephala*, Solid-phase micro-extraction, Gas chromatography-mass spectrometry.

INTRODUCTION

A tractylodes macrocephala Koidz is the dry root of the plants of chrysanthemum family. It is the widely-used tonic for vital energy, having the medical functions of tonic spleens, eliminating-dampness, alleviation of water retention and sweat-reduction. It is mainly used to treat the diseases of weak spleens, small appetite, distention of abdomen, diarrhea, edema, dizziness, dropsy, spontaneous sweating and painful fetal movement[1,2], *etc*. *A tractylodes macrocephala* Koidz is one of the so-called "characteristic medicines in Zhejiang of China". It has the characteristics of first-rate quality with high yield and good medical effect. Recent years of research has shown that it has the confirmed effect of regulating immune system, antidecrepitude, antitumor, antiinflammation, reducing the blood sugar level and urination-helping, *etc*. It is widely planted in Pan'an of Zhejiang Province, China, Anguo of He'bei Province, China and especially in Lin'an of Zhejiang Province, China with a large-scale, high-quality and standardized planting area. *A tractylodes macrocephala* Koidz has a strong aroma because of its content of a certain amount of volatile chemical constituents. Concerning the volatile constituents. in *A tractylodes macrocephala* Koidz, there are only some reports on its extraction by steam distillation and supercritical flow extraction (SFE)[3-6], but there have been no reports on its extraction by solid phase micro-extraction (SPME).

This paper adopts the method of steam distillation and SPME to extract the volatile chemical constituents from the root of *A tractylodes macrocephala* in different growing areas of China. Uses of the technique of gas chromatography-mass spectrometry to determine the volatile chemical constituents and the method of area normalization to determine the relative percentage content of chemical constituents of volatile oil, analyzes and compares the experiment result of the two kinds of extraction technique. In order to understand clearly that the plant type and distribution of volatile oils, further studies on

its active components and integrated development and utilization of medical resources for reference.

EXPERIMENTAL

Trace DSQ Finnigan gas chromatography-mass spectrometry series instrument American Finnigan Corporation), chromatographic column: OV-1701 (30 m × 0. 25 mm, 0.25 μm) capillary-tube column, manual solid-phase micro-extraction device (American Supelco Company), 100 μm PDMS the extraction fibre plug (American Supelco Company). The root of *A tractylodes macrocephala* was collected in the Pan'an of Zhejiang Province, China, Lin'an of Zhejiang Province, China and An'guo of He'bei Province, China in Nov. 2009 and was identified by Prof. Lu-Huan Lou, Department of Botany, Zhejiang Agriculture and Forestry University. The samples of this plant are mixed and washed clean and then made into powder for later use.

Extraction of the volatile oil: Steam distillation method: Pulverize the *A tractylodes macrocephala* Koidz produced in Pan'an of Zhejiang, Anguo of He'bei and Lin'an of Zhejiang into crude powder, respectively (through 20-hole sieve), then take 100 g of them, respectively into the round-flasks and add some distilled water into them and then put the volatile oil extractor into each of the flask. After this, extract the volatile oil according to the methods stipulated in first edition of Chinese Pharmacopoeia of 2005. During this process, control the speed of the distillation to 36 drops/min till all the volatile oil is extracted. Then collect the volatile oil and determine their volumes in parallel for three times and then figure out their average value and work out their oil extraction rate. The results show that the volatile oil present in *A tractylodes macrocephala* Koidz produced in Lin'an of Zhejiang Province is the pale-yellowish oily liquid having the thick fragrant smell, with the oil extracting rate of 1.92 % (mL/100 g). The volatile oil present in *A tractylodes macrocephala* Koidz produced in Pan'an of Zhejiang Province is the pale-yellowish oily liquid having the thick fragrant smell, with the oil extracting rate of 1.36 % (mL/100 g). The volatile oil present in *A tractylodes macrocephala* Koidz produced in An'guo of He'bei Province is the colourless oily liquid having only some fragrant smell, with the oil extracting rate of 0.84 % (mL/100 g).

Weigh 1 g of the dried and powdered the root of *A tractylodes macrocephala* and put it in the 15 mL sample bottle for solid-phase micro-extraction. Put the solid-phase micro-extraction fibre plug on the sample-feeding mouth of gas chromatography instrument for ageing and keep the ageing temperature at 250 ºC, the gas-loaded flow at 0.8 mL min^{-1} and the ageing time is 10 min. Put the 100 μm PDMS fibre plug into the sample bottle through the rubber washer of the bottle cap and extract for 0.5 h under the temperature of 50 ºC. Then take out the extraction plug from the sample bottle and put it immediately into sample-feeding mouth of gas chromatography instrument to get rid of sorption for 3 min under the temperature of 250 ºC. Meanwhile start up the instrument to collect the experimental data.

Analytical conditions of gas chromatography-mass spectrometry: Gas chromatographic conditions: OV-1701 (30 m × 0.25 mm, 0. 25 μm) capillary-tube chromatographic column, temperature-increasing procedure: keep the initial temperature 50 ºC for 2 min and then increase the temperature to 130 ºC at the rate of 10 ºC min^{-1} and increase the temperature to 190 ºC at the rate of 5 ºC min^{-1} again and continuous increase the temperature to 240 ºC at the rate of 12 ºC min^{-1} and keep it for 4 min the temperature at the sample-feeding entrance is 200 ºC, the temperature at the vaporizing room is 260 ºC, the loaded gas is high-purified (He) and the flowing rate is 0.8 mL min^{-1}, proportion of cleft sample-feeding: 30:1, capacity of sample-feeding: 0.1 mL. Mass-spectrometry conditions: the ion source of electron impact (EI), the ion energy is 70 eV, the temperature of the ion source is 200 º, the voltage of the checking device is 350 V, the scanning range is m/z: 33-450 AMU, the scanning speed is 0.5 s, the retrieving data-base is Willey and NIST standard mass-spectrometry data-base.

RESULTS AND DISCUSSION

Results of identification: Using OV-1701 (30 m × 0. 25 mm, 0. 25 μm) capillary-tube chromatographic column and taking out 0.2 mL volatile oil extracted by the method of steam distillation and SPME, we analyze and identify the volatile chemical consti-tuents by gas chromatography-mass spectrometry instrument. The ratio of the compounds is calculated by the method of area normalization. The calculation of the individual peak area is by Hewlett-Packard software treatment system. The calculation of the relative percentage content in each volatile compo-nent is by the method of peak area normalization. We analyzed the volatile chemical component in the root of *A tractylodes macrocephala* according to the above GC-MS conditions and achieved the total ion flow chart (Figs. 1-6).

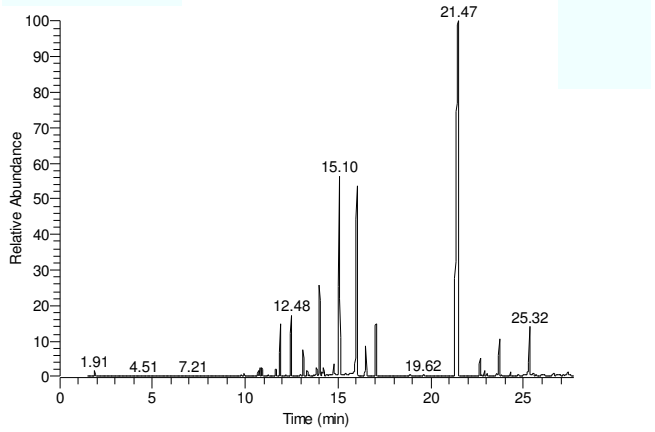

Fig. 1. GC-MS total ion current chromatogram of the volatile oil in *A tractylodes* of Lin'an by steam distillation

After scanning each peak value to the total ion flow chart by mass spectrometry and retrieving and contrasting[7] by mass spectrometry data system (NIST and Willey Standard Atlas Data Bank) and consult the relevant information[8] of MS combined with artificial analysis, we identified the chemical constituents of volatile oil in *A tractylodes macrocephala*. The result can be seen in Tables 1 and 2.

The traditional steam distillation method needs a large amount of samples and it takes longer time; while the SPME

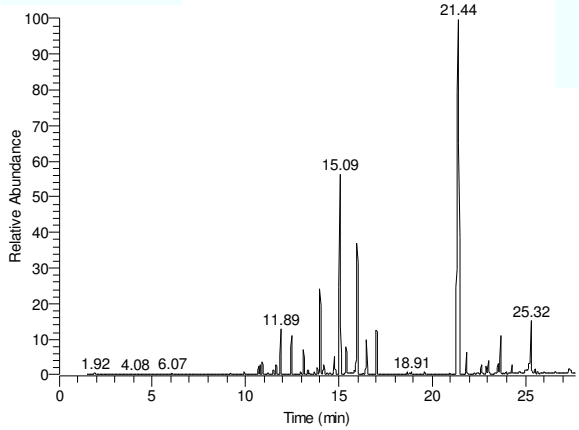

Fig. 2. GC-MS total ion current chromatogram of the volatile oil in *A tractylodes* of Pan'an by steam distillation

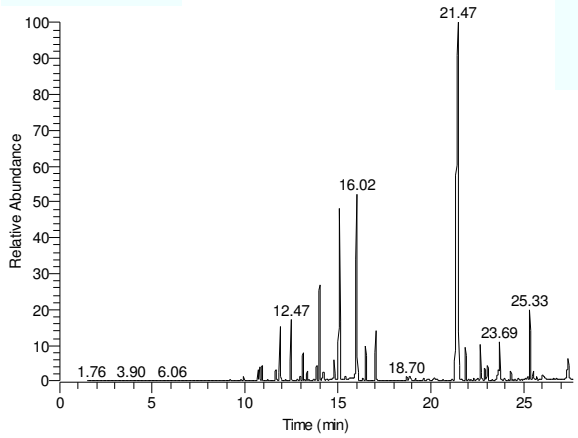

Fig. 3. GC-MS total ion current chromatogram of the volatile oil in *A tractylodes* of An'guo by steam distillation

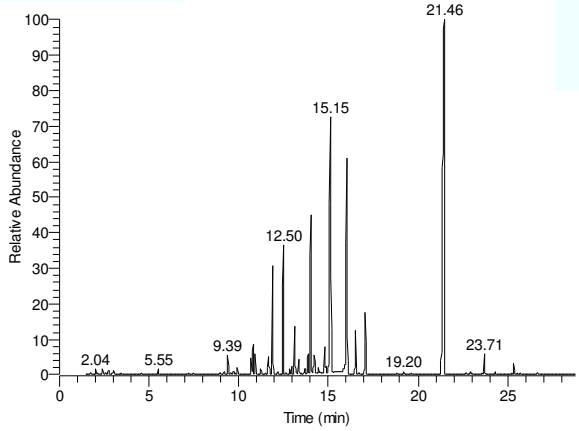

Fig. 4. GC-MS total ion current chromatogram of the volatile oil in *A tractylodes* of Lin'an by solid phase micro-extraction

method greatly reduces the pre-treatment procedure and thus increases the analytical speed and sensitivity. It has the advantages of short operating time, small sample mount and no need of extracting solution and it is suitable for the analysis of the volatile matter and it has good reproducibility. At present, SPME method has been widely used in medicine, environment protection, biological and food analysis[9-11] and the area of volatile oil in traditional Chinese medicine[12,13].

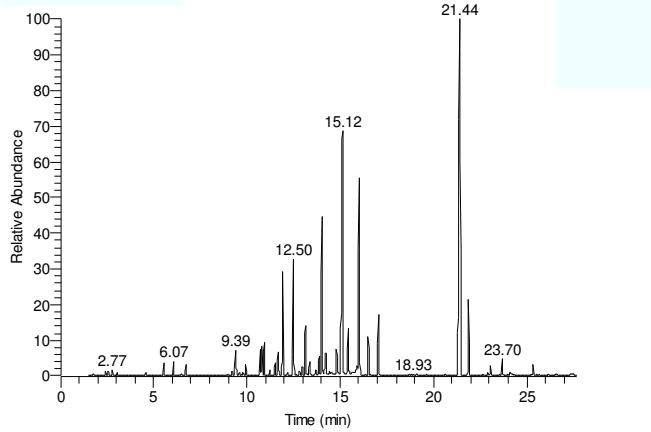

Fig. 5. GC-MS total ion current chromatogram of the volatile oil in *A tractylodes* of Pan'an by solid phase micro-extraction

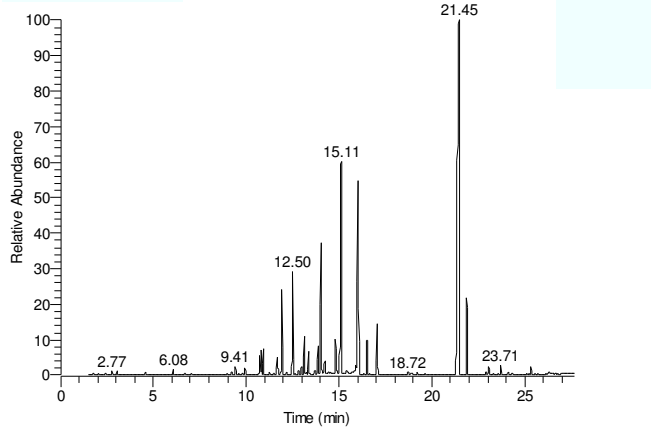

Fig. 6. GC-MS total ion current chromatogram of the volatile oil in *A tractylodes* of An'guo by solid phase micro-extraction

It can be seen from Table-1 that by using steam distillation to extract the volatile constituents in root of *A tractylodes macrocephala*, we identified 30 constituents, the relatively high content in root of *A tractylodes macrocephala* is atractylon (relatively content 43.96-44.60 %), valencene (relatively content 9.34-14.21 %), germacrene (relatively content 7.99-13.61 %), β-selinene (relatively content 4.20-4.55 %), 4,5-dehydro solongifolene (relatively content 2.11-2.57 %). In total volatile oil, the relatively content of atractylon above 40 %.

It can be seen from Table-2 that by using SPME to extract the volatile constituents in root of *A tractylodes macrocephala*, we identified 31 constituents, the relatively high content in root of *A tractylodes macrocephala* is atractylon (relatively content 30.56-35.37 %), valencene (relatively content 13.39-17.90 %), germacrene (relatively content 11.06-13.52%), β-selinene (relatively content 6.28-6.91 %), caryophyllene oxide (relatively content 3.62-3.86 %). In total volatile oil, the relatively content of atractylon above 30 %.

Experimental results show that the type of volatile components is basic similar by steam distillation and SPME method, content of atractylon of *A tractylodes macrocephala* are high prevalent in three different growing areas. In the steam distillation method, the yield of volatile oil was the highest in Lin'an of Zhejiang province, China, reached 1.92 %, the relative content of atractylon in total volatile oil was the highest,

TABLE-1
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF VOLATILE OIL BY STEAM DISTILLATION

Name of components	m.f.	m.w.	Area % *A tractylodes* of Lin'an	Area % *A tractylodes* of Pan'an	Area % *A tractylodes* of An'guo
Guaiene	$C_{15}H_{24}$	204	0.12	0.16	0.15
β-Elemene	$C_{15}H_{24}$	204	0.28	0.41	0.43
α-Gurjunen	$C_{15}H_{24}$	204	0.34	0.46	0.55
γ-Gurjunen	$C_{15}H_{24}$	204	0.10	0.10	0.08
ξ-Guaiene	$C_{15}H_{24}$	204	0.51	0.58	0.65
Caryophyllene	$C_{15}H_{24}$	204	2.24	2.13	2.12
Elemene	$C_{15}H_{24}$	204	2.68	1.79	2.34
Gurjunene	$C_{15}H_{24}$	204	0.10	0.14	0.18
α-Caryophyllene	$C_{15}H_{24}$	204	1.20	1.22	1.13
Turmericene	$C_{15}H_{24}$	204	0.25	0.24	0.14
Zingiberene	$C_{15}H_{24}$	204	0.38	0.35	0.62
β-selinene	$C_{15}H_{24}$	204	4.42	4.55	4.20
Aromadendrene	$C_{15}H_{24}$	204	0.12	0.00	0.10
Calamene	$C_{15}H_{24}$	204	0.36	0.65	0.34
γ-Muurolene	$C_{15}H_{24}$	204	0.61	1.00	0.82
Valencene	$C_{15}H_{24}$	204	13.69	14.21	9.34
Longifolene	$C_{15}H_{26}$	206	0.61	0.41	0.75
Germacrene	$C_{15}H_{26}$	206	13.67	7.99	11.81
Dehydro-aromadendrene	$C_{15}H_{22}$	202	1.44	1.86	3.76
3,7,11-Trimethyl-(E)-1,6,10-dodecatrien-3-ol	$C_{15}H_{26}$	206	0.10	0.13	0.12
Atractylon	$C_{15}H_{20}O$	216	46.60	43.96	44.28
Spathulenol	$C_{15}H_{20}O$	216	0.75	0.43	1.32
β-Eudesmol	$C_{15}H_{26}O$	222	0.24	0.33	0.42
β-Santalol	$C_{15}H_{24}O$	220	0.11	0.65	0.56
Cadinene	$C_{15}H_{26}O$	222	0.16	0.56	0.28
6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1*H*-naphthalen-2-one	$C_{15}H_{22}O$	218	1.53	1.84	1.53
Longiverbenone	$C_{15}H_{22}O$	218	0.14	0.37	0.32
Aromadendrene oxide	$C_{15}H_{20}O_2$	232	0.46	1.33	0.15
4,5-Dehydro solongifolene	$C_{15}H_{22}$	202	2.11	2.57	2.53
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.10	0.18	0.54

TABLE-2
ANALYTICAL RESULTS OF CHEMICAL CONSTITUENTS OF VOLATILE OIL BY SPME

Name of components	m.f.	m.w.	Area % *A tractylodes* of Lin'an	Area % *A tractylodes* of Pan'an	Area % *A tractylodes* of An'guo
Thujopsene	$C_{15}H_{24}$	204	0.23	0.21	0.28
β-Elemene	$C_{15}H_{24}$	204	0.16	0.33	0.26
α-Gurjunen	$C_{15}H_{24}$	204	0.44	0.64	0.59
Isocaryophillene	$C_{15}H_{24}$	204	0.25	0.43	0.18
ξ-Guaiene	$C_{15}H_{24}$	204	0.44	0.65	0.34
Caryophyllene	$C_{15}H_{24}$	204	1.33	1.68	1.66
Elemene	$C_{15}H_{24}$	204	1.88	1.22	1.98
Gurjunene	$C_{15}H_{24}$	204	0.16	0.18	0.33
α-Caryophyllene	$C_{15}H_{24}$	204	1.12	1.01	1.33
Aristolene	$C_{15}H_{24}$	204	0.66	0.36	0.55
(E)-3,7-Dimethyl-2,6-octadien-1-ol-acetate	$C_{12}H_{20}O_2$	196	0.56	0.63	0.77
β-Selinene	$C_{15}H_{24}$	204	6.91	6.86	6.28
Aromadendrene	$C_{15}H_{24}$	204	0.43	0.33	0.42
Calamene	$C_{15}H_{24}$	204	0.15	0.33	0.25
γ-Muurolene	$C_{15}H_{24}$	204	0.33	0.46	0.64
Valencene	$C_{15}H_{24}$	204	17.90	15.48	13.39
Longifolene	$C_{15}H_{26}$	206	0.78	0.93	0.63
Germacrene	$C_{15}H_{26}$	206	13.52	11.06	12.29
Dehydro-aromadendrene	$C_{15}H_{22}$	202	1.56	2.34	2.17
Caryophyllene oxide	$C_{15}H_{24}O$	220	3.79	3.86	3.62
Atractylon	$C_{15}H_{20}O$	216	32.55	30.56	35.37
Spathulenol	$C_{15}H_{20}O$	216	0.99	0.73	1.01
β-Eudesmol	$C_{15}H_{26}O$	222	0.36	0.39	0.44
1-Methanol-à,à,4,8-tetramethyl-3,7-yclodecadiene	$C_{15}H_{26}O$	222	1.55	0.58	0.35
β-Santalol	$C_{15}H_{24}O$	220	0.45	0.25	0.53
Cadinene	$C_{15}H_{26}O$	222	0.66	0.94	0.74
2-Methoxy-4-(1-propenyl)-phenol	$C_{10}H_{12}O$	164	1.10	2.15	1.34
Longiverbenone	$C_{15}H_{22}O_2$	218	0.14	0.37	0.32
Aromadendrene oxide	$C_{15}H_{20}O_2$	232	1.36	1.63	1.12
4,5-Dehydro solongifolene	$C_{15}H_{22}$	202	1.22	1.36	1.85
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.27	0.33	0.26

reached 46 %. This experiment result enhances present understanding of volatile constituents in *A tractylodes macrocephala* and content, the result provides further reference for the development and utilization of *A tractylodes macrocephala* of China.

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