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Molecular Characteristics of Three Extractives of *Cinnamomum camphora* Leaves

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***Cinnamomum camphora* was an woody medicinal plant containing many valuable active ingredients. However, the rich extractives from its leaves were invariably wasted for unclear chemical composition. To further utilize these resources, the three extractives were obtained, and analyzed by GC-MS. The three extractives contained 3, 11 and 3 components, respectively. The three extractives were suitable for the extraction of 1,5-hexadien-3-yne.. Furthermore, the extractives contained many active groups. It therefore suggested that the extractives could be used as biomedicines, antibacterial substances, etc.**

Key words: *Cinnamomum camphora* leaves, Woody biomedicine, Bioactive extractives.

Cinnamomum camphora, which was a large evergreen tree that grown up to 20–30 meters tall, was native to the south China, Taiwan, Japan, Korea, and Vietnam, and had been introduced to many other countries. *Cinnamomum camphora* was also a woody medicinal plant, and studied by a large number(Wei-qin *et al*, 2012). Linalool of *Cinnamomum camphora* grown in Taiwan and Japan was normally high up to 80%-85%. *Cinnamomum camphora* which was a dominant plant in India, Sri Lanka and Madagascar was high to 40%-50% in 1,8 Cineole(Qianfeng *et al*, 2007). *Cinnamomum camphora* had a long history of herbal use in the Orient with the treatment of hysteria as the essential oil. The wood and leaves were antispasmodic, analgesic, rubefacient, odontalgic, stimulant. The infusion was used as an inhalant in the treatment of colds and lungs diseases. The essential oil of *Cinnamomum camphora*, which could be obtained by distillation of the branches, trunk, leaves and wood, was anthelmintic, diaphoretic, antispasmodic, antirheumatic, carminative, cardiotonic, sedative and tonic, could treat joint and muscle pains, chapped lips, balms for chilblains, palpitations, cold sores, skin diseases, bronchial congestion, convulsions, digestive complaints and depression(Qianfeng *et al*, 2007) . *Cinnamomum camphora* wood was also used for making furniture, cabinets, the interior finish of buildings, and so on. So *Cinnamomum camphora* was a very important economic tree species.

Cinnamomum camphora was widely planted for many advantages. It wasn't large area cultivated, but scattered to the fields and villages along the road(Wei-qin *et al*, 2012). And *Cinnamomum camphora* biomass could not achieved in the large-scale industrial application. What's, *Cinnamomum camphora* tree had two

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times leaves a year and the huge biomass. However, the rich extractives of the leaves had traditionally been wasted because of inefficient extraction and separation processes, narrow use, and unknown compositions. In the current study, we had obtained three extractives using the differed stages extraction technique, analyzed and identified the active molecules from the three extractives using GC-MS with the aim of further utilizing the high quality reuse of *Cinnamomum camphora* leaves.

MATERIALS AND METHODS

The fresh *Cinnamomum camphora* leaves were collected from the Central South University of Forestry and Technology, P. R. China. The fresh leaves were pound to pieces and kept in vacuum. Benzene, methanol, petroleum ether (60-90°C), acetic ether and ethanol (chromatographic grade) were prepared for the subsequent experiments. Cotton bag and cotton thread were both extracted in benzene/ethanol solution for 12 h. The benzene-ethanol solution was mixed according to $V_{ethanol}$ /$V_{benzene}$ 2 double.

Experiment methods

Weighed 3 pieces of leaves, each was about 20g (0.1mg accuracy) and finally parceled by using the cotton bag and tied by using cotton thread, and signed. Benzene/ethanol extraction was carried out in 350ml solvent by the Foss method for 6 hours. BE- methanol extraction was carried out in 350ml petroleum ether/acetic ether ’! methanol solvents by the Foss method for 6 hours. BE- benzene/ethanol extraction was carried out in 350ml petroleum ether/acetic ether extraction, methanol ’! benzene/ ethanol solvents by the Foss method for 6 hours. The temperature of petroleum ether/acetic ether extraction, methanol extraction, and benzene/ ethanol extraction were 85°C, 65°C, 85°C, respectively. After extraction, the leaves extractives were obtained by evaporation in 60°C - 70°C air.

GC/MS condition

Each 0.5 mg leaves extractives was analyzed by online linked GC/MS (gas chromatograph/ mass spectrometer), respectively. The GC/MS analysis was carried out on a GC/MS-QP2010 (Shimadzu Corp., Japan), which was linked to a mass selective detector. An elastic fused silica capillary column DB-5 (30m×0.25mm) was used. The carrier gas was helium and the injection port temperature was 250°C. The split injection ratio was 10:1, the GC column temperature was programmed as follows: 10°C /min from 50 to 200°C, 5°C /min from 200 to 300°C. The program of MS was scanned over the 35-335AMU (m/z) respectively, with an ionizing voltage of 70eV and an ionization current of 150¼A of electron ionization (EI). The flow velocity of helium was 1.0ml/ min(Wanxi *et al*, 2013a; 2013b).

FTIR Analysis

The extractives samples were directly recorded on a Thermo Nicolet FT-IR spectrometer (Thermo Fisher Nicolet, 670FT-IR). Thirty-two scans were collected per sample at a spectral resolution of 4 cm^{-1}, and the collected spectra were normalized against air. The spectral range was from 4000 to 500 cm^{-1}(Yong-Chang *et al*, 2014).

RESULTS AND DISCUSSION

Components of three extractives from *Cinnamomum camphora* leaves

According to the optimum extraction method, three extractives (benzene/ethanol, BE-methanol, BE- benzene/ethanol) were respectively obtained. The total ion chromatograms of the three extractives by GC/MS were shown in Fig.1. The relative content of each component was counted by area normalization. Subsequent analysis of the MS data using the NIST standard MS map by computer, as well as open-published books and papers (Wanxi *et al*, 2013a; 2013b; Yong-Chang *et al*, 2014; Camurça-Vasconcelos *et al*, 2007; Oka *et al*, 2000; Zhang, 2005; Peng *et al*, 2012; Katrin *et al*, 2001), allowed for the individual components to be identified.

According to GC/MS result, 3 components were identified from the 3 peaks produced by the benzene/ethanol extractives from *Cinnamomum camphora* leaves. The results showed that the main components were 1,5-hexadien-3-yne (91.30%), eucalyptol (1.43%), 3-methylbut-2-enoic acid, 3,5-dimethylphenyl ester (7.26%).

According to GC/MS results, 11 components were identified from the 11 peaks produced by the BE-methanol extractives from *Cinnamomum camphora* leaves. The result showed that the main components were nerolidol

2 (3.19%), bromo-cyclohexane (15.98%), 1,2-bis(trimethylsilyl)benzene (2.27%), 1,2-benzisothiazol-3-amine tbdms (1.97%), 1,2-bis(trimethylsilyl)benzene (3.20%), tributylethylstannane (3.75%), 1,2-benzisothiazol- 3-amine tbdms (3.34%), 3-[methyl(4-nitrophenyl)amino]-propanenitrile (30.79%), 1,2-bis (trimethylsilyl) benzene (5.21%), hexamethyl-cyclotrisiloxane (13.71%), 3,5-bis-trimethylsilyl-2,4,6-cyclohep - tatrien-1-one (16.55%).

According to GC/MS result, 3 component was identified from the many peaks provided by the BE- benzene/ethanol extractives from the *Cinnamomum camphora* leaves. The results showed that the components was 1,5-hexadien-3-yne (98.44%), dibutyl phthalate (0.96%), 1,2-benzenedicarboxylic acid, diisooctyl ester (0.59%).

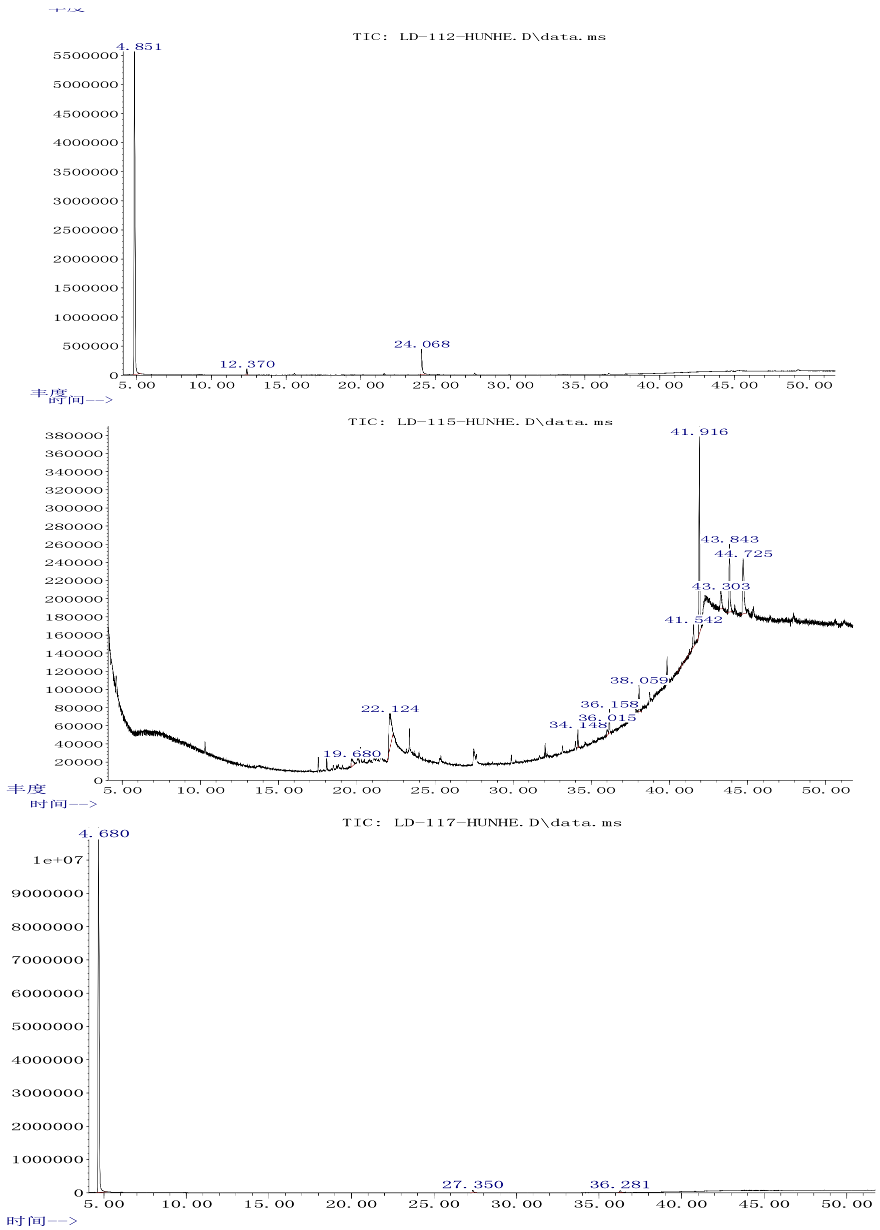

Fig. 1. Total ion chromatograms of three extractives from the *Cinnamomum camphora* leaves by GC/MS

Group Properties of *Cinnamomum camphora* Leaves Extractives

The FTIR spectra of extractives of *Cinnamomum camphora* leaves were shown in Fig.2.

The spectra in Fig.2 were assigned as follows. The signals observed at 3317 cm^{-1} related to -OH stretching vibrations. The peaks at 2917 cm^{-1}, 1728 cm^{-1}, 1607 cm^{-1}, 1515 cm^{-1}, 1460 cm^{-1}, 1370 cm^{-1}, 1235 cm^{-1}, 1205 cm^{-1}, 1160 cm^{-1}, 1050 cm^{-1}, and 1030 cm^{-1} were the $–CH_2$ stretching vibration of methylene, C=O stretching vibration of acetyl xylan, benzene skeleton C=C stretching vibration, C-H bending vibration of chitosan, CO-OR stretching vibration of acetyl xylan, O-H in-plane bending vibration of hemicelluloses, C-O-C stretching vibration of hemicelluloses, C-O stretching vibration of acetyl xylan, and the C-O stretching vibration of hemicelluloses, respectively. The absorbance of peaks at 3317-3375 cm^{-1} increased from 0.239 to 0.656 in LD-117 extractives. The absorbance of peaks at 2917-2933 cm^{-1} increased from 0.194 to 0.830 in LD-117 extractives. The absorbance of peaks at 2850 cm^{-1} and 1730 cm^{-1} were not only in LD-115 extractives. The absorbance of peaks at 1607–1612 cm^{-1} reduced from 0.409 to 0.176 in LD-117 extractives, the absorbance of peaks at 1515 cm^{-1} increased from 0.135 to 0.396 in LD-115 extractives, the absorbance of peaks at 1376 cm^{-1} was not in LD-115 extractives, the absorbance of peaks at 1261-1279 cm^{-1} reduced from 0.651 to 0.199 in LD-117 extractives, the absorbance of peaks at 1033-1073 cm^{-1} reduced from 0.259 to 0.746 in LD-117 extractives. And the extractives of *Cinnamomum camphora* leaves contained highly active groups.

Resource utilization of three extractives from *Cinnamomum camphora* leaves

Cinnamomum camphora was a medicinal woody plant, and its extractives could be used as novel lead compounds to create new drugs. Research in this area had shown that there were many rare bioactive components in the extractives of *Cinnamomum camphora* leaves. Given its officinal value, Eucalyptol, which could be used to reduce inflammation and pain, and destroy leukemia cells in vitro, had been used as a mouthwash and cough suppressant, as well as a treatment for headaches, sensitivity of the pressure points of the trigeminal nerve, nasal obstruction, impairment of general condition, and nasal secretions (Wanxi *et al*, 2013a; 2013b; Camurça-Vasconcelos *et al*, 2007; Oka *et al*, 2000; Zhang, 2005; Peng *et al*, 2012). Nerolidol, which was known as peruviol, was used as a flavoring agent and in perfumery, and as a skin penetration enhancer for the transdermal delivery of therapeutic drugs (Katrin *et al*, 2001). tributylethyl-stannane, which was an inorganic compound, decomposed slowly at room temperature to give metallic tin and hydrogen and ignites on contact with air. What's more, there were some antibacterial substances such as 1,2-bis(trimethylsilyl)benzene, 1,2-benzisothiazol-3-amine tbdms, 1,2-bis (trimethylsilyl) benzene, 1,2-bis(trimethylsilyl) benzene, 1,2-benzenedicarboxylic acid, diisooctyl

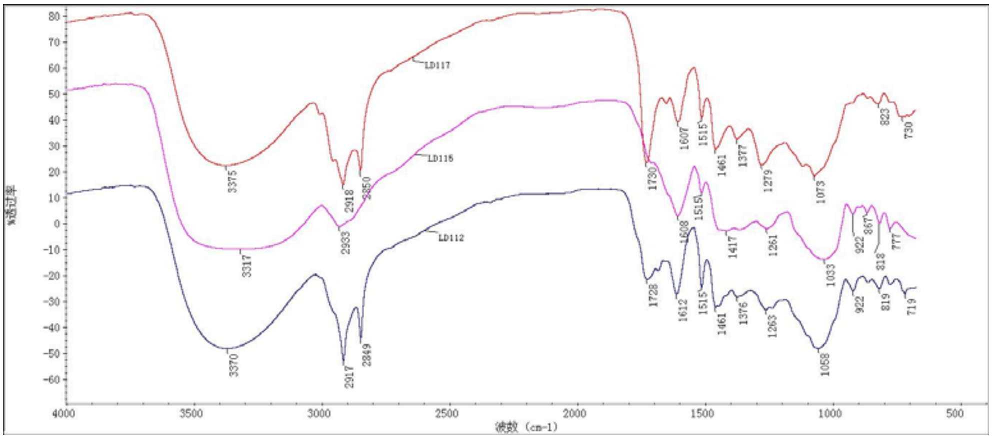

Fig. 2. Group characteristic of three extractives of *Cinnamomum camphora* leaves

ester, and so on. These antibacterial substances could act by interfering with ribosomal function, cell wall synthesis, nucleic acid synthesis, plasma membrane integrity, and folate synthesis in order to prevent the growth of fungi and other pathogensthat cause the plant's diseases..

CONCLUSIONS

The three extractives of *Cinnamomum camphora* leaves gave 3, 11, 3 components, respectively, that could be identified by GC-MS. The most abundant components of benzene/ ethanol extractives were 1,5-hexadien-3-yne (91.30%), whereas the most abundant components in the BE-methanol extractives were 3-[methyl (4-nitrophenyl) amino]-propanenitrile (30.79%) and 3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one (16.55%). The most abundant components in the BE- benzene/ethanol extractives were 1,5-hexadien-3-yne (98.44%). Furthermore, the three extractives were suitable for the extraction of 1,5-hexadien-3-yne.

The functional analytical result suggested that the three extractives of *Cinnamomum camphora* leaves was rich in bioactive components that could be used in biomedicines and antibacterial substances. Furthermore, the three extractives contained many highly active groups. There were some toxic compounds in the three extractives of *Cinnamomum camphora* leaves.

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