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Analysis of Volatile Organic Compounds of Pine Nodular Branch

XIAOBO WU, JIAN XUE* and LINLIN ZHANG

Department of Natural Medicine Chemistry Research Center, Institute of Medicinal Plant Development, Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100193, P.R. China

*Corresponding author: Fax: +86 10 57833097; Tel: +86 10 62895076; E-mail: xuejian5076@yahoo.com.cn

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In order to investigate the volatile composition of pine nodular branch, supercritical fluid extraction, steam distillation and solvent extraction was used. The volatile oil was analyzed by gas chromatography-mass spectrometry. In all, 41 volatile components were identified. It was established that the volatile components mainly include some terpenoids compounds, such as pinene, longifolene and caryophyllene. The volatile components of pine nodular branch which was collected from chinese pine (*Pinus tabulaeformis* Carr.) and masson pin (*Pinus massoniana* Lamb.) are similar.

Key Words: Pine nodular branch, GC/MS, Volatile oil.

INTRODUCTION

Pine Nodular Branch is widely used in Chinese medicine for dispels wind, dries damp, relieves pain. It is used for pain in the joints with stiffness due to cold damp, also for traumatic injury and pain. The earliest recordation of it in medical book (Ming Yi Bie Lu) is about 1800 years ago in Wei and Jin Dynasty of China. Several ancient classical medicinal books of dynasties, such as Ri-Hua-Zi-Ben-cao in Song Dynasty, Ben-Cao-Yan-Yi-Bo-Yi in Yuan dynasty, compendium of materia medica in Ming dynasty, have recorded the information about it. The volatile organic compounds are the activity compounds in this herb. The root, pine needles, pine bark, farina, cone, colophony of pine are all drug in Chinese traditional medicine[1].

Pine Nodular Branch[2] is the branch or strumae of chinese pine (*Pinus tabulaeformis* Carr.) mainly grow in the north of China and masson pine (*Pinus massoniana* Lamb.) is found in the south of China. Chinese pine is widely distributed in 12 provinces of north China, such as Liaoning province, Shandong provinces, Shanxi provinces, Qinghai provinces, Sichuan provinces. Masson pine, which has excellent characteristics on barren resistant, adaptable, fast-growing, high yield, is widely distributed in South China's 16 provinces of north latitude 22-32°[3].

In our study, supercritical fluid extraction, steam distillation and solvent extraction were used to get the volatile compounds. Supercritical CO_2 fluid extraction (SFE-CO_2), using CO_2 instead of organic solvent and possessing unusual properties including high compressibility, liquid-like density, high diffusivity, low viscosity and low surface tension, can be considered one of the most potentially useful new methods of sample preparation in pharmaceutical and food processing industry[4].

The aim of this paper, therefore, was to identify the volatile compounds of Pine Nodular Branch by GC-MS and compare the differences of the three extraction methods.

EXPERIMENTAL

α-Pinene, α-terpineol and caryophyllene were purchased from sigma, USA. Ethanol (analytically pure) was obtained from Beijing chemical plant, China.

Samples: Samples were collected from three mainly herb markets (An-guo of Hebei province, Bo-zhou of Anhui province and Kunming of Yunnan province) and local places of different provinces (Jiangxi, Anhui, Hunan, Henan, Shandong, Beijing) in 2008. Samples were identified by Zhang ben-gang, a professor of Institute of Medical Plant Development, Peking Union Medical College, Beijing, China.

Extraction methodologies

Ethanol extraction: About 2.0 g of sample was directly extracted for 15 min, with 20 mL of ethanol by ultrasonic extraction. Afterwards, the extract was filtered and then prepare for GC and GC-MS.

Steam distillation: Samples were dried at room temperature, powdered and submitted to steam distillation for 5 h. Then oils were dried over anhydrous sodium sodium sulphate (Na_2SO_4), then stored at 2-4 °C.

Supercritical CO_2 extraction: Supercritical CO_2-extraction of oil was carried out using a supercritical fluid extraction unit (Jasco LC-1500 SFE) at 60 ºC and at 450-bar pressure with a gas flow of 60 g/min for 3 h. The oil was collected in the cyclone separator and stored at 4 ºC until used.

Gas chromatography condition and gas chromatography-mass spectrometry analysis: Aglient 6890N gas chromatography equipped with FID was used to optimize the chromatography condition and further confirm identified compounds with standards. The capillary column was DB-5 (30 m × 0.25 mm, film thickness 0.32 μm). The oven temperature was 60 ºC (for 5 min), then increased at 5 ºC/min to 160 ºC, then increased at 20 ºC/min to 280 ºC and held for 10 min. N_2 was used as the carrier gas at a flow rate 1.5 mL/min. The injector temperatures was 200 ºC, the detector temperature was 300 ºC.

Volatile oil was analyzed using an Aglient 6890 gas chromatography equipped with a HP5673 mass selective detector and a NIST2.03 software version. The capillary column used for quantification analysis was HP-5ms (30 m × 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas at a flow rate 1 mL/min. The injector temperature was 250 ºC.

Identification was achieved by comparisons of mass spectra obtained from the sample, with those from pure standards injected in the same conditions; by comparing the mass spectra present in the NIST 2.03 MS Library Database and in the literature.

RESULTS AND DISCUSSION

A total of 41 volatile compounds were identified by gas chromatography-mass spectrometry in 20 samples of chinese pine and masson pine from South china to north China (Fig. 1). When the mass spectral fit values of compounds were ≥ 80 %, the volatile compounds were tentatively considered 'identified' (Table-1). α-Pinene, α-terpineol and caryophyllene were further confirmed by comparing their retention times with standards (Figs. 2-5).

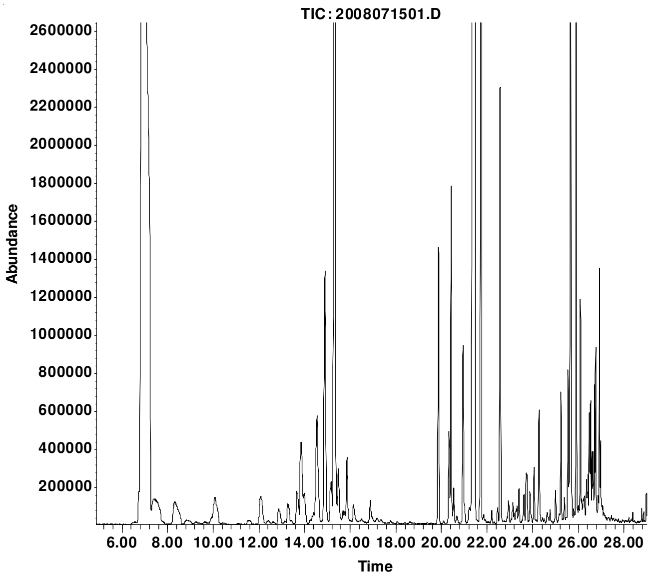

Fig. 1. Total ion GC-MS chromatogram of volatile oil in Pine Nodular Branch

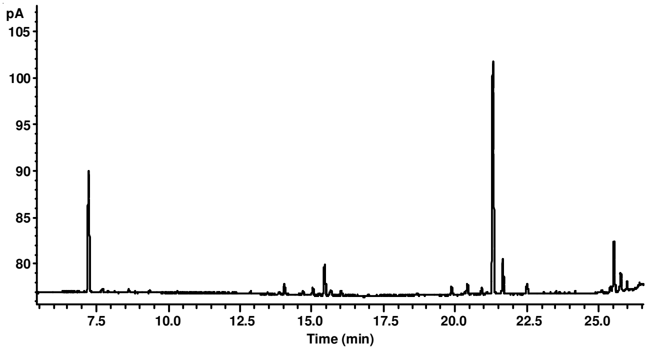

Fig. 2. Gas chromatogram obtained from the volatile oil of Pine Nodular Branch

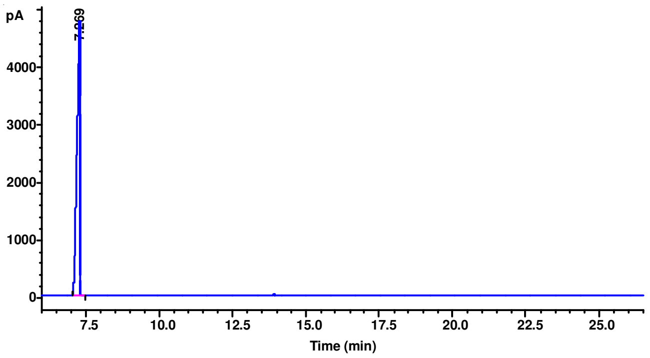

Fig. 3. Gas chromatogram of α-pinene

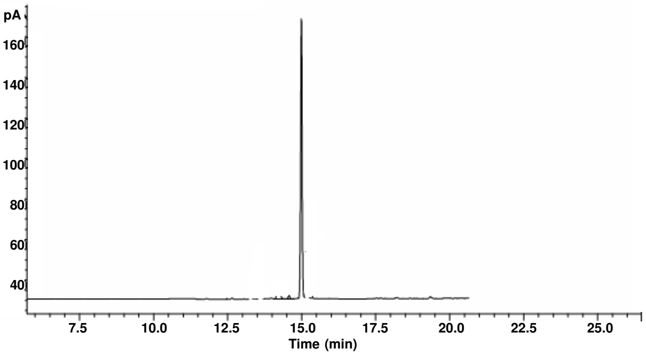

Fig. 4. Gas chromatogram of α-terpineol

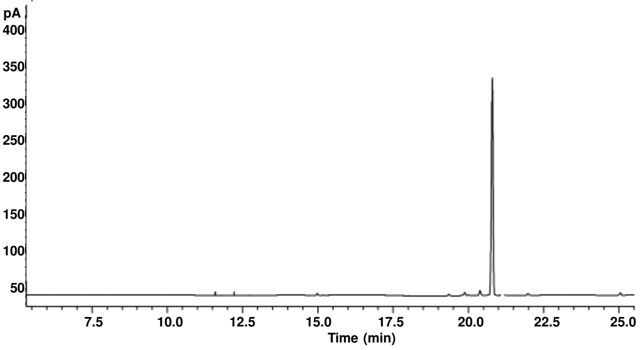

Fig. 5. Gas chromatogram of caryophyllene

The compound comprises different classes of compounds: terpenoids, heterocyclic compounds, aromatic compounds. The major compounds are caryophyllene, α-pinene, caryophyllene oxide, α-terpineol.

In this study, three different extraction techniques were used for analyzing volatile compounds. The constituents of

TABLE-1
IDENTIFICATION OR TENTATIVE IDENTIFICATION OF VOLATILE COMPOUNDS IN PINE NODULAR BRANCH BY GC/MS

No.	Compound	Retention time	Empirical formula	EIMS fragmentation	Relative contents (%)
1	α-Pinene	6.713	$C_{10}H_{16}$	41, 53, 65, 77, 93, 105, 121, 136	16.208
2	β-Phellandrene	8.385	$C_{10}H_{16}$	27, 41, 53, 69, 79, 93, 107, 121, 136	0.378
3	Cyclooctadiene,1,5-dimethyl -1,5	10.090	$C_{10}H_{16}$	27, 39, 51, 65, 77, 91, 103, 119, 134	0.368
4	D-Limonene	10.106	$C_{10}H_{16}$	27, 41, 53, 68, 79, 93, 107, 121, 136	0.143
5	Bicyclo[2.2.1]heptan-2-one,1,3,3-trimethyl	12.087	$C_{10}H_{16}$	27, 41, 53, 69, 81, 109, 137, 152	0.214
6	Oxatricyclo[4.1.1.0(2,4)]octane,2,7,7-trimethyl -3	12.734	$C_{10}H_{16}O$	27, 43, 55, 71, 81, 93, 111, 121, 139, 154	0.257
7	Bicyclo[2.2.1]heptan-2-ol,1,3,3-trimethyl	12.918	$C_{10}H_{18}O$	27, 41, 55, 69, 81, 85, 93, 97, 107, 111, 121	0.116
8	Cyclopentene,2,2,3-trimethyl -1-acetaldehyde -3	13.311	$C_{10}H_{16}O$	27, 41, 55, 67, 81, 93, 108, 119, 137	0.201
9	[1S-(1.α.,3.α.,5.α.)]-6.,6-dimethyl-2-methylene-bicyclo[3.1.1]heptan-3-ol,	13.722	$C_{10}H_{16}O$	41, 55, 70, 83, 92, 109, 119, 134, 152	0.513
10	Bicyclo[2.2.1]heptan-2-one,1,7,7-trimethyl,	13.900	$C_{10}H_{16}O$	27.41.55.69.81.95, 108, 137, 152	1.653
11	Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl	14.569	$C_{10}H_{18}O$	27, 41, 55, 67, 82, 95, 110, 121, 139	0.555
12	Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-3	14.909	$C_{10}H_{18}O$	43, 55, 71, 77, 86, 93, 111, 121, 136, 154	1.107
13	Benzenemethanol,.α.,.α.,4-trimethyl	15.162	$C_{10}H_{14}O$	43, 51, 59, 65, 77, 91, 105, 115, 135, 150	0.225
14	α-Terpineol	15.335	$C_{10}H_{18}O$	31, 43, 51, 59, 67, 81, 93, 107, 121, 136	4.739
15	Bicyclo[3.1.1]hept-2-ene-2-methanol,6,6-dimethyl	15.518	$C_{10}H_{16}O$	27, 41, 55, 67, 79, 91, 108, 119, 134, 152	0.248
16	Bicyclo[3.1.1]hept-3-en-2-one,4,6,6-trimethyl	15.880	$C_{10}H_{14}O$	27, 39, 55, 69, 84, 95, 109, 119, 137, 152	0.689
17	2-Cyclohexen-1-ol,2-methyl-5-(1-methylethenyl)-	16.150	$C_{10}H_{16}O$	27, 41, 55, 69, 84, 95, 109, 119, 137, 152	0.276
18	Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl	19.872	$C_{15}H_{24}$	41, 55, 69, 79, 93, 105, 119, 133, 147, 161, 189, 204	1.547
19	Cycloisosativene	20.320	$C_{15}H_{24}$	41, 55, 67, 79, 94, 105, 119, 133, 147, 161, 189, 204	0.449
20	1,2,4-Methenoazulene,decahydro-1,5,5,8a-tetramethy	20.428	$C_{15}H_{24}$	27, 41, 55, 69, 79, 94, 105, 119, 133, 147, 161, 189, 204	1.719
21	1*H*-Cycloprop[e]azulene,1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl,	20.504	$C_{15}H_{24}$	27, 41, 55, 67, 77, 91, 105, 119, 133, 147, 161, 189, 204	0.223
22	2-Cyclopenten-1-one,4-hydroxy-3-methyl-2-(2-propenyl)	20.649	$C_9H_{12}O_2$	29, 41, 55, 69, 79, 93, 105, 119, 134, 147, 161, 175, 189, 204	0.300
23	1,4-Methano-1*H*-indene,octahydro-4-methyl-8-methylene-7-(1-methylethyl),	20.925	$C_{15}H_{24}$	41, 55, 67, 79, 91, 108, 119, 133, 147, 161, 175, 189, 204	1.221
24	1*H*-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene,	21.184	$C_{15}H_{24}$	29, 41, 55, 69, 81, 91, 105, 119, 133, 147, 161, 175, 189, 204	0.114
26	Caryophyllene,	21.712	$C_{15}H_{24}$	27, 41, 55, 69, 79, 93, 105, 120, 133, 147, 161, 175, 189, 204	48.132
27	1*H*-Cyclopropa[a]naphthalene,1a,2,3,3a,4,5,6,7b-octahydro-1,1,3a,7-tetramethyl,	22.457	$C_{15}H_{24}$	29, 41, 55, 69, 79, 93, 105, 119, 134, 147, 161, 175, 189, 204	0.069
28	α-Caryophyllene	22.570	$C_{15}H_{24}$	41, 53, 67, 80, 93, 107, 121, 147, 189, 204	1.615
29	Bicyclo[7.2.0]undec-4-ene,4,11,11-trimethyl-8-methylene,	22.943	$C_{15}H_{24}$	27, 41, 55, 69, 79, 93, 107, 120, 133, 148, 161, 175, 189, 204	0.071
30	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	23.126	$C_{15}H_{24}$	27, 41, 55, 69, 79, 93, 105, 119, 133, 148, 161, 175, 189, 204	0.073
31	Eudesma-4(14),11-diene	23.385	$C_{15}H_{24}$	41, 55, 67, 81, 93, 105, 121, 133, 147, 161, 175, 189, 204	0.133
32	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl),(1.alpha.,4a.alpha.,8a.alpha.)	23.714	$C_{15}H_{24}$	41, 55, 67, 77, 91, 105, 119, 134, 147, 161, 189, 204	0.438
33	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)	23.881	$C_{15}H_{24}$	27, 41, 55, 69, 79, 93, 109, 119, 135, 147, 161, 189, 204	0.125
34	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	24.054	$C_{15}H_{24}$	29, 41, 55, 67, 79, 91, 105, 119, 133, 147, 189, 204	0.266
35	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	24.270	$C_{15}H_{24}$	27, 41, 55, 69, 81, 91, 105, 119, 134, 147, 161, 189, 204	0.578

36	(+)-Longicamphenylone	25.236	$C_{14}H_{22}O$	27, 41, 55, 67, 79, 91, 105, 121, 135, 145, 163, 175, 191, 206	0.599
37	1*H*-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylen,	25.581	$C_{15}H_{24}O$	32, 43, 55, 67, 79, 91, 105, 119, 131, 145, 159, 177, 189, 205, 220	0.779
38	Caryophyllene oxide	25.694	$C_{15}H_{24}O$	27, 43, 55, 69, 79, 93, 109, 121, 135, 149, 161, 177, 205, 220	6.865
39	1,4-Methanoazulen-9-ol, decahydro-1,5,5,8a-tetramethyl	25.996	$C_{15}H_{26}O$	41, 55, 69, 85, 95, 109, 119, 137, 151, 161, 189, 204	2.778
40	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,1,5,5,8-tetramethyl-,[1R-(1R*,3E,7E,11R*)]	26.234	$C_{15}H_{24}O$	27, 43, 55, 67, 81, 96, 109, 123, 138	1.020
41	Longifolenaldehyde	26.962	$C_{15}H_{24}$ O	29, 41, 55, 67, 81, 95, 109, 121, 135, 149, 103, 176, 187	0.136

oils are consistent, but the relative percentage obtained from peak area of compositions of different extraction techniques is different.

The extraction efficiency of steam distillation was equivalent with ethanol extraction, but the extraction efficiency of supercritical fluid extraction was much lower than two former. The reason would be that the polarity of CO_2 in supercritical state is not fit for low polarity components, such as caryophyllene.

Conclusion

Fourty one compounds were identified from pine nodular branch, which collected from *Pinus tabulaeformis* Carr. and *Pinus massoniana* Lamb.. The compositions of the volatile oil of pine nodular branch of 2 species were consistent. This result verify the 2 species have close genetic relationship.

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