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LETTER TO THE EDITOR

Diverse Bioactive Components from Cold-acclimated *Cinnamomum Camphora* Branches by Different Extraction

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Cinnamomum camphora has been used as an excellent tree during construction of beautiful China. In order to explore the utilization potential of bioactive components from the branches of cold-acclimated *C. camphora* in North China, the diverse bioactive components were successfully extracted by acetone, benzene and ethanol, and identified by GC-MS/MS and QTOF-LC-MS. Among the volatile organic compounds (VOCs) from three extractions of *C. camphora* branch, the benzene extract has the highest contents of bioactive components for biomedicine, bioenergy, food additive and spices, the acetone extract has highest contents of cosmetics components. Among the non-volatile organic compounds (non-VOCs), the benzene extract has the highest contents of bioactive components for food additive spices and cosmetics, the acetone extract has highest contents of biomedicine components. These findings suggested that the branch of cold-acclimated *C. camphora* has the potential to develop diverse bioactive products by different extractions.

I Introduction

Cinnamomum camphora is a kind of large evergreen tree of lauraceae. The height can reach 30 meters and the diameter can reach 3 meters. It is native to south and southwest China. It is not only an excellent greening tree species, but also an important economic timber tree species (Li et al. 2018, Ben Mariem and Chaieb 2017). Its whole body has high economic value. In addition, camphor bark and root, wood, fruit and leaf have medicinal value, which can prevent and treat some diseases (Singh et al. 2016, Chowdhury et al. 2019, Gao and Wang 2017). However, the researches on the branch of C. *camphora* are relatively few, and not fully understand its value in all aspects and apply it to production and life. So choosing the cold-acclimated *C. camphora* branch in Northern China to study, extract its bioactive components, and find more effective functions. The deep understanding and efficient utilization of *C. camphora* branch can be achieved.

The volatile compounds in *C. camphora* branch were studied by GC-MS/MS. FT-IR was used to analyze the changes of functional groups in *C. camphora* branch during heating. QTOF-LC/MS) was used to study non-volatile compounds in the cold acclimatized *C. camphora* branch.

II Materials and Methods

(1) Experimental Materials

The cold-acclimated *C. camphora* branch was collected in early June, and processed into powder before baking. The *C. camphora* branch was extracted by benzene, acetone, and ethanol.

(2) Extraction with the three solvents

C. camphora branch was extracted with benzene, acetone, or ethanol in the ratio of solid to liquid = 1:20. The sample mixed with solvent was distilled in water bath for 4 hours, followed by rapid filtration by vacuum pump. The benzene, acetone, and ethanol filtrates were evaporated by rotary evaporator in 0.01 mPa vacuum, and concentrated to 20 mL (Cheng et al. 2018).

(3) FT-IR analysis

The branch of *C. camphora* was analyzed by FT-IR after sieving in 200 mesh, and then extracted with benzene, ethanol, or acetone. Samples of 0.5-2 mg were added to 200 mg of potassium bromide and mixed quickly with a smooth surface mortar. The mixture was pressed in a tablet press and placed under heated light for 0.5 to 1 minute. The sample was FT-IR (NIOLET 5) from 4000 to 500 cm^{-1} (Yang et al. 2016; Lam et al, 2019).

(4) GC-MS/MS analysis

GC-MS/MS quartz capillary column was 30 m × 0.25 × m × 0.25 micron, starting at 50°C, without retention, then heated at the rate of 5°C/min to 130°C, without retention, at the rate of 2 °C/min to 180°C, and then at the rate of 30 °C/min to 300°C; holding time of 5 min, inlet temperature was 50°C, the column flow rate was 1 mL/min, the split ratio was 20:1, and the carrier gas is high purity helium. Under MS conditions, the ionization mode was EI, the electron energy was 70 eV, the ion source temperature was 230°C, quadrupole temperature of 150 C, and the scanning starting point is 30-600°C (Taha et al., 2018).

(5) Quantitative time-of-flight-liquid chromatography/mass spectroscopy (QTOF-LC/MS) analysis

High performance liquid chromatography: Agilent 1290 Infinity chromatograph. Sample size: the sample is diluted 100 times and 5 times, and the quality of sample is 5 L. Chromatographic column: Thermo Scientific Acclaim RSLC C18 100 x 2.1 mm (2.2 μm, 120) Mobile phase: A phase (0.1% FA, 99.9% H2O); B phase (0.1% FA, 99.9% ACN) (Chen et al. 2018).

Mass spectrum: Mass spectrometric type: Compact high resolution mass spectrometer. Ion source: ESI source. Scanning mode: positive ion mode scanning. Scanning range: m/z 100-1000. Correction: external standard method, sodium formate correction (He et al. 2018).

III Results

(1) Regulation of the changes in chemical groups in the C. camphora branch extracts

Analyzing the infrared spectra (Figure 1), the infrared spectra of the three extracts were similar to those of the original powder of *C. camphora* branch. The results showed that the characteristic group frequency of acetone extract was the closest to that of ethanol extract, indicating that the substances in acetone extract were similar to those in ethanol extract. The infrared spectrum of the original powder of *C. camphora* branch has four distinct peaks at the characteristic frequency, and the strongest peak appears at 3369.51 cm^{-1}, which is a wide absorption band (Chu et al. 2016). Similar to one of the characteristic peaks of acetone extract and ethanol extract, and similar to the characteristic peak of benzene extract 3431.71 cm^{-1}, this is caused by the stretching vibration of intermolecular hydrogen bond O-H, intermolecular association and the presence of alcohol and phenol (Zuo et al. 2018). In the range of 3033-2746 cm^{-1}, all four curves have a weak peak and a very weak peak. The peak at

2916.81 cm^{-1} is caused by the antisymmetric expansion of C-H group, indicating the existence of alkanes. The strongest peak of benzene extract appeared at 1664.27 cm^{-1}, which was caused by the expansion of C=O group, indicating the existence of esters and aldehydes (Liu et al. 2017).

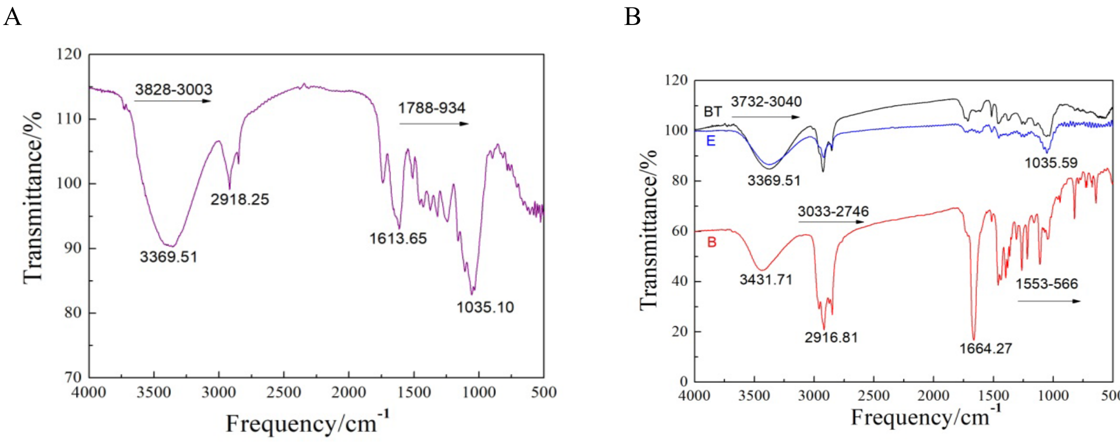

Figure 1. Group changes of the three extracts and the raw *C. camphora* branch powder via FT-IR.

(2) Diversity of the volatile organic carbon components in the different *C. camphora* branch extracts

Among the three extracts (acetone, benzene and ethanol) of C. *camphora* branch, the content of chemical raw materials is the highest, followed by the content of bioenergy. The total contents of chemical raw materials and bioenergy in acetone extract accounted for 52% of the total contents of the six functional substances, 55% in benzene extract and 69% in ethanol extract. The contents of five other functional substances except cosmetics in benzene extract were significantly higher than those in acetone extract and ethanol extract. The content of chemical raw materials in acetone extract is about 50% in benzene extract, the biomedical content in acetone extract is about 50% in benzene extract, the content of perfume in acetone extract and acetone extract is about 44% in benzene extract, and the content of bioenergy in benzene extract is more than that in acetone extract. The content is about 36%, which is about 28% higher than that of ethanol extract. The content of chemical raw materials and bio-energy in ethanol extract is higher than that in acetone extract, but it is opposite in bio-medicine, spice and food additive. The three extracts contain very few cosmetic ingredients, and acetone extract has the highest content (Figure 2A).

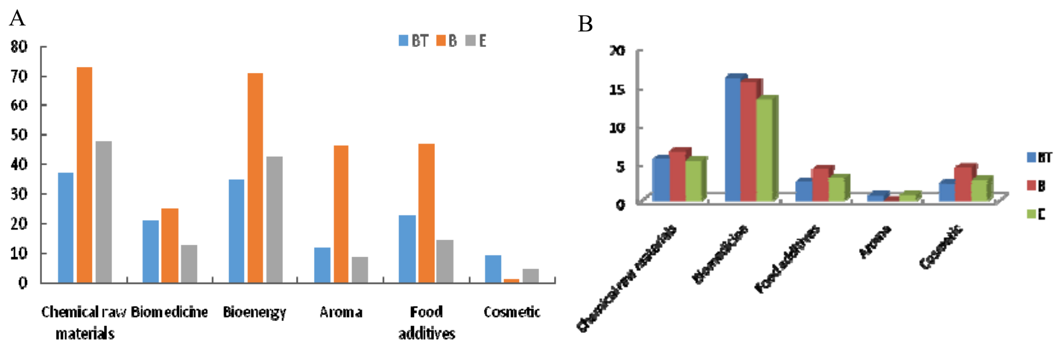

Figure 2. A: Comparison of functional categories of the C. *camphora* branch extracts by GC-MS/MS. B: Comparison of functional categories of the C. *camphora* branch extracts *by QTOF-LC/MS.* (The same substance may be repeated for various purposes.

(3) Non-volatile compounds in the different *C. camphora* branch extracts

Compounds used in medicine were found in acetone extract of C. *camphora* branch. Fargesin (2.52%) is used for pharmacological experiments and content determination (Yue et al. 2018). Bocconoline (1.20%) has the function of clearing away heat, detoxifying, killing insects and sterilizing bacteria. It can be used to treat various inflammations and also inhibit tumor cells. Cerevisterol (1.01%) has been widely used in the field of immunology. Decursidate (0.70%) is a moderate antipsychotic drug. Protopine (0.55%) has anti-histamine and obvious anti-choline effect, used for allergic asthma and rhinitis, treatment of cardiovascular diseases.

The benzene extract from C. *camphora branch* contains chemical substances that can be used in biomedicine. 7-Hydroxycoumarin (3.20%) is a pharmaceutical ingredient for the treatment of impaired neurotransmission diseases, Alzheimer’s disease, vascular and lymphedema cancer (Deng et al. 2018). Anemonin (0.72%) has antibacterial and analgesic effects. It has been used to treat dysentery, sedative and analgesic drugs.

By QTOF-LC/MS, the relative content of biomedicine was the highest among the three extracts of CCP, followed by chemical raw materials. Acetone contains the highest content of biological medicine, which is about 3% higher than that of ethanol. The relative content of food additives and cosmetics is relatively close. The relative content of food additives, cosmetics and chemical raw materials is the highest among benzene extracts. The content of the aroma components in the three extracts was very low and the highest was about 1% (Figure 2B).

IV Conclusion

The infrared transmittance of acetone, ethanol and benzene extracts from C. *camphora* branch were similar to that of the original powder. However, there are still some differences in the infrared spectra of the three extracts. The characteristic group frequencies of the ethanol extracts and the acetone extracts are the closest, indicating that the substances are similar. The detection signals of benzene extracts in the three extracts were weak, indicating that the extraction efficiency of benzene was not high.

The products detected by GC-MS/MS and QTOF-LC/MS have some high content but unknown functional uses. We can do further research to understand the functional uses of these substances and their related bioactive components, and apply them to life and production. At the same time, the other substances detected were compared with the substances in other genera and species to explore the existence of uniqueness. The cold-acclimated *C. camphora branch* in the north has these bioactive components which can promote the efficient utilization of *C. camphora branch*. As for the bioactive components of C. *camphora branch* in other parts of the world, further study is needed in order to make full use of them.

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References

Bae DS, Kim YH (2012) Protopine reduces the inflammatory activity of lipopolysaccharide-stimulated murine macrophages. BMB Rep 45(2).

Ben Mariem H, Chaieb M (2017) Climate change impacts on the distribution of stipa tenacissima l. Ecosystems in north african arid zone - a case study in Tunisia. Applied Ecology and Environmental Research 15(3):67-82.

Chen L, Wang Z, Chen R, He H, Ma J, Zhang D. Gene Cloning and Gene Expression Characteristics of Alcohol Dehydrogenase in Osmanthus fragrans var. semperfloren. Emirates Journal of Food and Agriculture, 2018, 30(10): 820-827

Cheng X, Yang T (2018) New method for effective identification of adulterated Camellia oil basing on Camellia oleifera-specific DNA. Arabian Journal of Chemistry 11 (6):815-826

Chowdhury MA, Islam KN, Hafiz N, Islam K (2019) Diversity of trees in a community managed forest: the case of komolchori vcf, Khagrachari, Bangladesh. Geology, Ecology, and Landscapes 3(2):95-103.

Chu J, Ma L, Zhang JH (2016) The Chemical Composition of Bamboo after Heat Pretreatment with Fourier Infrared Spectrum Analysis. Guang Pu Xue Yu Guang Pu Fen Xi 36(11).

Deng J, Guo L, Wu B (2018) Circadian Regulation of Hepatic Cytochrome P450 2a5 by Peroxisome Proliferator-Activated Receptor. Drug Metab. Dispos 46(11).

Gao W, Wang W (2017) New isolated toughness condition for fractional (g, f, n) - critical graph. Colloquium Mathematicum 147(1):55-65.

He H, Qin J, Cheng X, Xu K, Teng L, Zhang D (2018) Effects of exogenous 6-BA and NAA on growth and contents of medicinal ingredient of Phellodendronchinense seedlings. Saudi J Biol Sci 25(6):1189–1195.

Lam SS, Mahari WAW, Ma NL, Azwar E, Kwon EE, Peng WX, Chong CT, Liu ZL, Park YK (2019) Microwave pyrolysis valorization of used baby diaper. Chemosphere 230: 294-302

Li YR, Fu CS, Yang WJ, Wang XL, Feng D, Wang XN, Ren DM, Lou HX, Shen T (2018) Investigation of constituents from Cinnamomum camphora (L.) J. Presl and evaluation of their anti-inflammatory properties in lipopolysaccharide-stimulated RAW 264. 7 macrophages. J Ethnopharmacol 221:37-47.

Liu Y, Kim HJ. (2017) Fourier Transform Infrared Spectroscopy (FT-IR) and Simple Algorithm Analysis for Rapid and Non-Destructive Assessment of Developmental Cotton Fibers. Sensors (Basel) 17(7).

Qin J, Wang Y, He G, Chen L, He H, Cheng X, Xu K, Zhang D (2017) High-efficiency Micropropagation of dormant buds in spine base of red pitaya (Hylocereuspolyrhizus) for industrial breeding. Int J Agric Biol 19(1): 193-198.

Singh P, Srivastava B, Kumar A, Dubey NK (2008) Fungal contamination of raw materials of some herbal drugs and recommendation of Cinnamomum camphora oil as herbal fungitoxicant. Microb Ecol 56(3):555-60.

Szulczyński B, Armiński K, Namieśnik J, Gębicki J (2018) Determination of Odour Interactions in Gaseous Mixtures Using Electronic Nose Methods with Artificial Neural Networks. Sensors (Basel) 18(2).

Taha SM, Gadalla SA (2017) Development of an efficient method for multi residue analysis of 160 pesticides in herbal plant by ethyl acetate hexane mixture with direct injection to GC-MS/MS. Talanta 174.

Yang D, Zhang H, Peng K, Chen L, He H, Huang X, Qin J, Zhang D (2016) Differential Gene Regulation of Lipid Synthesis in the Developing Seeds of Two Biodiesel Tree Species, Jatropha and Vernicia. Int J Agric Biol 18(6):1143–1152.

Yue B, Ren YJ, Zhang JJ, Luo XP, Yu ZL, Ren GY, Sun AN, Deng C, Wang ZT, Dou W (2018) Anti-Inflammatory Effects of Fargesin on Chemically Induced Inflammatory Bowel Disease in Mice. Molecules 23(6).

Zuo Y, Deng X, Wu Q (2018) Discrimination of from Different Geographical Origin for Quality Evaluation Using Newly-Build Near Infrared Spectrum Coupled with Multivariate Analysis. Molecules 23(5).