Ekoloji 28(108): 87-91 (2019)

LETTER TO THE EDITOR

Diverse Essential Oils from Leaves of Three Chemotypes *Cinnamomum Camphora*

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Cinnamomum camphora is an important timber and economic tree species in southern China. The leaves of *C. camphora* are a renewable biological resource and are an important resource for the comprehensive development and utilization of eucalyptus. The composition and functional characteristics of ethanol extracts from leaves of three chemotypes *C. camphora* were elucidated by Gas Chromatography/Mass Spectrometer (GC/MS). The results showed that the known contents of volatile organic components (VOCs) detected in the three chemotypes leaves accounted for 98.428%, 45.185% and 97.907% of the total essential oil respectively, and two of them were identified, namely aromatic camphor (Linalool 66.774%) and camphor type (Camphor 47.483%). However, a special component with 53.070% content in the one chemotypes was not identified. Its second-rich component is trans-(+)-Nerolidol, with a content of 17.725%. More substances were detected in Camphor type than those of the other two chemotypes, and there were more VOCs at low distillation points. The unknown chemotypes of *C. camphora* are more abundant in VOCs than that of distillation points. Meanwhile, some bioactive components including 3-Hexen-1-ol, (Z)-, Eucalyptol, Caryophyllene, Humulene and Caryophyllene oxide were detected in three chemotypes of *C. camphora*.

I Introduction

Cinnamomum camphora is the dominant tree of evergreen broad-leaved forest in subtropical region of China. The scholars have studied the chemical composition of *C. camphora* leaves and found that the chemical composition of different species of *C. camphora* is very different. *C. camphora* can be divided into five main types according to the main components and combinations in their leaves. They are aromatic camphor, isoborneol-type, camphor-type, cineole-type and borneol-type (Guo et al. 2017). With the increasing demand of natural spices at home and abroad, the rapid development of *C. camphora* essential oil has been promoted (Guo et al. 2017, Gao et al. 2017, Jiang et al. 2016). All *C. camphora* parts (stems, leaves, branches, roots, fruits, etc.) can produce essential oil. Therefore, there is a significant way to use *C. camphora* leaves to product essential oil. *C. camphora* leaves is a renewable resource and the VOCs of *C. camphora* leaves are similar to roots and stems (Shu et al. 2018). It is updating fast and has large quantity total. In this study, the *C. camphora* leaves from Central South University of Forestry and Technology in Hunan Province were used as experimental materials. The chemical composition and content was analyzed by GC/MS. We hope to provide the theoretical basis for the resourcing of *C. camphora* leaves.

II Materials and Methods

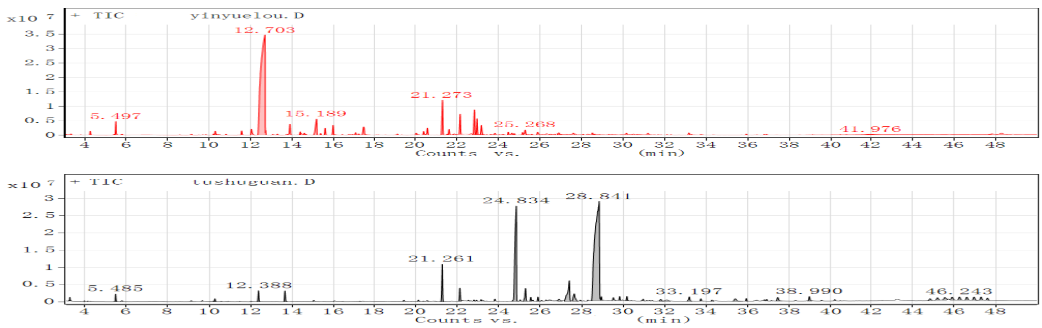
Materials and reagents: *C. camphora* leaves are picked at three different locations in Central South Forestry University. The age of each tree is more than 15 years old. *C. camphora* leaves were picked in January 2018. Smashing with a FZ102 pulverizer. (Tanjing Taisite Ins. Corp., China) The powder was screened with 40 orders. (Jin and Mi 2019) Ethanol extraction of *C. camphora* leaves: Take 10g *C. camphora* leaves powder and 200 ml ethanol, with the solid-liquid ratio of 1:20 (m/v). After immersing at room temperature for 1 h, the mixed solution were fully extracted by automatic FOSS Soxhlet Extracted apparatus (Agilent, USA) at 70°C for 3h, and then filtrated fast with filter paper which immersed in ethanol for 1min. The filtrated extraction was evaporated at 45 °C under a vacuum of 0.01 MPa and concentrated to 20 mL, were set in 4 °C refrigerator for the following determination (Liu et al. 2017; Lam et al. 2019).

VOCs analysis by GC/MS: 750 µL of supernatant was removed into the reagent bottle to be detected by GC/MS. GC/MS uses the Agilent5975C/6890N type produced by the American Agilent company. GC temperature program consisted of start temperature at 50°C held for 3 min, followed by a temperature ramp of 5°C min-1 to 250°C held for 4min, then 5°C min-1 to 260°C held for 1 min, while the volatiles of the extractive (1 µL) were released in a split mode (ratio of 1:3) to a capillary column (30 m×250 µm×0.25 µm); The flow velocity of helium was 1.0mL/min. the carrier gas was high purity He (99.999%), with a column pressure 57.4KPa; vaporization chamber temperature was 280°C. The program of MS was scanned over the 35-600 AMU (m/z), with an ionizing voltage of 70 eV and an ionization current of 150 µA of electron ionization (EI). Ion source temperature was 230°C, and quadropole temperature was 150°C, solvent delay 3 min (Peng et al. 2016, Liu et al. 2017). Identification of compounds through the GC/MS TIC (total ion chromatogram) and the NIST mass spectrometry database. The identification results retain the compounds with higher matching degree (more than 90%), and it in combination with the known compounds reported in the literature. The relative percent content of each component is calculated by the method of peak area normalization.

Statistical Analysis: The graphs and tables were done by Origin8.5 and Excel Microsoft 2010

III Results

In the range of 50°C to 260°C, the GC/MS TIC of VOCs from *C. camphora* leaves extractives is shown in Figure 1. (The chromatogram of the three *C. camphora* leaves extracts is slightly different from the peak position of the same substance, now the peak position data of the extraction chromatogram are only present at the earliest time. For the convenience of expression, three kinds of *C. camphora* leaves numbering A, B, C are now given)



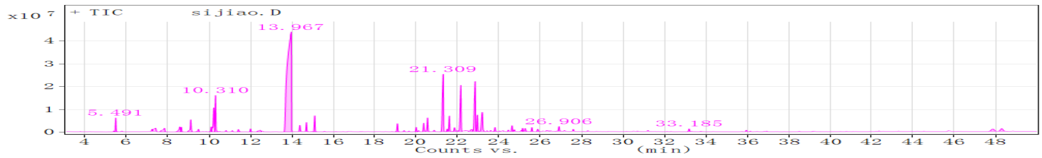


Figure 1 ***C. camphora*** **yinyuelou.D (A), tushuguan.D (B) and sijiao.D (C) are GC/MS TIC of VOCs from** ***C. camphora*** **leaves extractives at three different locations, respectively.**

The peak of A and C is basically completed before 28 minutes. Its corresponding temperature is 175°C. And the peak of B is after 28 minutes. This means that A and C essential oil is more volatile, while B essential oil is more difficult. The specific peak position and the corresponding material information are listed as follows.

Table 1 VOCs of three ***C. camphora*** **leaves extractives**

No	Retention time/min	Compounds	Relative content %		
			A	B	C
1	5.485	3-Hexen-1-ol, (Z)-	1.356	0.600	1.073
2	10.281	Cyclofenchene(6CI)	--	--	1.153
3	12.388	.beta.-Myrcene	--	--	1.401
4	13.907	D-Limonene	--	--	2.730
5	14.382	Eucalyptol	0.528	0.271	3.314
6	14.703	trans-Linalool oxide (furanoid)	1.623	--	--
7	15.189	Linalool	66.774	1.024	--
8	17.077	camphor	--	1.067	47.483
9	17.469	3-Acetyl-2-octanone	1.442	--	--
10	21.261	.alpha.-Terpineol	--	--	1.529
11	21.499	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	3.352	--	--
12	22.116	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	1.616	--	--
13	23.153	Caryophyllene	4.854	3.731	7.688
14	23.807	.gamma.-Elemene	1.688	--	2.894
15	24.632	Humulene	2.691	1.364	5.575
16	25.143	beta-Cubebene	3.259	--	6.339
17	25.268	(+)-b-Selinene	1.997	--	1.491
18	25.535	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	1.456	--	--
19	25.588	Isolepidozene	--	--	2.882
20	26.906	trans-(+)-Nerolidol	--	17.725	--
21	28.948	Caryophyllene oxide	1.361	6.711	0.721
22	41.976	9-Octadecenamide, (Z)-	--	2.667	--
23	44.279	13-Docosenamide, (Z)-	--	5.392	--

Note: Some substance CAS numbers could not be found, replaced by -- and partial isomers cannot find CAS numbers, replacing them with the most common ones.(more than 1% or be detected in three types of camphor trees were listed.)

VOCs of Three *C. camphora* leaves are shown in Table 1. According to chemical classification, Linalool accounted for 66.774% of total essential oils in A, so judge A as aromaticcamphor. Camphor accounted for 47.483% of total essential oils in C, so judge C as camphor-type (Guo et al. 2017). However, the main substance of B was not identified at 28.841 minutes, and it could not be judged which type it wa s. 23, 21, and 36 VOCs were detected in camphor A, *C. camphora* B and camphor-type C extracts, 98. 428%, 98.255%, and 97.907% of the total volatile oil, respectively. (*C. camphora* B counts undetected 2 8.841 minutes of material) All three have been extracted with alcohols, alkanes, organic acids and esters. 63 substances were identified by GC/MS in all three extracts. Among the three extracts, five substances

3-Hexen-1-ol, (Z)-, Eucalyptol, Caryophyllene, Humulene and Caryophyllene oxide were detected. And their peak position time is close. Linalool (A) has good antibacterial activity, as well as calming, killing insects (Mirghaed et al. 2016).The main substance of B was not detected at 28.841 minutes. However, the second-rich component, trans-(+)-Nerolidol (B) content reached 17.725%.Its isomers Nerolidol is used to make flavors. Lasting fragrance, it has a certain degree of coordination and aromatherapy (Lee et al. 2014). The main substance camphor in C is 47.483%. Camphor is also the main product of camphor tree essential oil (Lin et al. 2015).It has analgesic swelling, itching and insect repellent and other effects (Chen et al. 2013). In addition, the VOCswhich content of above1%are the following. In addition to be used as essential oils, they have applications in other fields such as medicine, chemicals, foods, etc. There are(Kim et al. 2017, Liu et al. 2010)beta-Cubebene(A3.259%,C6.339%), 3,7-Octadiene-2,6-diol, 2,6-dimethyl-(A 3.352%),(+)-b-Selinene(A1.997% ,C1.491%),9-Octadecenamide,(Z)- (B2.667%) , D-Limonene (C2.730%) , gamma.-Elemene(A1.688%,C2.894%),Isolepidozene(C 2.882%).Besides, there are some low-level but more cherished substances(Zhou et al. 2019, Chen et al. 2018). Such as beta-Pinene, beta.-Myrcene, alpha.-Phellandrene , a-Phellandren-8-ol, Terpinen-4-ol, alpha.-Terpineol, cis-b-Copaene, Spathulenol, alpha.-Bisabolene epoxide, Phytol, Vitamin E, etc.

IV Conclusion

23, 21 and 36 kinds of VOCs were detected in three types of *C. camphora* leaves extracts. They accounted for 98.428%, 98.255% and 97.907% of the total volatile oils, respectively. There is a huge difference in their relative content. According to the international demand for high quality essential oil, single component content should reach a certain standard. Therefore, the production of high-quality essential oils should choose the optimal strains and clonal breeding to afforest. The main substances known for the above three essential oils, Linalool, trans-(+)-Nerolidol and camphor rich in content. They are traditionally used compounds in *C. camphora* and have a wide range of applications. The experiment is randomly selected camphor tree, so the corresponding main material is not very high. In addition, the content of essential oils is not high, but VOCs with special uses are also targets for breeding. Such as (-)-Sabinene, beta-Pinene, beta.-Myrcene, alpha.-Phellandrene, Terpinen-4-ol,alpha.-Terpineol, cis-b-Copaene, D-Limonene, Spathulenol, Vitamin E etc. The relative contents of these VOCs in the three extracts of *C. camphora* leaves were not regular. Therefore, according to the different needs of VOCs, we should select or cultivate varieties with high content of single component in practical use.

Acknowledgements

This project was supported by the Science and Technology Project of Hunan Province, China (2016NK2154), talent project of Henan Agriculture University, China (Dangquan Zhang), and the Project of Henan Provincial Science Research, China (192102110174).

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