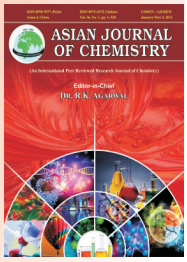
Asian Journal of Chemistry; Vol. 26, No. 11 (2014), 3388-3390

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17532



Chemical Components and Pharmacological Action of Ethanol/Benzene Extractives from Chinese Plantation *Pinus massoniana* Biomass

Z.F. ZHANG[1,*], K. HUANG[1], Y.J. YE[1] and X. ZHANG[2,*]

[1]Department of Furniture, Central South University of Forestry and Technology, Changsha, P.R. China
[2]Department of Forestry, Central South University of Forestry and Technology, Changsha, P.R. China

*Corresponding author: Tel: +86 731 85623630; E-mail: csfuzhangzf@163.com; csfuzx@126.com

Received: 17 February 2014; *Accepted*: 18 April 2014; *Published online*: 25 May 2014; AJC-15254

Pinus massoniana plantations have been planted extensively in China. To clarify whether *Pinus massoniana* wood can provide rich resources for woody extractive medicine or not, the ethanol/benzene extractives from *Pinus massoniana* wood were obtained by the method of ethanol/benzene extraction and their chemical components were identified with gas chromatography-mass spectrometry (GC-MS) to analyze the pharmacological activity of the contained biomedical molecules in this research. Seventeen chemical components were confirmed on the peaks of ethanol/benzene extractives and 81.01 % of which were esters and the main retention time of which was 20-30 min. Furthermore, the expounded pharmacological action of ethanol/benzene extractives from Chinese plantation *Pinus massoniana* showed *Pinus massoniana* wood would have a huge potential in bio-pharmaceuticals.

Keywords: *Pinus massoniana*, Ethanol/benzene extractives, GC-MS, Biomedical molecules, Chinese plantation.

INTRODUCTION

Pine resources are rich in China totally including 10 genera and about 230 species around the world and 10 genera and about 120 species of which are in China[1]. *Pinus massoniana* belongs to the family of Pinaceae and is widely planted in China with the area reaching to 30 million Mu which is mainly applied to make artificial board, converted timber, rosin and paper-making. For example, the chemical constituents of oleo resin form high-quality *Pinus massoniana* are analyzed with GC[2] and the chemical components in wood and pulp/paper-making properties of 14 natural populations covering over the distribution range of *Pinus massoniana* are analyzed[3] and the reason why and how the impregnating and bleaching process is influenced by the solvent extractive was obtained[4]. However, Pine is not only a kind of forest plants with a wide range of distribution and also has great medicinal values. Modern pharmacological studies have shown Pinaceae plants extractives have antitumor, antivirus (HIV), antibacterial, antioxidant and enhance immune function[5,6]. Now a days, in order to develop the medicinal values of Pinaceae plants, the researchers at home and abroad only studied on the chemical components and pharmacological activity of a few species of Pinaceae. Sakagami *et al.*[7] studied on the antitumor of Japanese white pine and found which had a strong antitumor activity and low toxicity for the first time. And the medicinal values of Pine roots, Pine pollen, Pine cones, Pine needles, Pine twigs have documented in the Chinese medical literature. Such as, Pine pollen and nodular branch are used as two traditional Chinese medicines, which can be used to treat traumatic hemorrhage, eczema, sprain and strain[8], Pine cone is reported to contain terpenes, lignins and polysacch arides, biologically active for its antineoplastic, antiviral, antibacterial and immunopotent actions and promising for the drug development[9] and 21 known compounds are isolated from the twigs of *Pinus massoniana* and four of which are identified as the antioxidant constituents[10]. Reviewing the existing literatures on the medicinal values of Pinaceae plants, the research on the medicinal values of woody extractives from *Pinus massoniana* is not reported. However, *Pinus massoniana* plantations have been planted extensively to provide rich resources for woody extractive medicine. So it's necessary to identify the chemical components of woody extractives of Chinese plantation *Pinus massoniana* and analyze their pharmacological action. In this research, the woody extractives from *Pinus massoniana* were obtained by the use of Soxhlet Extractor with the method of ethanol/benzene extraction (EB extraction) and the chemical components of which were identified by the use of gas

chromatography-mass spectrometry (GC-MS) and the pharmacological activities of biomedical molecules were analyzed, which can provide the theoretical basis for the development and utilization of woody extractive medicine from Chinese plantation *Pinus massoniana*.

EXPERIMENTAL

A 10-year-old *Pinus massoniana* with a diameter of 22 cm at breast height was obtained from the Zhuzhou Forest Farm, Hunan province, P.R. China in September 2013, the sample chips processed from which were dried to absolute dry with drying oven at 60 ºC, then the 50 mesh powder was sieved out by using AS200 Sieving Instrument (Made in America). Both ethanol and benzene ether (chromatographic grade) were prepared for the subsequent experiments. Quantitative filter paper, cotton bag and cotton thread were all extracted in Ethanol/ Benzene solution for 12h and the ethanol/benzene solution (EB solution) was mixed according to $V_{Ethanol}/V_{Benzene}$ = 2:1.

General procedure: The pieces of the above wood powder was weighed about 10 g (0.1 mg accuracy) and then parcelled by using the cotton bag and tied by using cotton thread and being marked. Extraction was gradually carried out by the Soxhlet extractor and extracted in 800 mL ethanol/benzene solution (EB solution) with $V_{Ethanol}/V_{Benzene}$ = 2:1. Extraction time was 7 h. Extraction temperature was 90 ± 1 ºC. After extraction, the obtained extractive solution was dried to 10 mL in Rotary Evaporator under the condition of 45 ºC and vacuum 0.05-0.07 MPa to obtain the ethanol/benzene extractives.

Detection method: The ethanol/benzene extractives were analyzed by online linked gas chromatograph/mass spectrometer (GC-MS). GC-MS analysis was performed in an Agilent 6890 A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) and Agilent 5973 N Mass Spectrometer (Agilent Technologies). The GC device was equipped with HP-5MS column (30 m × 0.25 mm × 0.25 μm) coated with a non-polar phase of 5 % phenyl methyl silox. The carrier gas was helium (99.999 %) at a constant flow rate of 3 mL/min. The temperature program was as follows: initial temperature 50 ºC held for 3 min, from 50-200 ºC at the rate of 8 ºC/min, from 200-300 ºC at the rate of 5 ºC/min and final temperature holding for 5 min. The ion source was operated in the electron ionisation mode (EI; 70 eV). Full scan mode data were acquired to determine appropriate masses for the later acquisition in selected ion monitoring mode (SIM) under the condition of mass range from 50-300 amu with the scan rate of 0.5 s/scan. Ion source temperature was 230 ºC and quadropole temperature was 150 ºC.

The identification of chemical components of ethanol/benzene extractives was based on computer matching with the reference mass spectra of the Wiley7, Mainlib and NIST08 libraries by comparing their retention time.

RESULTS AND DISCUSSION

The chemical components characteristics of ethanol/benzene extractives: The ethanol/benzene extractives were abtained with the method of ethanol/benzene extraction and the total ion chromatograms of which by GC-MS were shown in Fig. 1. The mass fractions of all compounds were calculated with the area normalization method.

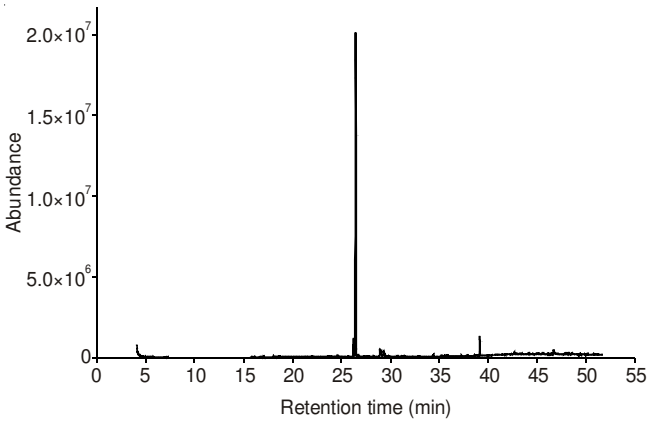


Fig. 1. Total ion chromatograms of ethanol/benzene extractives by GC-MS

According to the results of GC-MS, 17 chemical components were identified from 21 peaks of ethanol/benzene extractives from Chinese plantation *Pinus massoniana* including benzene dicarboxylic acid dibutyl ester (79.72 %), *n*-hexadecanoic acid (4.01 %), squalene (3.64 %), (Z)-2-pentadecen-4-yne (3.16 %), 9,12-octadecadienoic acid (Z,Z)- (1.88 %), octadecanoic acid (1.64 %), campesterol (1.38 %), eicosane (1.03 %), methyl linoleate (0.69 %), vitamin A aldehyde (0.59 %), 2,6,10,14-hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)- (0.39 %), 1-chloro-4-methoxybenzene (0.35 %), 4-hexenylbenzene (0.34 %), pentacosane (0.32 %), palmitic acid ethyl ester (0.31 %), *bis*(2-ethylhexyl) phthalate (0.29 %), butyl-(trimethylsilyl) ether (0.26 %).

The GC-MS analysis results showed the chemical components distribution of ethanol/benzene extractives from Chinese plantation *Pinus massoniana.* Relative content of esters, acids, hydrocarbons, ethers, alcohols and other compounds occupied 81.01, 7.53, 8.49, 0.61, 1.38 and 0.98 % of ethanol/benzene extractives. What's more, the retention time of ethanol/benzene extractives showed a particular rule that the chemical components with the retention time of ≤ 10, ≤ 20, ≤ 30, ≤ 40 and ≤ 40 min was 0.00, 0.69, 91.41, 5.28 and 2.62 %, respectively.

Pharmacological action of ethanol/benzene extractives: There are many biomedical molecules in the 17 identified chemical components of ethanol/benzene extractives from Chinese plantation *Pinus massoniana*. Squalene, a kind of non-toxic marine bioactive substance with the function of disease prevention, can increase superoxide dismutase (SOD) activity, enhance body immunity and improve sexual function, antiaging, antifatigue and antitumor *etc*.. And 9,12-octadecadienoic acid (Z,Z)- can be used for the treatment of high blood lipids and atherosclerosis embolism. And octadecanoic acid has the emulsification to make skin care products, such as vanishing cream, cold cream *etc*.. And campesterol can be used for the synthesis of steroid drugs which has the effects of cholesterol-lowering, antiinflammatory, antipyretic, antiulcer and anti-tumor effects. And methyl linoleate has preventive effect on acute and chronic inflammation. And vitamin A aldehyde, an intermediate metabolite of natural vitamin A acid, can be applied to reduce the possibility of lung cancer. 1-Chloro-4-methoxybenzene can be use to make spices and insect repellents. According to the relative content of biomedical molecules of which, ethanol/benzene extractives from Chinese plantation

Pinus massoniana will become the high-grade biomedical resources in future.

Conclusion

The chemical components of woody extractives of Chinese plantation *Pinus massoniana* are identified for the first time with the method of ethanol/benzene extraction and the use of GC-MS. 17 chemical components are obtained on the peaks of ethanol/benzene extractives including benzenedi carboxylic acid dibutyl ester (79.72 %), *n*-hexadecanoic acid (4.01 %), squalene (3.64 %), (Z)-2-pentadecen-4-yne (3.16 %), 9,12-octadecadienoic acid (Z,Z)- (1.88 %), octadecanoic acid (1.64 %), campesterol (1.38 %), eicosane (1.03 %), methyl linoleate (0.69 %), vitamin A aldehyde (0.59 %), 2,6,10,14-hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)- (0.39 %), 1-chloro-4-methoxybenzene (0.35 %), 4-hexenyl-benzene (0.34 %), pentacosane (0.32 %), palmitic acid ethyl ester (0.31 %), *bis*(2-ethylhexyl) phthalate (0.29 %), butyl-(trimethylsilyl) ether (0.26 %), 81.01 % of which are esters and the main retention time of which is between 20 and 30 min. Furthermore, the pharmacological action of biomedical molecules in the 17 identified chemical components is clarified, respectively, which demonstates the ethanol/benzene extractives of Chinese plantation *Pinus massoniana* contain rich pharmaceutical components including squalene, 9,12-octadecadienoic acid (Z,Z)-, campesterol, methyl linoleate and vitamin A aldehyde *etc*. and have a huge potential in bio-pharmaceuticals.

ACKNOWLEDGEMENTS

This work was financially supported by the National Department Public Benefit Re-search Foundation, China (No. 201404519), the Program for New Century Excellent Talents in University, China (No. NCET-12-0725) and the Outstanding Youth Projects of Hunan Province Education Department, China (No. 12B134).

REFERENCES

1. X.G. Liu and M.M. Chen, *J. Chem. Ind. Forest Prod*., **37**, 29 (2003).
2. Z.Y. Liang, H.Y. Chen, M.Y. Shen and H. Liu, *J. Chem. Ind. Forest Prod*., **36**, 8 (2002).
3. Z.C. Zhou, G.F. Qin, G.G. Li, G.L. Huang, B.X. Chen and C.Y. Cheng, *For. Res*., **8**, 1 (1995).
4. X.L. Lin, The Effect of Organic Extractive Content on the Properties of *Pinus massoniana*, Shanxi University of Science and Technology, 2 (2012).
5. H.T. Cheung, T. Miyase, M.P. Lenguyen and M.A. Smal, *Tetrahedron*, **49**, 7903 (1993).
6. Y.S. Li, G.M. Liu and D.M. Li, *Shizhen Med. Mater. Med. Res*., **23**, 726 (2012).
7. H. Sakagami, K. Takeda, Y. Makino and K. Konno, *Jpn. J. Cancer Res*., **77**, 59 (1986).
8. Pharmacopoeia of People's Republic of China, Chinese Pharmacopoeia Commission, Chin. Med. Sci. Technol. Press, p. 191 (2010).
9. X.M. Lv, Y.F. Hu, G.M. Liu and F. Peng, *Med. Recapitulate*, **16**, 1071 (2010).
10. Y.M. Zhang, Q.F. Gong, J.L. Yang, G.Z. Zeng and N.H. Tan, *Plant Diversity Resour*., **35**, 209 (2013).