Biomedical molecular of woody extractives of *Cunninghamia Lanceolata* biomass

Xu Zhang[1], Kai Huang[1,2], Yong-jun Ye[1], Jun-you Shi[3] and Zhong-feng Zhang[1]*

[1]Central South University of Forestry and Technology, Changsha, China
[2]Jiangmen Polytechnic, Jiangmen, China
[3]Beihua University, Jilin, China

Abstract: Extractives, important compounds from wood, provide abundant resources for woody medicine. In this study, the three extractives from *Cunninghamia lanceolata* wood were removed by method of three-stage extraction with alcohol, petroleum ether, and alcohol/petroleum ether and their chemical components were analyzed by gas chromatography-mass spectrometry (GC-MS). Thirteen chemical components were discovered in the first-stage extractives, including: 4-((1e)-3-hydroxy-1-propenyl)-2-methoxyphenol (36.80%), *α*-(2-phenylethenyl)-1-piperidineacetonitrile (15.39%). One-hundred chemical components were discovered in the second-stage extractives, including: [1s-(1α,4aα,10aβ)]-1, 2,3,4,4a,9,10,10a-octahydro-1,4a- dimethyl-7-(1-methylethyl)-1- phenanthrenecar-boxylic acid (15.16%), 1,3-dimethoxy-5-[(1e)-2- phenylethenyl]-benzene (6.99%). Seven chemical components were discovered in the third-stage extractives, including: 1,3-dimethoxy -5-[(1E)-2-phenylethenyl]-benzene (32.88%), stigmasta-4,6,22-trien-3α-ol (17.83%). And both the main retention time of the first-stage and which of third-stage extractives are 20-30 minutes, and the main retention time of the second-stage extractives is ≤10 minutes. Besides, the three extractives contained many biomedical molecular, such as [1s-(1*α*,4a*α*,10a*β*)]-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecar-boxylic acid, squalene, stigmast-4-en-3-one and *γ*-sitosterol and so on, which means that the three extractives from *Cunninghamia lanceolata* wood have huge potential in biomedicine.

Keywords: *Cunninghamia lanceolata,* biomedical molecules, woody extractives, woody biomedicine.

INTRODUCTION

The study of Chinese medicine, especially the development of modern Chinese medicine, has important significance in the field of medicine study and life science study, and which has become a unique system with a history of several thousand years (Liu, 2007). Nowadays, Innovative pharmaceuticals of Chinese medicine are the important direction of modern pharmaceuticals in China (Tu *et al.*, 2007). The pharmaceuticals of Chinese medicine mainly come from herbaceous plant (Liang *et al.*, 2008; Peng *et al.*, 2004). However, the outputs of herbaceous plant are too little to meet the current market demands. Nowadays plantations of woody plant have been planted extensively to provide rich resources for woody medicine and extraction industries of woody medicine are emerging.

Cunninghamia lanceolata, planted only in China, has become the dominating species of plantation forest in Southern China, and an area of over 0.6 million ha[2] *Cunninghamia lanceolata* forest can produce 2.4 billion m[3] wood at present (Yun *et al.*, 2000). With the rapid development of wood market, *Cunninghamia lanceolata* resources are also developing greatly. However, most products made of *Cunninghamia lanceolata* wood have lower value (Bachir *et al.*, 2008). On the other hand, *Cunninghamia lanceolata* wood is rich in the drug compositions (Ashour, 2008), and which has too many extractives to be purified (Rahimi-Nasrabadi *et al.*, 2012). In order to obtain high value products, the three extractives were removed by method of three-stage extraction and analyzed by GC-MS to discover the high value biomedicines in this study.

MATERIALS AND METHEDS

Materials

An 8-year-old *Cunninghamia lanceolata* with a diameter of 22cm was retrieved from Zhuzhou Forest Farm, Hunan province, P.R. China September in 2013, the sample chips were dried to absolute dry with a drying oven at a temperature of 55°C, and then the 40 mesh powder was sifted out by using an AS200 Sieving Instrument Made in America. Both alcohol and petroleum ether (chromatographic grade) were prepared for the subsequent experiments. Quantitative filter paper, cotton bag and cotton thread were all extracted in a alcohol/ petroleum ether solution (according to $V_{alcohol}$ / $V_{petroleum\ ether}$ =2:1) for 12hours.

Extraction

Three pieces of the wood powder weighing about 5g (1.0 mg accuracy) were parcelled by using the cotton bag tied with the cotton thread and then marked. Extraction was gradually carried out by the Soxhlet Extractor and extracted in 800ml of the alcohol, petroleum ether and

**Corresponding author:* e-mail: csfuzzf@163.com

alcohol/petroleum ether solution for 5h at a temperature of 80±1°C, respectively. After extraction, the obtained three extractives solutions were dried to 10ml in a rotary evaporator at 45ºC and in a vacuum 0.05-0.07MPa to obtain the alcohol, petroleum ether and alcohol/petroleum ether extractives, respectively.

GC-MS

The obtained extractives were determined by online linked gas chromatograph/mass spectrometer (GC-MS), respectively. 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 a HP-5MS column (30m× 0.25mm× 0.25μm) coated with a neutral phase of 5% phenyl methyl silox. The carrier gas was helium (99.999%) at a constant flow rate of 3ml/min. The temperature program was as follows: initial temperature 50°C held for three minutes, from 50°C to 250°C at the rate of 8°C/min, from 250°C to 300°C at the rate of 5°C/min, and the final temperature for 10 minutes. The ion source was operated in the electron ionisation mode (EI; 70eV). Full scan mode data were acquired to determine the appropriate masses for the later acquisition in selected ion monitoring mode (SIM) in the conditions of mass range from 50 to 300amu with the scan rate of 0.5s/scan. The ion source temperature was 230°C and the quadropole temperature was 150°C.

RESULTS

During the three-stage extraction, the three extractives (alcohol, petroleum ether, alcohol/petroleum ether) were obtained respectively. The total ion chromatograms of three extractives by GC-MS were shown in fig. 1. Related content of each component was counted by area normalization. The identification of the extractives was based on computer matching with the reference mass spectra of the Wiley7, Mainlib and NIST05 libraries by comparing their retention time.

According to the GC-MS results, 13 components were identified from 14 peaks of alcohol extractives of *Cunninghamia lanceolata* wood. The main components were 4-((1e)-3-hydroxy-1-propenyl)-2-meth oxyphenol (36.80%), *α*-(2-phenyletheny l)-1-piperidineacetonitrile (15.39%), 1-(2, 4,6-trihydroxy-3-methylphenyl)-1-butanone (9.82%), 1,7-diethyl-2,3,6,7-tetrahydro-3-met hyl-1h-purine-2,6-dione (7.49%), 4-hydroxy-benzeneethanol (5.85%), *γ*-sitosterol(5.47%), 4-methoxy-4',5'-methylenedioxy biphenyl -2-carboxylic acid (4.06%), 3-(4-hydroxy-3- methoxyphenyl)-2-propenal (3.32%), stigma sta-4,6,22-trien-3*α*-ol(2.98%), 4-hydroxy-3- methoxy-benzeneacetic acid methyl ester (2.63%), 4-hydroxy-3,5-dimethoxy-ben zald ehyde (2.58%), 2-cyclohexylimino-3-methylb utane (2.00%), 3,5-dimethoxy-4-hydroxycinn amaldehyde (1.61%).

100 components were identified from 119 peaks of petroleum ether extractives of *Cunninghamia lanceolata* wood. The main components were [1s-(1α,4aα,10aβ)]-1,2,3, 4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecar-boxylic acid(15.16%), 1,3-dimethoxy-5-[(1e)-2-pheny lethenyl]-benzene (6.99%), dehydroabietic acid (6.89%), 1-fluoro-indolo[2,3-b]quinoxal ine (5.46%), undecane (3.28%), p-dimethyla minobenzylidene p-anisidine (2.94%), decane (2.88%), methyl dehydroabietate (2.78%), 15-hydroxydehydroabietic acid methyl ester(2.77%), 7-methylene-2,4,4- trimethyl-2-vinyl-bicyclo[4.3.0]nonane (2.53%), dodecane (2.30%), 1,1',3,3'-tetrakis (1,1-dimethyl ethyl)-ferrocene (2.26%), α-(2- phenylethenyl)-1-piperidineace-tonitrile (1.94%), [1r-(1α,4aβ,4bα,7β,10aα)]-7-ethen yl-1,2,3,4,4a,4b,5,6,7, 9,10,10a-dodecahydro-1,4a, 7-trimethyl-1-phenanthrene-carboxal dehyde (1.74%), tridecane (1.45%), nonane (1.40%), 1,2,3-trimethyl –benzene (1.28 %), α-pinene (1.18%), γ-sitosterol (1.04%), p-xylene (0.99%), etc. Others were o-cymene, 7-oxo dehydroabietic acid methyl ester, naphthalene, 10,10-diethoxy-9-oxa-10-sila -9,10-dihydro phenanthrene, decahydro- naphthalene, 2-phenyl-4-(propen-1-yl)- pyrimidin-5-carboxamide, mesitylene, 4-methyl-decane, (5β)-pregn-14-ene, octane, 2- methyl-trans-decalin, 4-methyl-heptane, 5- methyl-nonane, butyl-cyclohexane, 1-ethyl- 3-methyl-benzene, 2-methyl-decane, 2- methyl-nonane, [1r-(1α, 4aβ,4bα,7α,10aα)]-7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-1,4a,7-trimethyl-1- phenanthr-enecarbo xylic acid methyl ester, squalene, [s-(e)]-2,6- dimethyl-4-octene, decahydro-2-methyl- naphthalene, (1r)-2,6,6-trimethyl bicyclo[3.1.1]hept-2-ene, 2-methyl-undecane, stigmast-4 -en-3-one, cis-decahydro-naphthalene, 2-carene, 3,5,6,7-tetrahydro-3,3,4,5,5,8-hexa methyl-s-indacen-1(2h)-one, 1-ethyl-1-methyl-cyclohexane, 3-ethyl-2-methyl-heptane, propyl-cyclohexane, 2,6-dimethyl-undecane, (1s)-4,6,6-trimethyl-bicyclo [3.1.1]hept-3-en -2-one, pentyl-cyclohexane, stigmastan- 3,5,22-trien, 2-ethylhexyl sulfurous acid hexadecyl ester, dibutyl phthalate, 4-methyl-undecane, hexyl-cyclohexane, (1s,3r)-(+) -m-menthane, 1-methyl-3-propyl-benzene, benzeneacetald-ehyde, 1-ethyl-2-methyl- cyclohexane, 1-phenyl-1-butene, 3-amino-5- methoxycarbonylmethyl -sulfanyl-4-trifluoromethyl-thiophene-2-carboxylic acid methyl ester, 3-methyl-undecane, 3-methyl-decane, [4as-(4aα,4bβ, 7β,10aβ)]- 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10, 10a-dodecahydro-1,1,4a,7-tetra-methyl-phenanthrene, 1,1,3-trimethyl-cyclohexane, tetradeca ne, cis, trans-1,6-dimethylspiro[4.5] decane, 1,2,4,5-tetramethyl-benzene, toluene, 1,2,3, 3a,4,5,6,7,8,9,9a,10, 11,12-tetradecahydro-perylene, 1-ethyl-2,4-dimethyl-benzene, 8-propoxy-cedrane, 1,2,3,4-tetramethyl -benz ene, 2-methyl-naphthalene, 1,2,4-trimeth yl-cyclohexane, propanol-

cyclohexane, 2-me thyl-dodecane, (4as-trans)-1,2,3,4,4a,9,10, 10a-octahydro-1,1,4a-trimethyl-7- (1-methylethyl)-phenanthrene, oxalic acid 1-menthyl pentyl ester, ethyl-cyclohexane, 3,7- dimethyl-decane, cis-octahydro-1h-indene, cyclohexylmethyl sulfurous acid octadecyl ester, ethyl oleate, 3-methyl-nonane, hexyl-cyclopentane, cis-1-ethyl-3-methyl- cyclohexane, 2,3-dihydro-5-methyl-1h-indene, (1α,2β,3α)-1,2,3-trimethyl-cyclohexane, hexadecanoic acid ethyl ester, hydroquinone bis(trimethylsilyl) ether, 2,7-diacetyl-3,6- dimethyl-1,8-naphthalenediol, 1,2,3,4- tetrahydro-5,7-dimethyl-acridin-9-amine, cis -1,3-dimethyl-cyclohexane, 4-ethyl-1,2-dime thyl-benzene, 2-methyl-octane, trans-1,2- dimethyl-cyclohexane.

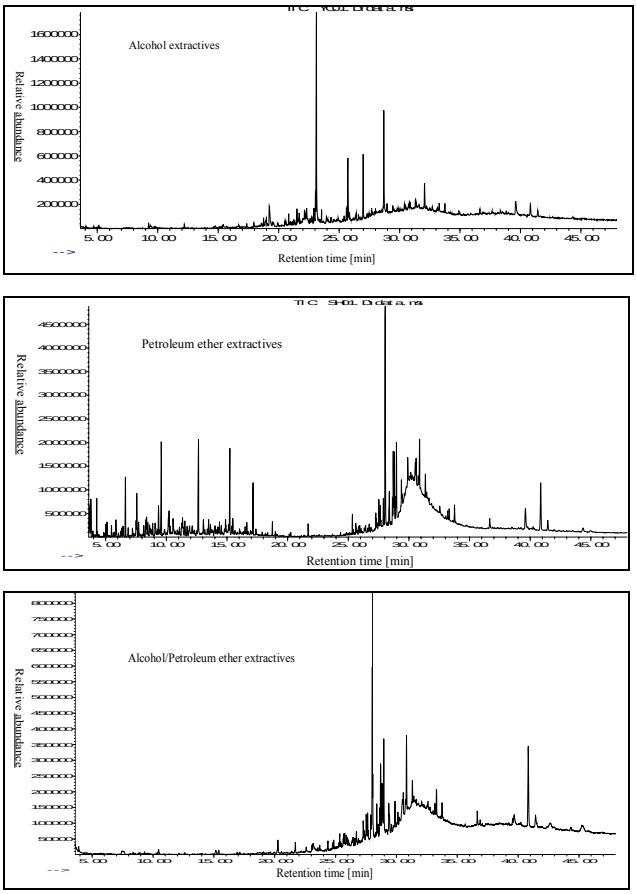

Fig. 1: Total ion chromatogram of woody extractives of *Cunninghamia lanceolata* wood by GC/MS

7 components were identified from 7 peaks of alcohol/petroleum ether extractives of *Cunninghamia lanceolata* wood. The main components were 1,3-dimethoxy -5-[(1e)-2-phenylethenyl]-benzene (32.88%), stigmasta-4,6,22-trien-3α-ol (17.83%), meth yl dehydroabiet-ate (12.42%), n,n-dimethy lindoaniline (11.81%), [1r-(1r*,3e,7e, 11r*, 12r*)]-4,8,12,15,15-pentamethyl–bicyclo [9. 3.1]pentadeca-3,7-dien-12-ol (10.76%), 15- hydroxy dehy droabietic acid methyl ester (8.75%), [1r-(1α, 4aβ,10aα)]-1,2,3,4,4a,9,10, 10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenan-threnecarboxal-dehyde (5.55 %).

DISCUSSION

Molecular distribution of extractives

The GC-MS analysis results showed the molecular distribution of three extractives from *Cunninghamia lanceolata* wood. The richest components of first-stage extractives (alcohol extractives) were 4-((1e)-3-hydroxy -1-propenyl)-2-methoxyphenol (36.80%), *α*-(2-phenylethenyl)-1-piperidine-acetonitrile (15.39%), etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ketones, acid/esters and other compounds occupied 9.51%, 51.10%, 17.31%, 6.67% and 15.39% of alcohol extractives, respectively. The richest components of second-stage extractives (petroleum ether extractives) were [1s-(1α,4aα,10aβ)]-1,2,3, 4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthre-necarboxylic acid (15.16%),1,3-dimethoxy-5-[(1e)-2-phenyleth-enyl]-benzene (6.99%) etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ ketones, acid/esters and other compounds occupied 17.69%, 21.31%, 34.40%, 23.87% and 2.73% of petroleum ether extractives, respectively. The richest components of third -stage extractives (alcohol/ petroleum ether extractives) were 1,3-dimethoxy-5-[(1E)-2-phenylethenyl]-Benzene (32.88%), Stigmasta-4,6,22-trien-3α-ol (17.83%), etc. Relative content of hydrocarbons, alcohols (phenol alcohols), aldehydes/ ketones, acid/esters and other compounds occupied 38.43%, 28.59%, 0.00%, 8.75% and 24.23% of alcohol/ petroleum ether extractives, respectively. The results suggested that the three extractives were suitable to extract hydrocarbons and alcohols (phenol alcohols).

The retention time of each stage extractives of *Cunninghamia lanceolata* wood showed a particular rule. Among the first-stage extractives, the molecules with the retention time of ≤10min, ≤20min, ≤30min, ≤40min and ≥40min were 0.00%, 5.85%, 81.64%, 9.53% and 2.98%, respectively. Among the second-stage extractives, the molecules with the retention time of ≤10min, ≤20min, ≤30min, ≤40min and ≥40min were 62.33%, 1.20%, 1.36%, 27.88% and 6.93%, respectively. Among the third-stage extractives, the molecules with the retention time of ≤10min, ≤20min, ≤30min, ≤40min and ≥40min were 0.00%, 0.00%, 73.42%, 8.75% and 17.83%, respectively. The results showed that the first-stage and third-stage extractives had a main retention time between 20-30min, and the second-stage extractives had a main retention time between 0-10min.

Biomedical resource utilization of extractives

There are many biomedical components in the woody extractives of *Cunninghamia lanceolata* biomass. Because of their officinal value, [1s-(1.alpha.,

4a.alpha.,10a.beta.)]-1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1- Phenanthre- necarboxylic acid is the active ingredient of skin care to heal facial peeling (Wang *et al.*, 2002). 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, anti-aging, anti-fatigue and anti-tumor etc. (Kim *et al.*, 2012). Tridecane is the main active ingredient of Anemone Altaica Fisch. BothStigmast-4-en-3-one, stigmasta-4,6,22-trien-3*β*-ol and γ-sitosterol are the natural active materials with the physiological actions of protecting the cardio-cerebral-vascular system, having effects on suppressing tumor and Regulating hormone levels (Zhang *et al.,* 2011). According to the relative content of biomedicine components of which, *Cunninghamia lanceolata* woody extractives will become the high-grade medicine resources in future.

CONCLUSION

The chemical compositions of woody extractives of *Cunninghamia lanceolata* were investigated for the first time with the method of three-stage extraction and the use of GC-MS. We have identified 13, 100 and 7 components on the peaks of three-stage extractives from *Cunninghamia lanceolata* wood respectively. The functional analytical results suggest that the three-stage extractives of *Cunninghamia lanceolata* wood which contain rich pharmaceutical components have a huge potential in biomedicine, especially including [1s-(1*α*,4a*α*,10a*β*)]- 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-1-phenanthrenecar boxylic acid, squalene, stigmast-4-en-3-one and *γ*-sitosterol, and so on.

ACKNOWLEDGEMENTS

This work 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

Ashour HM (2008). Antibacterial, antifungal and anticancer activities of volatile oils and extracts from stems, leaves and flowers of *Eucalyptus sideroxylon* and *Eucalyptus torquata*. *Cancer Biol. Ther.,* **7**: 399-403.

Bachir RG and Benali M (2008). Antibacterial activity of leaf essential oils of Eucalyptus globulus and Cunninghamia lanceolata. *Afr. J. Pharm. Pharmaco.,* **2**: 211-215.

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

Liang MX and Fan Y (2008). Research on four properties of Chinese herb: Current status, problems and countermeasures. *China J. Tradit. Chinese Med. & Pharm.,* **23**: 565-568.

Liu K (2007). Review and thought on modern research and development of traditional Chinese medicine. *Chinese J. Nat. Med.,* **5**: 87-91.

Peng WX, Zhu TL, Zheng ZZ, Fan ZC, Li KF and Li NC (2004). Present situation and developing tendency of extracts from wood. *China Forest. Sci. & Technol.,* **18**: 6-9.

Rahimi-Nasrabadi M, Ahmadi F and Batooli H (2012). Essential oil composition of eucalyptus procera dehnh. leaves from central Iran. *Nat. Prod. Res.,* **26**: 637-642.

Tu PF and Jiang Y (2007). New drug discovery and development of traditional Chinese medicines. *Chinese J. Nat. Med.,* **5**: 81-86.

Wang GH, Song ZQ and Wang LS (2002). Study on preparat ion and adsorpt ion charact erist ics of chemically modified barks. *Chem. and Ind. of Forest Prod.,* **22**: 12-16.

Yun BS, Lee IK, Kim JP, Chung SH, Shim GS and Yoo ID (2000). Lipid per oxidation inhibitory activity of some constituents isolated from the stem bark of *Eucalyptus globules*, *Arch. Pharm. Res.,* **23**: 147-150.

Zhang ZF, Zhang ML, Wu FJ, Peng WX (2011). Analysis on function components and biohealth function of *Pinus massoniana Lamb* biomass. *Appl. Mech. Mater.*, **55**: 157-160.