ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 13 (4): 171-173, 2014
ISSN 1682-296X / DOI: 10.3923/biotech.2014.171.173

Determination of the Chemical Components and its Biological Activity of EB Extractives from *Buxus microphylla*

Zhong-Feng Zhang, Rui-Lin He, Yong-Jun Ye and Xu Zhang
Central South University of Forestry and Technology, Changsha, 410004, China

Abstract: The EB extraction, from *Buxus microphylla* were obtained by the method of ether/benzene extraction and their chemical components were identified with Gas Chromatograph-Mass Spectrometry (GC-MS) in this study. The result showed that the principal constituents were 1, 5-Hexadien-3-yne (82.993%), 1, 4-Methanoazulene, decahydro-4, 8, 8-trimethyl-9-methylene-, [1S-(1.α., 3a.β., 4.α., 8a.β.)] (2.427%), dibutyl phthalate (6.473%), 1-Phenanthrenecarboxal dehyde, 7-ethenyl-1, 2, 3, 4, 4a ,4b, 5, 6, 7, 9, 10, 10 a-dodecahydro-1, 4a, 7-trimethyl-, [1R-(1.α., 4a.β., 4b.α., 7.β., 10a.α.)] (1.165%), acetic acid, (3, 4-dimethoxyphenyl) (trimethylsiloxy), methyl ester (2.204%), 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane (4.738%). The identification of the biological activity of *Buxus microphylla* shows that its main ingredients can be used in making chloroprene rubber as the raw material of synthetic resin, synthetic perfume, flotation agent and organic synthesis and also appropriate for the reagent of general chemical analysis, insecticide, pesticide adjuvants and paint additives, etc.

Key words: *Buxus microphylla*, EB extractives, GC-MS, chemical componential analysis, biological activity components

INTRODUCTION

Buxaceae microphylla (Zhen and Min, 1980) belongs to the plant as evergreen shrub or small arbor. (Type genus: *Buxus* L.). There are 5 genuses, 60 species of *Buxaceae* around the world which are mainly distributed in the old world, the North temperate zone extending to the torrid zone, tropical Africa and Southern Africa, Madagascar and the Antilles. And there exists 1 genuse in the following areas; East Asia, North America, South America, Southeast Asia and Africa, from Mexico to California in North America, China, India and Malaya. China keeps 3 genuses, 35 species (Qiu and Li, 2002) which mostly come from Anhui province, Zhejiang province, Henan province, Jiangsu province and Changmei flower base, etc., For the time being, the main functions of *Buxaceae microphylla* lays in landscape planting, bonsai, arts and crafts, furniture (Hu, 2010) and medical (Li *et al*., 1984) and so on. *Buxaceae microphylla* as medicinal plant has already been recorded in Compendium of Materia Medica. *Buxaceae microphylla* has medicinal effects; activating vital energy, promoting the circulation, removing dampness, unblocking collaterals, clearing away heat and toxic material, dispelling wind, stanching bleeding, etc. (Wu, 1988). Among the folk, it was used to treat malaria, syphilis, rheumatism, dermatitis, rabies (Qiu and Nie, 1992) and coronary disease (Liang and Deng, 1980). The predecessors have made relative studies and reports about its chemical components (Liang and Mao, 2009; Zhang and Li, 2012) and alkaloid (Hu, 2010; Du *et al*., 1996) of *Buxus Microphylla*. For the further development and utilization of its resources, the EB extraction (Peng *et al*., 2004) from *Buxaceae microphylla* were obtained by the method of ether/benzene extraction and their Chemical components were identified with Gas Chromatograph-Mass Spectrometry (GC-MS), to provide the theoretical principle for the use of *Buxaceae microphylla* in some fields such as medicinal, engineered wood.

MATERIALS AND METHODS

A 20 year old *Buxus microphylla* was obtained from the Wuhan Forest Farm, Hubei province, P. R. China in October 2013. The sample chips processed were dried to absolute dry with drying oven at the tempreture of 65°C, then the 50 mesh powder was sieved out by using AS200 Sieving Instrument (Made in America). Both ether and benzene ether (chromatographic grade) were prepared for the subsequent experiments. Quantitative filter study, cotton bag and cotton thread were

Corresponding Author: Zhong-Feng Zhang, Central South University of Forestry and Technology, Changsha, 410004, China

all extracted in ether/benzene solution for 7 h and the ether/benzene solution (EB solution) was mixed according to $V_{ether}/V_{benzene}$ (2:1).

Extraction: A 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 ether/benzene solution (EB solution) with $V_{Ether}/V_{Benzene}$ (2:1). Extraction time was 7 h. Extraction temperature was 90±1°C. After extraction, the obtained extractive solution was dried to 10 mL in rotary evaporator under the condition of 45°C and vacuum 0.05-0.07 MPa to obtain the EB extractives.

GC-MS: The EB 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 nonpolar phase of 5% phenyl methyl silox. The carrier gas was helium (99.999%) at a constant flow rate of 3 mL min^{-1}. The temperature program was as follows: Initial temperature 50°C held for 3 min, from 50-200°C at the rate of 8°C min^{-1}, from 200-300°C at the rate of 5°C min^{-1} 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 $scan^{-1}$. Ion source temperature was 230°C and quadropole temperature was 150°C.

The identification of chemical components of EB extractives was based on computer matching with the reference mass spectra of the Wiley 7, Mainlib and NIST 08 libraries by comparing their retention time.

RESULTS AND DISCUSSION

Chemical components characteristics of EB extractives: The EB extractives were abtained with the method of ether/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.

According to the results of GC-MS, 6 chemical components were identified from 20 peaks of EB extractives from the Boxwood Powder including 1, 5-Hexadien-3-yne (82.993%), 1, 4-Methanoazulene, decahydro- 4, 8, 8-trimethyl-9-methylene-, [1S-(1.α., 3a.β., 4.α., 8a.β.)]-(2.427%), dibutyl phthalate (6.473%), 1-Phenanthrenecarboxal dehyde, 7-ethenyl-1, 2, 3, 4, 4a, 4b, 5, 6, 7, 9, 10, 10 a-dodecahydro-1, 4a, 7-trimethyl-, [1R-(1.α., 4a.β., 4b.α., 7.β., 10a.α.)] (1.165%), Acetic acid, (3, 4-dimethoxyphenyl) (trimethylsiloxy)-, methylester (2.204%), 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane (4.738%).

Determination of biological activity components of EB extractives from *Buxus microphylla*: 1, 5-Hexadien-3-yne is mainly used in producing chloroprene rubber. Special chemical activity exists in 1, 4-Methanoazulene, decahydro-4, 8, 8-trimethyl-9 -9methylene-, [1S-(1.α., 3a.β., 4.α., 8a.β.)], in which there are raw material of synthetic resin, synthetic perfume, flotation agent and organic synthesis. It can manufacture Isolongifolene, Isolongifolenone and of such products. Moreover, it can take the place of some expensive flavor in making

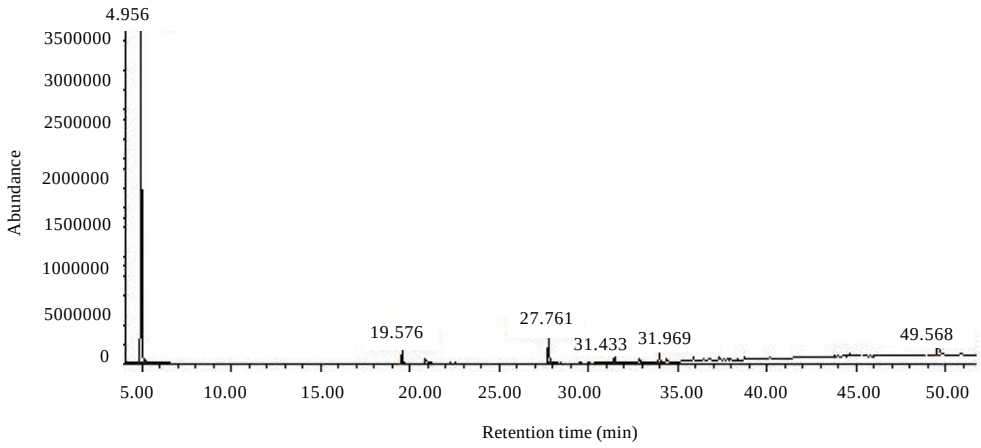

Fig. 1: Total ion chromatograms of EB extractives by GC-MS

perfume. Dibutyl phthalate is mainly used in reagent for general chemical analysis, insecticide, GC stationary liquid, solvent, insecticide, plasticizer, etc. As plasticizer, it makes products more flexible but larger in volatileness and water extractable, therefore, its durability is poor. 1, 1, 1, 3, 5, 5, 5-Hepta methyl trisiloxane is the basic raw material for synthetising polyether modified trisiloxane.

CONCLUSIONS

The chemical components of woody extractives of *Buxus microphylla* are identified for the first time with the method of ether/benzene extraction (EB extraction) and the use of GC-MS. Six chemical components are obtained on the peaks of EB extractives including 5-Hexadien-3-yne (82.993%), 1, 4-Methanoazulene, decahydro-4, 8, 8-trimethyl-9-methylene-, [1S-(1.α., 3a.β., 4.α., 8a.β.)] (2.427%), dibutyl phthalate (6.473%), 1-Phenanthrenecarboxal dehyde, 7-ethenyl-1, 2, 3, 4, 4a, 4b, 5, 6, 7, 9, 10, 10 a-dodecahydro-1, 4a, 7-trimethyl-, [1R-(1.α., 4a.β., 4b.α., 7.β., 10a.α.)] (1.165%), Acetic acid, (3, 4-dimethoxyphenyl) (trimethylsiloxy)-, methylester (2.204%), 1, 1, 1, 3, 5, 5, 5-Heptamethyltrisiloxane (4.738%). The identification of the biological activity of *Buxus microphylla* shows that its main ingredients can be used in making chloroprene rubber, as the raw material of synthetic resin, synthetic perfume, flotation agent and organic synthesis and also appropriate for the reagent of general chemical analysis, insecticide, pesticide adjuvants and paint additives, etc.

ACKNOWLEDGEMENTS

This study was financially supported by the National Department Public Benefit Research 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

Du, J., M.H. Qiu and R.L Nie, 1996. Two new *Buxus* alkaloids from *Buxus microphylla* sieb. ET ZUCC. Acta Botanica Sinica, 38: 483-488.

Hu, D., 2010. The timber of traditional hardwood furniture (II) Al-Ittihad, 10: 1-1.

Li, J.X., D.H. Zheng and S. Chen, 1984. Observing the curative effect of buxaceae double blind method in the treatment of coronary heart disease. Res. Chin. Patent Med.,

Liang, B.W. and C. Deng, 1980. The medicine for coronary disease-study on *Buxus microphylla* (The third report) II, III, VI Study on Isolating and structure of alkaloid from *Buxus microphylla*. Trad. Chinese Med. Res., 6: 37-38.

Liang, R. and T.Z. Mao, 2009. The study on chemical components of *Buxus microphylla*. Guangxi Plant Guihaia, 29: 703-706.

Peng, W.X., T.L. Zhu, Z.Z. Zheng, Z.C. Fan, K.F. Li and N.C. Li, 2004. Present situation and developing tendency of extracts from wood. China For. Sci. Technol., 18: 6-9.

Qiu, M.H. and D.H. Li, 2002. Study on chemotaxonomy of *Buxaceae*. Chin J. Applied Environ. Biol., 8: 387-391.

Qiu, M.H. and R.L. Nie, 1992. Alkaloid and plant resources of *Buxus microphylla*. Nat. Prod. Res. Dev., 4: 41-41.

Wu, Z.Y., 1988. Xinhua Materia Medica Outline. Shanghai People's Publishing House, Shanghai, China.

Zhang, S.J. and C.Y. Li, 2012. The progress on the chemical constituent of *Buxaceae*. Contemp. Med., Vol. 18.

Zhen, M. and T.L. Min, 1980. Flora Sinensis. Science Press, Beijing, China, pp: 19-59.