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Study on Terpenoid Compounds of Different Tissues of Camphor Tree

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Abstract. Based on the current research value of biomass and terpenoids, in this paper, the terpenoids contented in the different organizational of the camphor tree were analyzed by SDE extract method and GC-MS analysis method. The results showed that the contents of terpenoids contained in camphor leaves were the richest. The highest content of terpenoids in three tissues was camphor, especially the relative content of camphor in leaves got to 71.96%.In addition, except camphor, the higher terpeneoid compounds of the leaves respectively were carypohyllene (5.29%), α-pinene (4.06%) and limonene (2.87%). The higher terpeneoid compounds of the branches respectively were α-pinene (2.33%) and carypohyllene (1.78%). The terpenoids contained in the flowers were ketone terpenes, respectively, 2, 3-acetylacetone (1.33%) and propyl methyl ketone (1.25%). This paper provides the basis for the extraction and utilization of terpenoids in camphor trees, which lays a foundation for the further study of the application value of natural terpenoids in camphor trees.

Introduction

Terpenoids are the hydrocarbons that contain multiples of isoprene units and oxygen-containing derivatives, are widely found in nature. They are the relatively important kinds of biomass products, which have many medicinal function, such as deworming, anti-cancer, anti-aging, expectorant cough, sweating, antibacterial, analgesic, etc. The terpenoids have been applied in the pharmaceutical, spices, food and health industry and the oil industry. Scholars at home and abroad have shown the active attention on the terpene compounds [1-3].

Cinnamon is a special economic tree species with important application in subtropical regions. It is rich of volatile oil in root, stem, leaf, seed, skin, and flower. In Chinese traditional medicine, the camphor tree has the effect onremoving rheumatism, recuperating blood, and the joints. That can be used to treat epigastric pain, athlete's foot, gout, scabies, and bruises [4]. The study of the composition of compounds in camphor tissue has also become a hotspot. Camphor tree is as shown in Fig.1.

Fig.1.camphor tree

G Q, Xu et al [5] studied the anti-corrosion properties of camphor tree leaves extract on bamboo wood, and put forward the application characteristics of good biogenic preservative of camphor leaf benzene-alcohol extract. JXuet al [6] studied the GC-MS analysis of liposoluble components in different parts of camphor, and identified some of the components of liposoluble compounds in various parts of camphor trees by traditional percolation extraction method. RY Yeh et al. [7] discussed the antimicrobial properties of the extracts of the camphor leaves on the marine organisms such as white shrimp, abalone and fish. It was suggested that the extract of camphor extract could be the substitutes of the synthetic chemicals to inhibit the pathogen growth and enhance shrimp immunity. Venkatraman S et al. [8] considered the differences in the compositions of the extracts of the camphor leaves grown in two different areas with the medium of steam distillation. Although there are many studies on the chemical structure of camphor trees, the research on terpenoids in volatile components has yet to be deepened. In this paper, the extraction methods of camphor terpene compounds were optimized, and the related aspects of different tissue analysis were explored.

Materials and Methods

Materials

Air driedbranches, leaves and flowers of camphor trees are obtained from Guangdong Province, China. They were crushed into small pieces and filtered to get the powder. The materials were further dried in a vacuum oven for 8 h for further use. Then small pieceswere treated with petroleum etherat 60-90℃, purchased from Guangzhou Congyuan Instrument Co., Ltd. with simultaneous distillation extraction glass equipment (Fig.2). The water phase was heated by electric heating sets (Nantong Lihao Experimental Instrument Co., Ltd.) and the petroleum ether phase was heated by HH-S digital thermostatic water bath (Jiangsu Zhengji Instrument Co., Ltd.). The extracts dealt with the rotary evaporator (Guangzhou Branch of the Instrument Co., Ltd.). Gas Chromatography - Mass Spectrometer (GCMS-QP2010Plus, Shimadzu Corporation) was used for analysis.

Experimental Method

Extraction Method

All the branches, leaves and flowers of the camphor tree are extracted with simultaneous distillation extraction (SDE) method, the device shown in Fig.2.

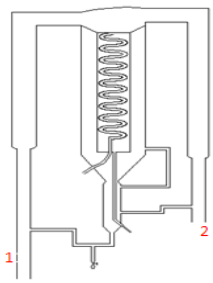

Fig.2. Simultaneous distillation extraction device

1 g of material and 100ml of distilled waterareadded into the 500ml round bottom flask 1 and connected to the interface 1, 30ml petroleum ether added into the 500ml

round bottom flask 2 and connected to the interface 2. The flask 1 is heated by the conventional electric heating and the flask 2 is heated by the water bath with 90℃. To keep the better extraction, the water bath is heated to 60℃ first. Then start the electric heating oven heating to keep the flask 1 in a slightly boiling state. The extracts processed under the rotary evaporator. Collect the extract to be tested.

Analysis Method

GC conditions served for analysis: Rxi®-5ms (30m × 0.25mm × 0.25μm), He (purity 99.999%) as the carrier gas, inlet temperature at 250℃ 1μl injection volume, using split less injection method. Heating program: Initial temperature was maintained at 40℃ for 2 min; then increased to 150℃ at the rate of 5℃/min, kept 1 min, raised the temperature to 200℃ at 2℃/min, kept for 1 min; the nraisedit to 250℃ at 10℃/min, kept for 2 min.

Mass spectrometry (MS) conditions: Interface temperature: 250℃, ion source temperature: 200℃, scan mode: Scan, scanning range: 40-600amu, solvent removal time: 4.0 min.

Result and Analysis

Total GC-MS spectra of the terpenoids were acquired based on the GC-MS analysis of the extracts from the leaves, the branches and the flowers of camphor tree(Fig. 3).

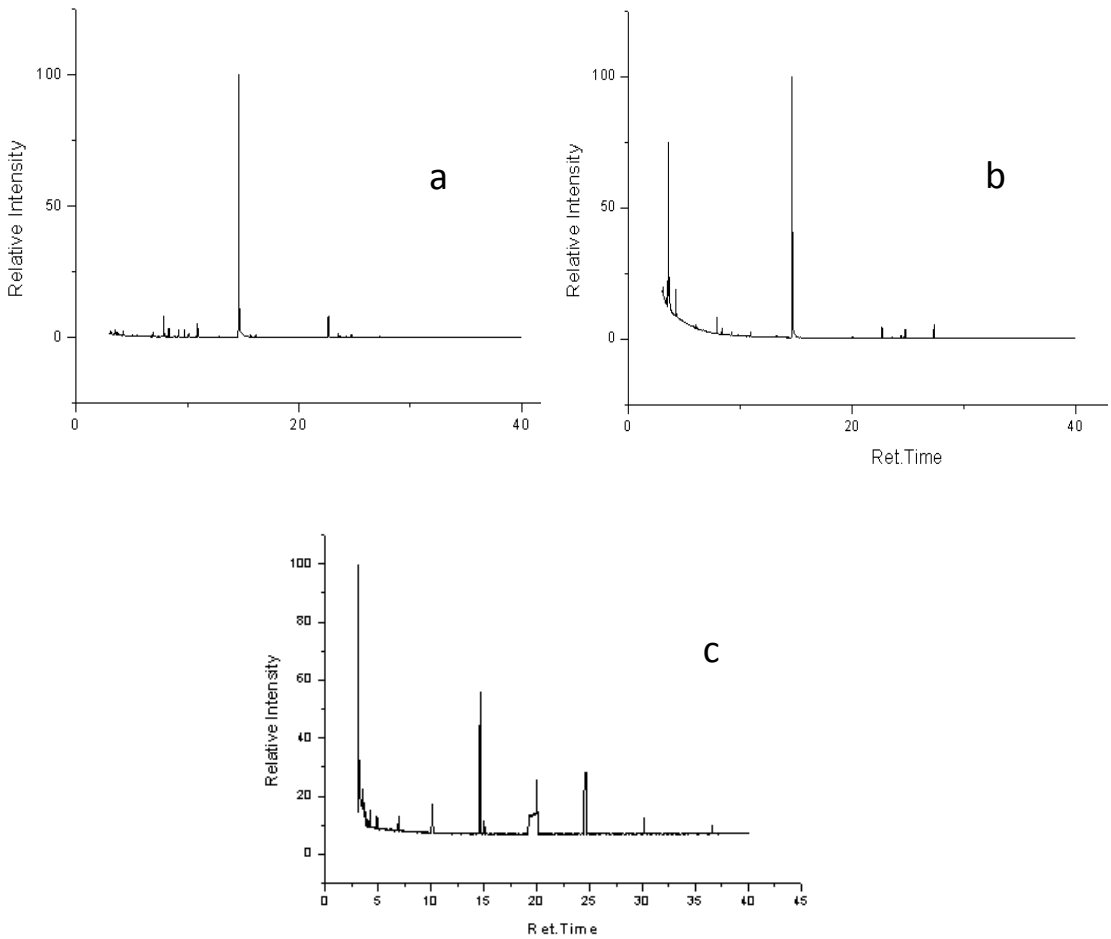

Fig. 3. Total GC-MS spectra of the camphor tree extracts (a. leaves, b. branches, c. flowers)

Compared with the different tissues extracts of the camphor tree, the leaves extract was relatively pure with the best extraction effect of terpene compounds. The extraction effect of branches was obvious, but the composition was slightly complicated. The composition of the flowers extract was the most complex with the worst extract effect. The total GC-MS spectra of the three different tissues of camphor tree were calculated by the normalized area of the chromatogram. The relative contents of each component were calculated. The data were as shown in Table 1.

The data showed that the species of terpenoids contained in camphor leaves was the most abundant, and the extraction of terpenoids by SDE was more pure (92.81%). The highest content of terpenoids in three kinds of raw materials was camphor, especially the relative content of camphor in leaves got to 71.96%. The highest content of the leaves and branches are terpenoids, In addition, except camphor, the higher terpeneoid compounds of the leaves respectively were carypohyllene (5.29%), α-pinene (4.06%) and limonene (2.87%). The higher terpeneoid compounds of the branches respectively were α-pinene (2.33%) and carypohyllene (1.78%). The terpenoids contained in the flowers were ketone terpenes, respectively, 2, 3-acetylacetone (1.33%) and propyl methyl ketone (1.25%).

Compared with the related literature [9-11], SDE method used in this paper, has high purity of terpene compounds, especially the camphor leaves. The result provides a good basis for the extraction and purification of camphor leaves of the terpene compounds research.

Table 1-1 Comparison of Chemical Constituents of Volatile Oil from Different Tissues of Camphor Tree

NO	Ret time	name	Molecular formula	Relative content(%)		
				lea	branch	flow
1	3.067	Dimethylsilanediol	$C_2H_8O_2Si$			33.7
2	3.071	Hexane, 2,3,4-trimethyl-	C_9H_20		0.63	
3	3.133	Cyclopentane, 1,2,4-trimethyl-	C_8H_{16}	0.7		
4	3.486	2,3-Dimethylhexane	C_8H_{18}		0.74	
5	3.506	2,3-Pentanedione	$C_5H_8O_2$			*1.33*
6	3.584	Toluene	C_7H_8		21.29	
7	3.594	2-Methylheptane	C_8H_{18}	1.0	2.97	
8	3.661	Allyl methyl ketone	C_5H_8O			*1.25*
9	3.751	3-Methylheptane	C_8H_{18}	0.5	4.49	
10	3.767	Cyclohexane, 1,3-dimethyl-, cis-	C_8H_{16}	0.9	0.55	
11	3.783	(S)-l-Alanine ethylamide, (S)-	$C_5H_{12}N_2O$			1.48
12	4.234	2-Nitropropane	$C_3H_7NO_2$			0.94
13	4.337	Octane	C_8H_{18}	0.6	3.28	
14	4.815	4-Methyl-2,4-bis(4'-trimethylsilyloxy phenyl)pentene-1	$C_{24}H_{36}O_2Si_2$			1.10
15	4.875	2,5-Dimethoxy-4-(methylsulfonyl)am				0.99
16	4.933	4-Methyl-2,4-bis(4'-trimethylsilyloxy				0.67
17	6.048	o-Xylene	C_8H_{10}		0.61	
18	6.900	Vinyl acetone	C_5H_8O			*0.59*

Table 1-2 Comparison of Chemical Constituents of Volatile Oil from Different Tissues of Camphor Tree

N	Ret	name	Molecular	Rel lea	branch	flow
20	8.055	alpha.-Pinene	$C_{10}H_{16}$	*4.0*	*2.33*	
21	8.516	Camphene	$C_{10}H_{16}$	1.7	0.84	
22	9.244	Sabinene	$C_{10}H_{16}$		0.38	
23	9.700	.beta.-Myrcene	$C_{10}H_{16}$	1.2		
24	10.06	Ethyl3-(6-methoxy-3-methyl-2-benzof	$C_{22}H_{24}O_5$			1.55
25	10.13	alpha.-Phellandrene	$C_{10}H_{16}$	0.7		
26	10.94	Cyclobutane, 1,3-diisopropenyl-, trans	$C_{10}H_{16}$		0.57	
27	11.150	Limonene	$C_{10}H_{16}$	*2.8*		
28	12.83 5	(+)-4-Carene	$C_{10}H_{16}$	0.2 1		
29	14.87	Camphor	$C_{10}H_{16}O$	*71.*	*53.66*	*7.89*
30	14.98	4,5-Octanediol	$C_8H_{18}O_2$			1.55
31	16.03	4-Terpineol	$C_{10}H_{18}O$	0.5		
32	16.52	alpha.-Terpineol	$C_{10}H_{18}O$	0.7		
33	19.24 2	Tetrasiloxane,3,5-diethoxy-1,1,1,7,7,7 -hexamethyl-3,5-bis(trimethylsiloxy)-	$C_{16}H_{46}O7Si_6$			3.72
34	19.34	Diethylenetriamine	$C_4H_{13}N_3$			5.05
35	19.51	-1,3-Dioxolane, 2-heptyl-	$C_{10}H_{20}O_2$			3.86
36	19.68	1,3-Dioxolanel	$C_3H_6O_2$			2.29
37	19.80	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si$			4.64
38	19.94	Formamide, N,N-dimethyl-	C_3H_7NO			2.47
39	22.68	Caryophyllene	$C_{15}H_{24}$	*5.2*	*1.78*	
40	23.56	alpha.-Caryophyllene	$C_{15}H_{24}$	1.1		
41	23.75 7	1H-Cycloprop[e]azulene,decahydro-1, 1,7-trimethyl-4-methylene-,	$C_{15}H_{24}$	0.4 7		
42	24.29	Germacrene D	$C_{15}H_{24}$	0.3		
43	24.37 0	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5, 5-tris(trimethylsiloxy)tetrasiloxane	$C_{16}H_{48}O_{10}Si_9$		0.68	
44	24.54	Tetrakis(trimethylsiloxy)silane	$C_{12}H_{36}O_4Si$			8.09
45	24.65	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-	$C_{16}H_{48}O_{10}$		0.46	
46	24.76	Germacrene B	$C_{15}H_{24}$	0.8	1.68	
47	27.83	Spathulenol	$C_{15}H_{24}O$	0.3	*2.75*	

Conclusions

In this paper, the relative contents of terpenoids in different components of Camphor tree were discussed, and the SDE method was used to extract the pure and higher

terpene compounds extracts of the camphor leaves effectively. The results are drawn as follows:

(1) In the camphor tree biomass resources, the camphor tree is rich of terpenoids and the leaves are more suitable for the development and application of camphor terpenoids.

(2) SDE is a better method for the extract of terpenoid compounds of camphor leaves for its pure and higher terpene compounds extract ability.

Acknowledgements

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