Study on antibacterial molecular drugs in *Eucalyptus granlla* wood extractives by GC-MS

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Abstract: *Eucalyptus granlla,* which was one of dominant plantations in south China, was deemed as the important wood bio resources. However, the small molecules of *Eucalyptus granlla* wood weren't effectively reused. Thus the molecules of wood extractives in *Eucalyptus granlla* were extracted and studied so as to further utilize the resources. The result suggested that the optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 5h, 7h and 4h, respectively. The wood extractives included hexanedioic acid, bis (2-ethylhexyl) ester, 3,3,7,11-tetramethyltricyclo[5.4.0.0(4,11)] undecan-1-ol, squalene, etc. and wood extractives of *Eucalyptus granlla* was suitable for extraction of 1,5-hexadien-3-yne and squalene.

Keywords: Antibacterial molecules, wood activities, *Eucalyptus granlla*, GC-MS.

INTRODUCTION

Traditional Chinese medicine, which was used as herbal medicines and body practices and various mind to treat or prevent health problems, originated in ancient China (Ko, 2004; Vickers *et al*, 2012). Traditional Chinese medicine had played a key role in maintenance of the health of Chinese people for over thousands of years. It was an essential part of the healthcare system in many Asian countries, and was deemed an alternative or complementary medical system in most Western countries (Chan *et al*, 2010). And thus it was widely applied in Asia, even in Europe and America, because of the characteristics of extensive application, significant curative effect, and little side effect (Lijun *et al*, 2007). More and more drugs came from the active constituents from Traditional Chinese medicine. However, the main bio resources of Traditional Chinese medicine were extracted from herbaceous plant and became so deficient to supply (Zhao *et al*, 2007; Birdee *et al*, 2009). Thus it was very important and urgent to innovate the new pathway of Traditional Chinese medicine source.

Eucalyptus was mostly native to Australia. Now it was widely cultivated in the tropical and temperate world, including the America, Africa, Europe, the Mediterranean Basin, the Middle East, China and Indian (Eschler *et al*, 2000; Grierson *et al*, 2000). Eucalypts were both lauded for their economic benefits to poor populations (Grierson *et al.*, 2000; Aggangan *et al.*, 1999). *Eucalyptus granlla* was firstly cross-bred from *Eucalyptus urophylla* and *Eucalyptus grandis* in China. At present, plantations of *Eucalyptus granlla* had been widely established in Yunnan, Guangxi, Guizhou province of south China. *Eucalyptus granlla* became one of the fastest growing tree species in south China, which could be used as industrial bio resources including pulping, wood-based panels, flooring and furniture. Therefore, the wood extractives of *Eucalyptus granlla* would be obtained and analyzed by the way of GC-MS (gas chromatograph/ mass spectrometer) in order to utilize the wood resources.

MATERIALS AND METHODS

Materials

Fresh *Eucalyptus granlla* wood were collected from the Nanning Forest Zone, Guangxi Province, China. The fresh wood was powdered and kept in vacuum. All of acetic ether, benzene, methanol, petroleum ether and ethanol were chromatographic grade for the subsequent experiments. Cotton thread and bag were both extracted in benzene/ethanol for 12h. $V_{ethanol}/V_{benzene}=2$.

Experiment methods

Single extraction

The above powder of wood were extraction in 350ml solvents for 1, 3, 4, 5, 6 and 7 hours, respectively. Ethanol/methanol ($V_{ethanol}/V_{methanol}=2$) extraction, petroleum ether/acetic ether ($V_{petroleum\ ether}/V_{acetic\ ether}=2$) extraction and benzene/ethanol ($V_{ethanol}/V_{benzene}=2$) extraction were done at the temperature of 75°C, 90°C and 95°C, respectively. Other treatment was the same as the document (Li *et al*, 2014; Peng *at al*, 2014).

Sequential extraction

The above powder of wood was the same as the document (Li *et al*, 2014; Peng *at al*, 2014). The extraction orders were petroleum ether/acetic ether → benzene/ethanol → ethanol/methanol (PABEEM), benzene/ethanol → ethanol/methanol → petroleum ether/acetic ether (BEEMPA), ethanol/methanol → petroleum ether/acetic ether → benzene/ethanol (EMPABE), respectively.

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GC/MS condition

Among the above extractives, wood BE extractives of single extraction (LD-002), wood EM extractives of single extraction (LD003), wood PA extractives of PABEEM extraction (LD164), wood EM extractives of EMPABE extraction (LD166), wood paBE extractives of PABEEM extraction (LD168), wood beEM extractives of BEEMPA extraction (LD169), wood empaBE extractives of EMPABE extraction (LD170), wood pabeEM extractives of PABEEM extraction (LD172) were analyzed, respectively. Each extractives was respectively analyzed by as the same as the documents (Li *et al*, 2014; Wanxi *et al*, 2013; 2014).

RESULTS

The LRs (full name: leaching rates) of single extractions were listed in table 1. During the sequential extraction, the ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were done for 5h, 7h, and 4h, respectively. The wood LD002, LD003, LD164, LD166, LD168, LD169, LD170, and LD172 extractives were obtained, respectively. The total ion chromatograms of eight extractives by GC/MS were shown in fig. 1.

Table 1: LRs of single extractions (%)

Extraction time [h]	ethanol/ methanol	petroleum ether/ acetic ether	benzene/ ethanol
1	4.17	4.53	7.61
3	8.91	8.37	9.71
4	6.00	6.97	10.44
5	11.68	5.66	9.33
6	11.23	8.54	2.52
7	9.06	8.56	9.67

DISCUSSION

Leaching rule of wood extractives of eucalyptus granlla

The LR trend of *Eucalyptus granlla* wood extractives in different solvents was described in table 1. It was found that during ethanol/methanol extraction, the LR of wood extractives fluctuated, and reached the maximum (11.68%) when extraction time was 5h. During petroleum ether/acetic ether extraction, the LR of wood extractives also fluctuated, and reached the maximum (8.56%) when extraction time was 7h. During benzene/alcohol extraction, the leaching rate of stem extractives fluctuated, and reached the maximum (10.44%) when extraction time was 4h. Moreover, the optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 5h, 7h, and 4h, respectively.

Molecular properties of eucalyptus granlla wood extractives

Relative content was counted by each peak area normalization. Based on the MS data, the NIST standard MS map by computer and the documents, the components and their contents were identified.

Based on GC/MS result, 21 components were identified from LD002 wood extractives of *Eucalyptus granlla*. The result showed that the components were heptane, 3-methyl- (17.5776%), heptane, 2-methyl- (13.6536%), phthalic acid, isobutyl octyl ester (11.9207%), hexane, 2,4-dimethyl- (8.6316%), toluene (7.4325%), dibutyl phthalate (5.2446%), (S)-(+)-5-methyl-1-heptanol (5.0871%, octane (4.8889%), pentane, 3-ethyl-2-methyl- (4.4235%), hexane, 2,3-dimethyl- (3.9001%), pentane, 2,2,4- trimethyl- (2.9692%), 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)]undecan-1-ol (2.4754%), cyclohexane, methylene- (1.8651%), cyclohexane, 1,2-dimethyl-, trans- (1.8032%), cyclotrisiloxane, hexamethyl- (1.6169%), cyclopentane, ethyl- (1.4149%), hexadecanoic acid, methyl ester (1.2767%), cyclopentane, 1,2,3-trimethyl-, (1.alpha.,2.alpha.,3.beta.)- (1.26%), cyclopentane, 1-ethyl-3-methyl-, trans- (0.9847%), 1,2- benzene - dicarboxylic acid, mono (2-ethylhexyl) ester (0.8318%), cyclopentane, 1-ethyl-3-methyl-, cis- (0.7418%).

The 13 components were identified from LD003 wood extractives. The result showed that the components were 4-cyclohexene-1,2-dicarboximide, n-butyl-, cis- (44.989%), testosterone (10.427%), n-hexadecanoic acid (8.728%), eicosane (6.099%), heptadecane (5.235%), 1h-indene, 2,3-dihydro- 1,1-dimethyl- (4.911%), cyclotrisiloxane, hexamethyl- (4.768%), octadecanoic acid (3.87%), 2,4,6- cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- (3.316%), octadecane (2.866%), 4,6-di-tert-butylresorcinol (2.244%), squalene (1.474%), phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester (1.073%).

The 3 components were identified from LD164 wood extractives. The result showed that the components were 1,5-hexadien-3-yne (86.114%), dibutyl phthalate (1.501%), 4-dehydroxy-N-(4,5- methylenedioxy-2-nitrobenzylidene)tyramine (12.384%). The 1 component was identified from LD166 wood extractives. The result showed that the one was 1,5-hexadien-3-yne.

The 1 component was identified from LD168 wood extractives. The result showed that the one was 1,5-hexadien-3-yne.

The 4 components were identified from LD169 wood extractives. The result showed that the components were 1,2- benzenedicarboxylic acid, butyl cyclohexyl ester (35.806%), 3-phenyl-2H-chromene (10.749%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (36.557%), 1,2-benzenediol, 3,5-bis (1,1-dimethylethyl)- (16.887%).

The 2 components were identified from LD170 wood extractives. The result showed that the components were 1,2- benzenedicarboxylic acid, butyl 2-methylpropyl ester

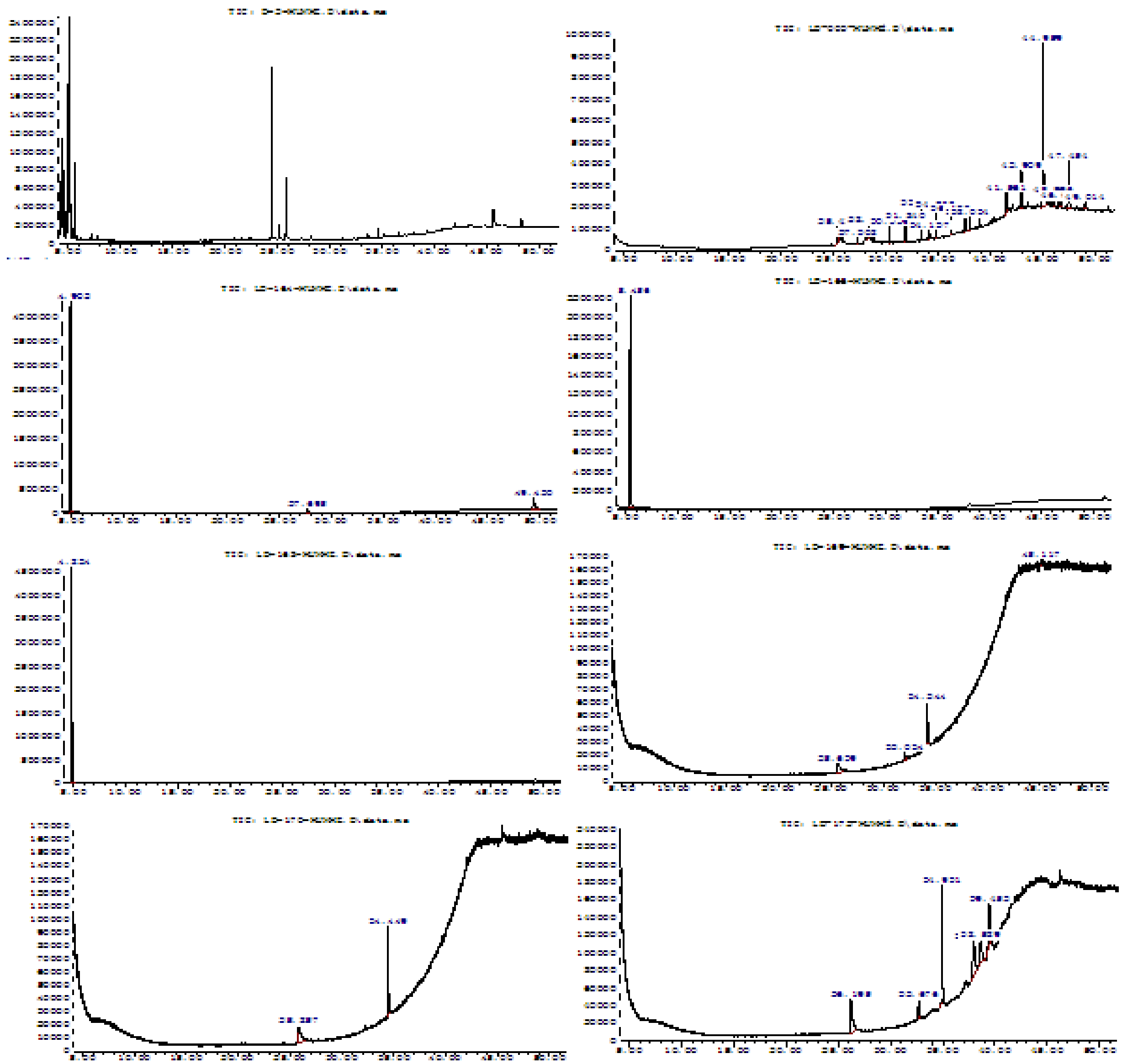

Fig.1: Total ion chromatograms of eight wood extractives by GC/MS

(48.661%), 1,2- benzene -dicarboxylic acid, mono (2-ethylhexyl) ester (51.339%). The 6 components were identified from LD172 wood extractives. The result showed that the components were 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (18.919%), hexanedioic acid, bis (2-ethylhexyl) ester (3.974%), 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (19.329%), 2,4-cyclohexadien-1-one, 3,5-bis(1,1- dimethylethyl)-4-hydroxy- (22.833%), 1,2-benzisothiazol-3-amine tbdms (14.349%), 5-methyl-2-phenylindolizine (20.596%).

Resource properties of wood extractives of eucalyptus granlla

There were many drugs components in the wood extractives of *Eucalyptus granlla*. For its officinal value, dibutyl phthalate was a pesticide to protect the environment homeostasis (Peng *et al*, 2014). Hexanedioic acid, bis(2-ethylhexyl) ester could be used in Canada in various products such as cosmetics, auto interior protectant, and lubricant (www. chemicals ubstances. gc.ca). 3,3,7,11-tetramethyltricyclo [5.4.0.0 (4,11)]undecan-1-ol was the primary alcohols and could lure Ips typographus Linnaeus (Ruijie, 2012). Phthalic acid derivatives were used to cure chronic cardiovascular and cerebrovascular diseases and had anti-tumor, anti-inflammatory, antibacterial functions (Hao *et al*, 2006). Squalene, which was deemed as the important substances in practical and clinical appliations with a huge potential in nutraceutical and pharmaceutical industries, could resist fatigue, protect liver, and strengthen the body's resistance, and improve human immunity (Kim *et al*, 2012). Thus the wood extractives of *Eucalyptus granlla* were rich in the drug and biomedical activities. According to the relative content, *Eucalyptus granlla* wood was fit to extract rare 1,5-hexadien-3-yne and squalene.

CONCLUSION

The leaching rule of wood extractives from *Eucalyptus granlla* was obvious. The optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 5h, 7h, and 4h, respectively. What's more, the extractives of *Eucalyptus granlla* wood rich in hexanedioic acid, bis(2-ethylhexyl) ester, 3,3,7,11- tetra- methyl-tricyclo [5.4.0.0 (4,11)]undecan-1-ol, squalene, phthalic acid derivatives, and other drug and biomedical activities According to the relative content, *Eucalyptus granlla* wood was very fit to extract rare 1,5-hexadien-3-yne and squalene.

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