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Analysis on molecular characteristics of wood extractives from *Eucalyptus urophydis* biomass

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ABSTRACT

Eucalyptus urophydis is the main dominant Eucalyptus plantation species in China. However, rich extractives in E. urophydis wood leads to serious processing defects and the extractives is considered as the key negative factors. In order to further utilize the wood resources, the molecular components and leaching characteristics of wood extractives of E. urophydis was investigated. The result showed that: (1) Among three extracting solvents including methanol, benzene/ethanol and acetic ether, the optimal extraction time to reach the largest leaching rate of E. urophydis wood extractives was 3h, 3h and 12h, respectively; during the different sequential extractions, benzene/alcohol-methanol-acetic ether displayed the best extracting effect whose leaching rate was up to 12.39%., meanwhile, each sequential extraction displayed gradually increased leaching rate, whose total leaching rate was larger than that of single extraction. (2) The methanol extractives of E. urophydis wood had a main retention time between 20-30 min and contained 47 components including γ-sitosterol (17.41%), (z)-9-octadecenoic acid methyl ester (16.33%),, etc; the acetic ether extractives had a main retention time below 20 min and contained 73 components including cedarwood oil (25.68%), ethyl propionate (22.34%), etc; the benzene/ethanol extractives had a main retention time between 10-20 min and contained 49 components including cyclohexylbenzene (37.56%), 3-methylcyclopentanebenzene (4.06%), etc. (3) The functional analytical results suggested that E. urophydis wood extractives had huge potential in biomedicine industry.

KEYWORDS

Eucalyptus urophydis;
Wood extractives;
Molecular characteristics;
GC/MS.

INTRODUCTION

Eucalyptus has been gradually introduced from Australia to all over the world since 1770[1]. Especially, *Eucalyptus* has been planted in large area in South China since 1980's. Now the area of *Eucalyptus* plantation reached more than 300 million ha. Among *Eucalyptus* plantations, *Eucalyptus urophydis* is one of the fastest growing tree species in China. *E.urophydis* has many uses which has made them economically impor-

tant trees in mountain area. And *E. urophydis* wood is mainly used in pulp, paper, fiberboard, plywood, and so on. The products are lower value added. What's worse, rich extractives in *E. urophydis* wood leads to serious processing defects and the extractives is considered as the key negative factors[2]. However, Recent researches showed that *E.urophydis* wood extractives could be developed into good medicinal resources[3]. Therefore, the molecular components and leaching characteristics of wood extractives of *E. urophydis* was investigated and analyzed in order to further utilize the wood resources.

MATERIALS AND METHODS

Materials

Three 5-year-old *Eucalyptus urophydis* were collected from Yangjiang Forest Farm, Guangdong province, P. R. China. The sample chips were processed from fresh material, and dried to absolute dry with rotary evaporator in 55°C and negative 0.01MPa. About 40 mesh powder was sieved out using AS200 Sieving Instrument (Made in America). Benzene, methanol, acetic ether and ethanol (chromatographic grade) were prepared for the subsequent experiments. Quantitative filter paper, cotton bag and cotton were all extracted in benzene/ethanol solution for 12 h. The benzene-ethanol solution was mixed according to $V_{ethanol}/V_{benzene}$ 2 double.

Methods

(1) Single extraction

Weighed 24 pieces of wood powders, each was about 2g (0.1mg accuracy) and finally parceled by using the quantitative filter paper and tied by using cotton thread, and signed. Extraction was carried out in 150ml solvent by the Foss method. Samples were removed at different times (3h, 6h, 9h, 12h), Solvents were methanol, acetic ether and benzene/ethanol solution ($V_{ethanol}/V_{benzene}$=2), respectively. Methanol extraction, acetic ether extraction and benzene/ethanol extraction were done under the condition of 75°C, 85°C and 95°C, respectively Parallel sample number was 2. After extraction, samples were baked to be absolute dried and weighed. Finally, the amount of dissolving extractives was calculated.

(2) Sequential extraction

Weighed 18 pieces of wood powders, each was 10g (1.0mg accuracy), and finally parceled by using the cotton bag and tied by using cotton thread, and signed. Extraction was carried out by large-caliber Soxhlet according to different orders combined by methanol, benzene/ethanol and acetic ether. Parallel sample number was 2. After extraction, samples were baked to be absolute dried under 105°C and weighed. Finally, the amount of dissolving extractives was calculated.

(3) GC/MS condition

Each 1.0 mg wood extractives of *E. urophydis* was analyzed by online linked GC/MS (gas chromatograph/mass spectrometer), respectively. The GC/MS analysis was carried out on a GC/MS-QP2010 (Shimadzu Corp., Japan), which was linked to a mass selective detector. An elastic fused silica capillary column DB-5 (30m×0.25mm) was used. The carrier gas was helium and the injection port temperature was 250°C. For methanol extractives and acetic ether extractives, thesplit injection ratio was 5:1, the GC column temperature was programmed as follows: 8°C /min from 50 to 200°C, 5°C/min from 200 to 300°C. For benzene-alcohol extractives, thesplit injection ratio was 3:1, the GC column temperature was programmed as follows: 20°C/min from 50 to 90°C, 2°C/min from 90 to 200°C, 12°C/min from 200 to 300°C. The program of MS was scanned over the 35-335AMU (m/z) respectively, with an ionizing voltage of 70eV and an ionization current of 150μA of electron ionization (EI). The flow velocity of helium was 1.0ml/min. The program of MS was scanned over the 35-335AMU (m/z) respectively, with an ionizing voltage of 70eV and an ionization current of 150μA of electron ionization (EI). The flow velocity of helium was 1.0ml/min.

RESULTS AND DISCUSSION

Leaching characteristics of *E. urophydis* wood extractives

The leaching rate trend of *E. urophydis* wood extractives in different solvents was described in TABLE

1. It was observed that during methanol extraction, the leaching rate of wood extractives first decreased and then increased, and reached the maximum (7.48%) when extraction time was 3h. During acetic ether extraction, the leaching rate of wood extractives decreased, and reached the maximum (6.58%) when extraction time was 3h. During benzene/alcohol extraction, the leaching rate of wood extractives first increased, then decreased and increased, and reached the maximum (7.78%) when extraction time was 12h. The observed result was not fully in accordance with the theoretical time-adding increase of leaching rate during Soxhlet extraction. It was that during solvent extraction, extractives could leach out from wood to increase leaching rate, but solvent could react with wood to decrease leaching rate. Andalcohol, methanol and ethanol could all react withwood to form ether bond or ester bond, which wouldddecrease the leaching rate.So the optimal extraction time of methanol extraction, acetic ether extraction, and benzene/alcohol extraction were 3h, 3h, and 12h, respectively.

TABLE 1 : Leaching rate of each single extraction[%]

Extraction time[h]	Methanol extraction	Acetic ether extraction	Benzene/alcohol extraction
3	7.48	6.58	6.25
6	6.67	6.22	6.20
9	7.40	6.05	6.52
12	7.45	5.68	6.66

Leaching mode of sequential extraction on *E. urophydis* wood

Based on the respective optimal extraction time, different sequential extractions on *E. urophydis* wood were carried out by combining methanol, benzene/alcohol and acetic ether, which showed different leaching rates (TABLE 2).

TABLE 2 : Theleaching rates of each sequential extraction[%]

Solvent of Sequentialextraction	Stage of extraction		
	1st	2nd	3rd
Methanol- Acetic ether- Benzene/alcohol	2.54	3.58	4.50
Benzene/alcohol- Methanol- Acetic ether	2.64	4.49	5.26
Acetic ether-Benzene/alcohol-Methanol	2.38	3.25	4.21

The statistical results showed that the leaching rates of *E. urophydis* wood extractives by methanol-acetic ether-benzene/alcohol sequential extraction was 10.62%, 12.39% by benzene/alcohol-methanol-acetic ether, and 9.84% by acetic ether-methanol-benzene/alcohol. Comparing with TABLE 1, it was observed that the leaching rate of each single extraction was less than that of TABLE 2, suggesting that the solvent effect also existed in the leaching periods of wood extractives. TABLE 2 also showed that both the three sequential extractions displayed gradually increased leaching rates of wood extractives, which were larger than that of any single extraction. Wood extractives existed mainly in cell gap, cell cavity, grain pore, etc, this would jam permeability channels of inner extractives. During the sequential extraction, wood extractives would be easily leached out by widening permeability channels, and finally to increase leaching rate. So benzene/alcohol-methanol-acetic ether extraction was the optimum extraction mode for the leaching rate was 12.39%.

Components of *E. urophydis* wood extractives

During methanol- acetic ether- benzene/alcohol sequential extraction, three solvent extractives (methanol extractives, acetic ether extractives, benzene/alcohol extractives) were obtained respectively. The total ion chromatograms of three solvent extractives by GC/MS were shown in Figure 1, Figure 2 and Figure 3, respectively. Relative content of each component was 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 were identified.

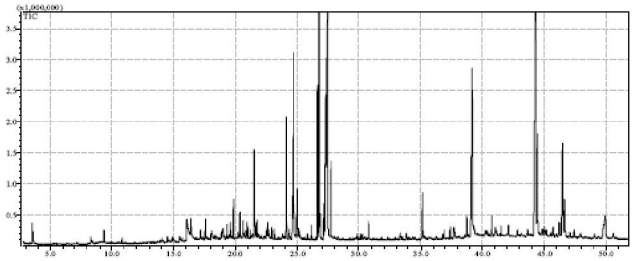

Figure 1 : Total ion chromatogram of methanol extractives from *E. urophydis* wood by GC/MS

According to GC/MS result, 47 components were identified from methanol extractives of *E. urophydis* wood. The result showed that the main components were γ-sitosterol (17.41%), (z)-9-octadecenoic acid methyl ester (16.33%), 9,12-trans octadecadienoic methyl (7.74%), trans-9-octadecenoic acid (5.9%),

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palmitic acid (5.21%), sitosterone (3.6%), methyl linoleate (2.55%), methyl hexadecanoate (1.73%), 1,2,3-phenol (1.1%), dibutyl phthalate (0.71%), methyl octadedanoate (0.55%), methyl tridecanoate (0.54%), oleamide (0.42%), 10-methyl oleate (0.41%), azabenzene (0.36%), 4-hydroxy-3,5-dimethoxy acetylbenzene (0.36%), and so on.

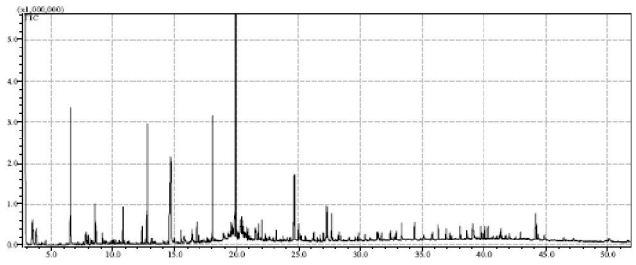

Figure 2 : Total ion chromatogram of acetic ether extractives from *E. urophydis* wood by GC/MS

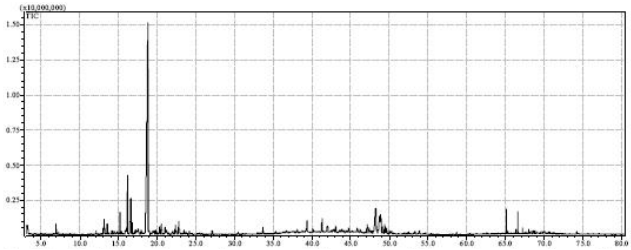

Figure 3 : Total ion chromatogram of benzene/ethanol extractives from *E. urophydis* wood by GC/MS

73 components were identified from acetic ether extractives of *E. urophydis* wood according to GC/MS result. The result showed that the main components were cedarwood oil (25.68%), ethyl propionate (22.34%), isosorbide (5.69%), 2-butoxyethanol (3.04%), 2,6-di-tert-butyl-4-methylphenol (2.28%), (-)-alpha-terpineol (2.25%), palmitic acid (2.11%), 3-methyl-2-pentanone (1.21%), γ- sitosterol (1.12%), 9,12-methyl linoleate (1.03%), trans-9-octadecenoic acid (0.95%), isobutyl acetate (0.94%), 1,2,4-trimethylbenzene (0.94%), stearic acid (0.71%), n-hendecane (0.67%), 4-hydroxy-3,5-dimethoxybenzaldehyde (0.65%), α-cadinol cedrelanol (0.56%), 3,4,5-trimethoxy- phenol (0.47%), α- cedrene (0.44%), and so on.

49 components were identified from benzene/ethanol extractives of *E. urophydis* wood according to GC/MS result. The result showed that the main components were cyclohexylbenzene (37.56%), 3-methylcyclopentane –benzene (4.06%), acetal (1.47%), diisobutyl phthalate (0.95%), bis (2-ethylhexyl) adipate (0.8%), 1-methylheptyl-benzene (0.73%), diisooctyl phthalate (0.61%), 1,2,3- trimethyl-1h-indene (0.58%), cedarwood oil (0.54%), phenol (0.51%), 2,5-diphenylhexane (0.4%), 1-methylphenyl- pentane (0.39%), 4-phenyloctane (0.34%), and so on.

Chemical distribution characteristic of *E. urophydis* wood extractives

Therichest components of methanol extractives were γ-sitosterol (17.41%), (z)-9-octadecenoic acid methyl ester (16.33%), etc; and there were 16 kinds of water-soluble compounds (35.84% of the total peak area), 12 kinds of acid compounds (18.17%), 13 kinds of resin compounds (44.24%), 3 kinds of hydrocarbons (0.80%), 3 other substances (0.95%).

Therichest components of acetic ether extractives were cedarwood oil (25.68%), ethyl propionate (22.34%), etc; and there were 25 kinds of water-soluble compounds (46.32%), 7 kinds of acid compounds (4.4%), 10kinds of resin compounds (26.02%), 28 kinds of hydrocarbons (5.01%), 3 other substances (0.76%).

Therichest components of benzene/ethanol extractives were cyclohexylbenzene (37.56%), 3-methylcyclopentane- benzene (4.06%),, etc; and there were 23 kinds of water-soluble compounds (46.17%), 2 kinds of acid compounds (0.28%), 5 kinds of resin compounds (2.67%), 16 kinds of hydrocarbons (2.1%), 3other substances (0.84%).

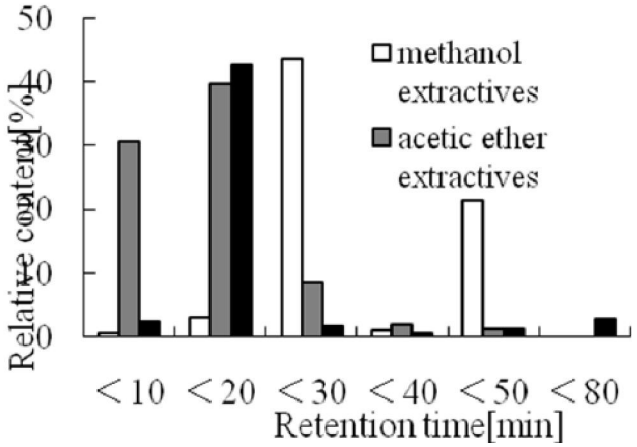

Figure 4 : Chemical distribution characteristic of *E. urophydis* wood extractives

The retention time of each solvent extractives of *E. urophydis* wood showed a particular rule. The methanol extractives of *E. urophydis* wood had a main re-

tention time between 20-30 min, which accounts for 43.65% of the total relative content; however, the relative content of below 10 min retention time was only 0.59%. The acetic ether extractives had a main retention time below 20min, the relative content of below 20 min retention time was 70.62%, but that of beyond 30min was only 3.27%. The benzene/ethanol extractives had a main retention time between 10-20 min, which accounted for 42.77% of the total relative content, but the other retention time displayed a wide distribution of components and their relative contents were very few (Figure 4).

Resource utilization of *E. urophydis* wood extractives

There were many biomedicine components in the wood extractives of *E. urophydis* biomass. Because of its officinal value, vanillin was used as a flavoring agent in foods, beverages and pharmaceuticals. α-Cadinol, which acted as anti-fungal and as hepatoprotective, was proposed as a possible remedy for drug-resistant tuberculosis[4-6]. Vitamin E brought many benefits to human body and promises amazing cosmetic results, and its series products were widely used in food, medicine, cosmetics and feed industry[7]. Cedarwood oil had a calming and soothing effect on the mind, and astringent effect on acne, oily skin, hair and dandruff[8]. α- cedrene and β- cedrene were used for the raw materials of advanced odorants[9]. Squalene could resist fatigue and strengthen the body's resistance, protect liver, and improve human immunity. squalene was used in nutraceutical and pharmaceutical industries[10]. And squalene was used in cosmetics and vaccines. Especially, γ-sitosterol could reduce serum cholesterol and had effect on atherosclerotic lesion development[11]. According to the relative content of biomedicine components, the methanol extractives of *E. urophydis* wood was suitable for the extraction of γ-sitosterol, and the acetic ester extractives was suitable for the extraction of cedarwood oil.

What's more, the others from wood extractives of *E. urophydis* biomass also contain many components of bioenergy, solvent, and so on. For example, Cyclohexylbenzene was a fine chemical raw materials and pharmaceutical intermediates[12]. In addition, the extractives were used for add bioenergy. Generally, the extractives of *E. urophydis* biomass are not only fine bioenergy but also high value-added chemical raw materials.

CONCLUSIONS

During single extraction, among three extracting solvents including methanol, benzene/ethanol and acetic ether, the optimal extracting time to reach the largest leaching rate of *E. urophydis* wood extractives was 3h, 3h and 12h, respectively. During the sequential extraction, each sequential extraction displayed gradually increased leaching rate, whose total leaching volume was larger than that of single extraction; benzene/alcohol-methanol-acetic ether extraction displayed the best extracting effect whose leaching rate was up to 12.39%.

The methanol extractives of *E. urophydis* wood had a main retention time between 20-30 min and contained 47 components, especially including γ-sitosterol (17.41%), (z)-9-octadecenoic acid methyl ester (16.33%) and 9,12-trans octadecadienoic methyl (7.74%). The acetic ether extractives had a main retention time below 20 min and contained 73 components, especially including cedarwood oil (25.68%), ethyl propionate (22.34%) and isosorbide (5.69%). The benzene/ethanol extractives had a main retention time between 10-20 min and contained 49 components, especially including cyclohexylbenzene (37.56%), 3-methylcyclopentanebenzene (4.06%) and acetal (1.47%).

The functional analytical result suggested that *E. urophydis* wood extractives contained rich pharmaceutical components which had huge potential in biological medicine industry, especially including vitamin-e, vanillin, cedarwood oil, γ-sitosterol, and so on.

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