Molecular characteristics of woody extracts of *Buxus microphylla*

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Abstract: As one of famous shrubs in China, *Buxus microphylla* is considered as the important wattle. However, the constituents of *Buxus microphylla* stem extracts aren't used effectively. Therefore, the molecules of stem extracts in *Buxus microphylla* are analyzed to further utilize the resources. The results show that the optimal extraction time of ethanol/methanol extraction, petroleum ether/ benzene extraction, and benzene/alcohol extraction are 7h, 7h, and 5h, respectively. The HK-61, HK-63, HK-73, HK-81, HK-82 stem extracts are obtained 1, 9, 1, 27, and 1 components, respectively. The stem extracts of *Buxus microphylla* is rich in drug and biomedical activities. *Buxus microphylla* stem is fit to extract 1,5-hexadien-3-yne, squalene, and dibutyl phthalate.

Keywords: Antibacterial Molecules, Stem Activities, *Buxus microphylla*, GC-MS.

INTRODUCTION

Buxus microphylla, which is native to Japan and Taiwan, is arguably the most popular shrub for low hedging. It is an evergreen shrub or small tree growing to 2~3 m tall (Jong-Hwoa *et al.,* 1993; Yu-Xin *et al.,* 2009). The leaves are bright green, 10~25 mm long, oval with a rounded or notched tip; the flowers are monoecious and bloom in spring for pleasant aroma is more noticeable than the flowers; the fruit is small capsule; the distinctive stems are flattened, with an angular and square feel; the root rot are a problem in poorly-drained soils (Shim *et al.,* 1990; Chengjun *et al.,* 1995). In most cases, *Buxus microphylla* is ornamental, rare, useful and valuable.

Buxus microphylla is widely used. The advanced equipments and instruments have largely used for the research on *Buxus microphylla* biomass. Buxmicrophylline B, C and D, which are isolated from the leaves and stems of *Buxus microphylla*, are elucidated by extensive analysis of the spectral data (Du *et al.,* 1999). Nakano et al (2006) found the methyl syringate, betulic acid, lupeol, and betulin of the nonalkaloidal constituents from the leaves and stems of *Buxus microphylla*. Hui-qin *et al* (2012). Discovered that ABML relaxes thoracic aorta VSM cells by suppressing influx of extracellular Ca^{2+} via voltage-dependent Ca^{2+} channel and receptor-operated Ca^{2+} channel. The five new triterpenoid alkaloids, buxmicrophyllines E−I (1−5), and six known ones (6−11) are isolated from the leaves and stems of Buxus microphylla whose structures are elucidated by NMR and MS spectroscopic analysis (Yu-Xin *et al.,* 2009). *Buxus microphylla* have an ability to form compression wood (Yoshizawa *et al.,* 1992). The Buxus microphylla leaf ethyl acetate extract have obvious effect on the levels of serum total cholesterol and triglyceride (Yong-wen *et al.,* 2009). Cyclobuxine is a steroidal alkaloid which is extracted from *Buxus microphylla*, is used as folk remedies for several diseases, including malaria and venereal diseases (Jong-Hwoa *et al.,* 1993). YuXin *et al* (2009) analyzed the cytotoxic triterpenoid alkaloids from *Buxus microphylla*. Zhuping *et al* (2013) identified and analyzed some active compounds (Wanxi *et al.,* 2011; 2012a; 2012b). My group also studied analyzed many woody extracts, herbal extracts (Wanxi *et al.,* 2009; 2012c; 2013a; 2013b; 2013c; 2013d; 2014a; 2014b; 2014c), and chemical compositions of wood biomass (Hongchen *et al.,* 2012; Yong-Chang *et al.,* 2014; Qiu *et al.,* 2014; Le *et al.,* 2014a; 2014b; Lansheng *et al.,* 2014; Lansheng *et al.,* 2013a; 2013b), and then found many important research efforts. However, the researches on the active ingredients in *Buxus microphylla* stem are less done. Therefore, the molecular characteristics of the stem extracts are investigated and analyzed by the optimized extraction techniques so as to further utilize *Buxus microphylla* resources.

MATERIALS AND METHODS

Materials

Buxus microphylla stems are collected from Wuling Mountain, Hunnan Province, China. The fresh stems are shaved, powdered and kept in vacuum. Methanol, benzene, petroleum ether and ethanol (chromatographic grade) are prepared for the subsequent experiments. Cotton thread and cotton bag are extracted by benzene/ethanol solution for 12 h under the $V_{ethanol}$ /$V_{benzene}$ of 2.

Experiment methods

Single extraction: Weighed 18 pieces of stem powder, each is about 15g (0.1mg accuracy) and then parceled by the cotton bag and tied by cotton thread, and signed. Extraction is carried out in 300ml solvents by the Foss method for 1, 3, 4, 5, 6, 7 hours. Solvents are ethanol/ methanol ($V_{ethanol}/V_{methanol}$ = 2), petroleum ether/ benzene ($V_{petroleum\ ether}/V_{benzene}$ = 2), and benzene/ethanol solution,

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respectively. Ethanol/methanol extraction, petroleum ether/benzene extraction, and benzene/ethanol extraction are done at the temperature of 75°C, 95°C and 95°C, respectively. After extraction, the one piece is taken out, dried at 105°C and weighed. The extracts are obtained by evaporation at 60~70°C.

Three-step extraction: Weighed 27 pieces of stem, each is 15g (1.0mg accuracy), and then parceled into cotton bag tied by cotton thread, and signed. Three-step extraction is carried out by large-caliber Soxhlet according to the different solvents of ethanol/methanol (HK-61) → petroleum ether/benzene (HK-62) → benzene/ethanol (HK-63) (EMPBBE), petroleum ether/benzene (HK-71) → benzene/ethanol (HK-72) → ethanol/methanol (HK-73) (PBBEEM), benzene/ethanol (HK-81)→ethanol/methanol (HK-82) → petroleum ether/benzene (HK-83) (BEEMPB), respectively. After every step extraction, the one piece is taken out, dried at 105°C and weighed. The extracts are obtained by evaporation at 60~70°C. Extraction time is 2.5h.

GC/MS condition: Among the above test, the HK-61, HK-63, HK-73, HK-81, HK-82 stem extracts are analyzed, respectively. Each 0.5 mg extracts is analyzed by a GC/MS-QP2010 (Shimadzu Corp., Japan). The GC/MS analysis is done as the same as the documents (Wanxi *et al.,* 2013a; 2013b; 2013c; 2013d; Lansheng *et al.,* 2014).

RESULTS

The leaching rates of single extractions are listed in table 1. The HK-61, HK-63, HK-73, HK-81, HK-82 stem extracts are obtained respectively. The total ion chromatograms of four extracts by GC/MS are shown in Figure 1. Relative content of each component is counted by area normalization. Analyzing the MS data, the NIST standard MS map by computer, open-published books and papers, then components and their contents are identified.

Results analyses

Leaching rule of stem extracts of Buxus microphylla

The leaching rate trend of *Buxus microphylla* stem extracts in different solvents is described in table 2. It is observed that during ethanol/methanol extraction, the leaching rate of stem extracts fluctuated, and reached the maximum (15.99%) when extraction time is 7h. During petroleum ether/ benzene extraction, the leaching rate of stem extracts also fluctuated, and reached the maximum (12.48%) when extraction time is 7h. During benzene/alcohol extraction, the leaching rate of stem extracts fluctuated, and reached the maximum (14.29%) when extraction time is 5h. And the optimal extraction time of ethanol/methanol extraction, petroleum ether/ benzene extraction, and benzene/alcohol extraction are 7h, 7h, and 5h, respectively.

Molecular properties of Buxus microphylla stem extracts

According to GC/MS result, one component is identified from HK-61 stem extracts of *Buxus microphylla*. The result showed that the main components are 1,5-hexadien-3-yne.

The 9 component is identified from HK-63 stem extracts. The result showed that the main components are 1,3-hexadien-5-yne (27.446%), 6-methyl-4-indanol (0.086%), naphthalene, 2-methyl- (0.21%), 4(1h)-pyrimidinone, 2-phenyl- (0.588%), phthalic acid, isobutyl nonyl ester (0.29%), 1,2-benzenedicarboxylic acid, dipropyl ester (70.966%), dibutyl phthalate (0.038%), hexanedioic acid, bis(2-ethylhexyl) ester (0.179%), phthalic acid, 2-ethylhexyl hexyl ester (0.196%).

The one component is identified from HK-73 stem extracts. The result showed that the main components are dibutyl phthalate.

The 27 components is identified from HK-81 stem extracts. The result showed that the main components are dibutyl phthalate (82.784%), squalene (3.462%), n-hexadecanoic acid (2.23%), cis-13 - octadecenoic acid (2.211%), 9,12-octadecadienoic acid (z,z)- (2.054%), 3-methyl-2-phenylindole (1.286%), 9-eicosyne (0.756%), hexadecanoic acid, ethyl ester (0.472%), phenol, 6-methyl-2- [(4-morpholinyl) methyl]- (0.45%), octadecane (0.443%), eicosane (0.42%), 2,6-dicyanotoluene (0.393%), z,z-3,13- octadecedien-1-ol

Table 1: Leaching rate trend of each single extraction [%]

Extraction time [h]	Ethanol/methanol	Petroleum ether/benzene	Benzene/ethanol
1	15.13	12.41	13.50
3	15.20	11.92	13.92
4	15.60	11.87	13.77
5	14.35	12.17	14.29
6	13.95	11.88	13.53
7	15.99	12.48	14.13

(0.386%), styrene (0.386%), 4-((1e)-3-hydroxy-1-propenyl)-2-methoxyphenol (0.343%), 1,2-benzenedicarboxylic acid, diisooctyl ester (0.295%), naphthalene, 1-methyl- (0.268%), (r)-(-)-14-methyl-8-hexadecyn-1-ol (0.254%), bendazol (0.237%), naphthalene, 1,4-dimethyl- (0.199%), 1,2,5-oxadiazol-3-amine, 4-(4-methoxyphenoxy)- (0.193%), 1,2,5-oxadiazol-3-amine, 4-(4-methoxy -phenoxy)- (0.137%), 6-methyl-4-indanol (0.136%), hexanedioic acid, bis(2-ethylhexyl) ester (0.133%), heptadecane, 8-methyl- (0.123%), 9,12-octadecadien-1-ol, (z,z)- (0.089%), 3',4'-difluoroacetophenone (0.028%).

The ong component is identified from HK-82 stem extracts. The result showed that the main components are dibutyl phthalate.

Resource properties of stem extracts of Buxus microphylla

There are many biomedical components in the stem extracts of *Buxus microphylla*. Because of its officinal value, dibutyl phthalate is a pesticide to keep internal environment homeostasis (Wanxi *et al.,* 2014b). 9,12-octadecadien-1-ol, and 9,12-octadecadienoic acid (Z,Z)- have been identified as the main medical component of dried worms, and has diuretic and detoxification properties (Guo *et al.,* 2006; Wanxi *et al.,* 2013a; 2013b; 2013c). The n-hexadecanoic acid might help in designing of specific inhibitors of phospholipase A(2) as anti-inflammatory agents whose binding energy is calculated by in silico method and compared with known inhibitors (Aparna *et al.,* 2012). Hexanedioic acid, bis(2-ethylhexyl) ester, which is an industrial chemical, is used in various products such as cosmetics and some personal care products, auto interior protectant, heavy-duty hand cleanser and lubricant (www.chemicalsubstances.gc.ca). Phthalic acid derivatives are the main constituents of *Elaeagnaceae* plant which could cure chronic cardiovascular and cerebrovascular diseases and have anti-tumor, anti-inflammatory, antibacterial functions (Hao *et al.,* 2006). Squalene, which could protect liver, resist fatigue and strengthen the body's resistance, and improve human immunity, is considered as important substances in practical and clinical uses with a huge potential in nutraceutical and pharmaceutical industries (Kim *et al.,* 2012). And there are many drug and biomedical activities in the stem extracts of *Buxus microphylla*. According to the relative content, *Buxus microphylla* stem is fit to extract 1,5-hexadien-3-yne, squalene, and dibutyl phthalate.

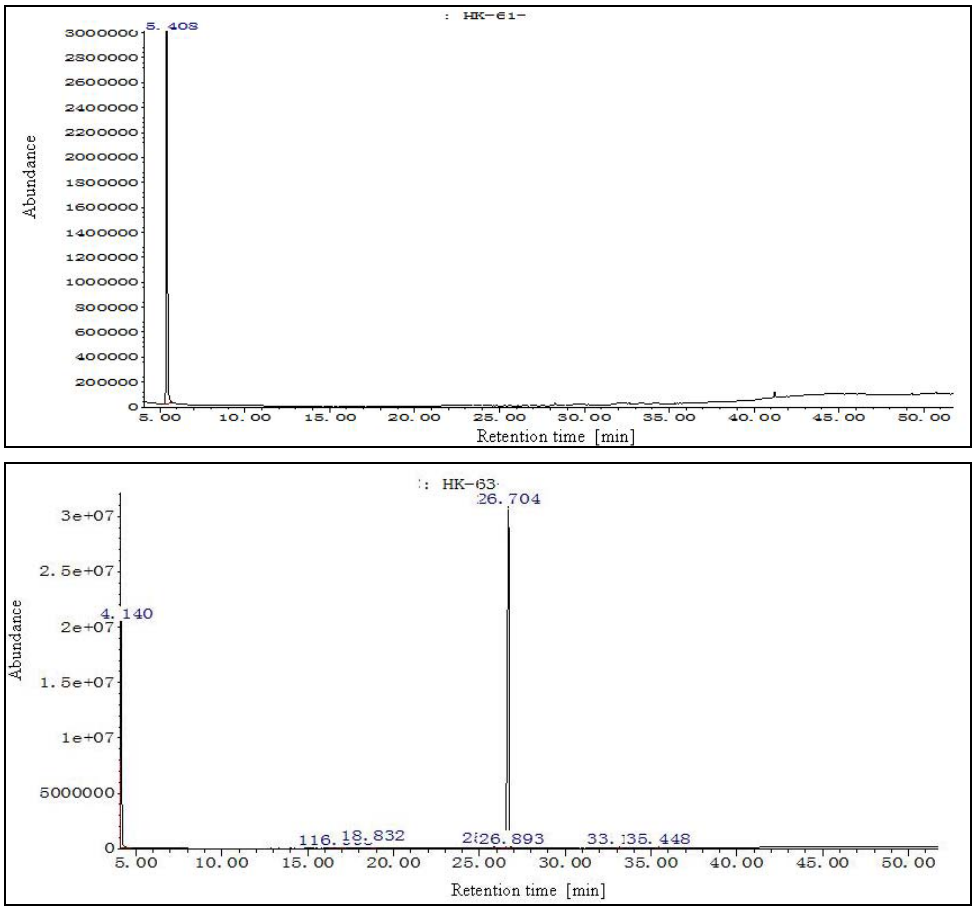

Fig. 1: Total ion chromatograms of stem extracts by GC/MS (continued…)

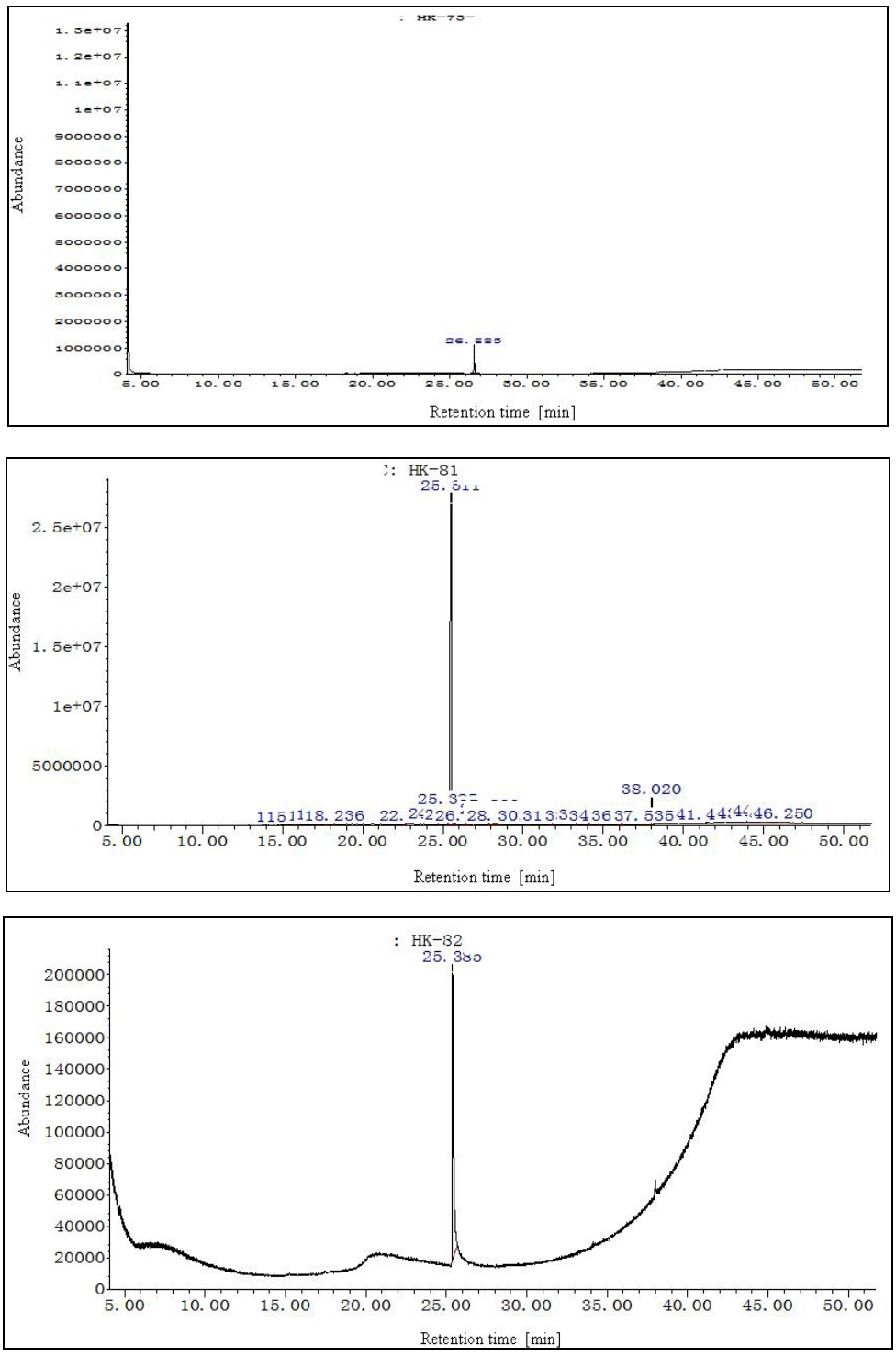

Fig. 1(continued): Total ion chromatograms of stem extracts by GC/MS

CONCLUSION

The leaching rule of stem extracts from *Buxus microphylla* stem is obvious. The optimal extraction time of ethanol/methanol extraction, petroleum ether/benzene extraction, and benzene/alcohol extraction are 7h, 7h, and 5h, respectively. The HK-61, HK-63, HK-73, HK-81, HK-82 stem extracts are obtained 1, 9, 1, 27, and one components, respectively. What's more, the stem extracts of *Buxus microphylla* is rich in biodrug and biomedical activities, including hexanedioic acid, bis(2-ethylhexyl)

ester, squalene, and dibutyl phthalate. According to the relative content, *Buxus microphylla* stem is fit to extract 1,5-hexadien-3-yne, squalene, and dibutyl phthalate.

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REFERENCES

Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C and Haridas M (2012). Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chem. Biol. Drug Des*., **80**(3):434~439.

Chengjun J and Zhenghai H (1995). Morphological and anatomical studies on the nectaries of *Buxus microphylla*. *Acta Botanica Boreali-Occidentalia Sinica*, **15**(6): 28-31.

Du J, Chiu M and Nie R (1999). Three steroidal alkaloids from *Buxus microphylla*. *J. Asian. Nat. Prod. Res.,* **1**(4): 239-244.

Guo C and Wei D (2006). Analysis of fatty acid composition from *Gryllotalpa orientalis* Burmeister by gas chromatography and its orthogonal design experiment of supercritical fluid extraction. *Chin. J. Anal. Chem*., **34**: 15-18.

Hao H, Xin Z and Biao J (2006). Survey in study on chemical constituents from plants of *Elaeagnaceae*. *Chinese Tradit. & Herb. Drugs*, **37**(2): 307-309.

Hongchen Q, Wanxi P, Yiqiang W, Shubin W and Ganjun X (2012). Effects of alkaline extraction on micro/nano particles of *Eucalyptus camaldulensis* biology. *J. Comput. Theor. Nanos*., **9**(9): 1525-1528.

Hui-qin Z, Yan-yan L, Yong-wen L, Li L and Zhi-qing C (2012). Effects of total alkaloids in *Buxus microphylla* leaves on aorta smooth muscle of rats and their Mech. *Herbal Med.,* **4**(2): 136-141.

Jong-Hwoa L, Jun-Tack K, Byung-Heon C and Chang KM (1993). Cyclobuxine protects the isolated rat heart from the myocardial injuries produced by ischemia and reperfusion. *Planta Med*., **59**(4): 296-301.

Kim SK and Karadeniz F (2012). Biological importance and applications of squalene and squalane. *Adv. Food & Nutr. Res.*, **65**: 223-233.

Lansheng W, Wanxi P, Dongli L, Liwen S and Yueping T (2014). Molecular pharmacology characteristics of immunology wood extractives of *Eucalyptus camaldulensis* Biomass. *Pak. J. Pharm. Sci.,* (in press)

Lansheng W, Wanxi P, Minglong Z and Zhi L (2013a). Separation characteristics of lignin from *Eucalyptus lignincellulose* for medicinal biocellulose preparation. *J. Pure Appl. Microbio.*, **7**(SE): 59-66.

Lansheng W, Wanxi P, Zhi L and Minglong Z (2013b). Molecule characteristics of *Eucalyptus hemicelluloses* for medical microbiology. *J. Pure Appl. Microbio*., **7**(2): 1345-1349.

Le C, Wanxi P, Zhengjun S, Lili S and Guoning C (2014a). Weibull statistical analysis of tensile strength of vascular bundle in inner layer of Moso bamboo culm In molecular parasitology and vector biology. *Pak. J. Pharm. Sci.,* **27**(Supp.4): 1083-1087.

Le C, Wanxi P, Zhengjun S, Huangfei L and Guoning C (2014b). Variability of macroscopic dimensions of Moso bamboo. *Pak. J. Pharm. Sci. ,* (in press)

Nakano T, Terao S and Lee KH (2006). Nonalkaloidal constituents of *Buxus microphylla* var. suffruticosa. *J. Pharm. Sci.,* **54**(8): 1201-1203.

Qiu X, Wanxi P and Makoto O (2014). Molecular bonding characteristics of self-plasticized bamboo composites. *Pak. J. Pharm. Sci*., **27**(Supp.4): 975-982.

Shim KK, Ha YM, Kang YH and Seo BK (1990). A study on new winter-green cultivar of Korean boxwood *Buxus microphylla* var. Koreana Nakai. *J. Korean Soc. Hort. Sci.,* **31**(4): 405-413.

WanXi P, YiQiang W, HongChen Q, ZhongFeng Z and HePing D (2009). Effect of extraction methods on micro/nano particles leaching from *Eucalyptus urophlla* wood. *Appl Electrom*(II), **13**: 373-374.

Wanxi P, Lansheng W, Fengjuan W and Qiu X (2011). 3-(4-Bromophenyl)-4- (4-hydroxyanilino) furan-2 (5H)-one. *Acta Crystallog. E*, **67**(9): O2329- U206.

Wanxi P, Fengjuan W, Lansheng W and Qiu X (2012a). Crystal structure of 3-(4-bromophenyl)- 4-(4-chlorophenylamino)furan-2(5H)-one, $C_{16}H_{11}BrClNO_2$. *Z. Kristallogr. NCS*, **227**(1): 61-62.

Wanxi P and Le C (2012b). Crystal structure of 3-(3-bromophenyl)-4- (3,5-dichloro- phenylamino) furan-2 (5H)-one, $C_{16}H_{10}BrC_{l2}NO_2$. *Z. Kristallogr. NCS*, **227**(2): 267-268.

Wanxi P, Lansheng W, Qiu X, Qingding W and Shilong X (2012c). TD-GC-MS analysis on thermal release behavior of poplar composite biomaterial under high temperature. *J. Comput. Theor. Nanos.,* **9**(9): 1431-1433.

Wanxi P, Lansheng W, Zhi L, Minglong and Minglong Z (2013a). Identification and chemical bond characterization of wood extractives in three species of Eucalyptus Biomass. *J. Pure Appl. Microbio*., **7**(SE): 67-73.

Wanxi P, Zhi L, Junbo C, Fangliang G, Xiangwei Z and Zhongfeng Z (2013b). Immunology molecular characteristics of JYBS Eextractives from *Illicium verum* Biomass. *J. Pure Appl. Microbio.,* **7**(2): 1237-1243.

Wanxi P, Zhongfeng Z, Zhi L, Ohkoshi M, Junbo C,

Fangliang G and Xiangwei Z (2013c). Molecular characteristics of biomedical and bacteriostasis extractives of *Illicium verum* fruit. *J. Pure Appl. Microbio.*, **7**(3): 2017-2024.

Wanxi P, Zhi L, Junbo C, Fangliang G and Xiangwei Z (2013d). Biomedical molecular characteristics of YBSJ extractives from *Illicium verum* fruit. *Biotechnol. Biotec. Eq.,* **27**(6): 4311-4316.

Wanxi P, Lansheng W, Minglong Z and Zhi L (2014a). Separation characteristics of lignin from *Eucalyptus camaldulensis* lignincelluloses for biomedical cellulose. *Pak. J. Pharm. Sci.*, **27**(Supp.3): 723-728.

Wanxi P, Qiu X and Makoto O (2014b). Immune effects of extractives on bamboo biomass self-plasticization. *Pak. J. Pharm. Sci.,* **27**(Supp. 4): 991-999.

Wanxi P, Shengbo G, Dongli L and Daochun Q (2014c). Molecular characteristics of three extractives of *Cinnamomum camphora* leaves. *Pak. J. Pharm. Sci*. , (in press)

Yong-wen L, Cheng-fang Y, Hui-qin Z and Li L (2009). Study on the antihyperlipidemia effect of *Buxus microphylla* leaf extract. *Lishizhen Med. Mater. Med. Res.*, (8): 1-3.

Yoshizawa N, Satoh M, Yokota S and Idei T (1992). Formation and structure of reaction wood in *Buxus microphylla* var. insularis Nakai. *Wood Sci. Technol.*, **27**(1): 1-10.

Yong-Chang S, Zhi L, Wan-Xi P, Tong-Qi Y, Feng X, Yi-Qiang W, Jing Y, Yang-Sheng W and Run-Cang S (2014). Chemical changes of raw materials and manufactured binderless boards during hot pressing: Lignin isolation and characterization. *BioResources,* **9**(1): 1055-1071.

Yu-Xin Y, Xiao-Dong H, Jian-Chao C, Yun S, Xian-Min Z, Chen Q and Ming-Hua Q (2009). Cytotoxic Triterpenoid alkaloids from *Buxus microphylla. J. Nat. Prod.,* **72**(2): 308-311.

Zhu-Ping X, Zhi-Yun P, Jing-Jun D, Rui-Cheng D, Xu-Dong W, Hui O, Pan Y, Juan H, Yuan-Feng W, Man Z, Xiao-Chun P, Wan-Xi P and Hai-Liang Z (2013). Synthesis, molecular docking and kinetic properties of β-hydroxy-β-phenylpropionyl-hydroxamic acids as *Helicobacter pylori* urease inhibitors. *Eur. J. Med. Chem*., **68**: 212-221.