

Analysis on active molecules in *Populus nigra* wood extractives by GC-MS

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Abstract: *Populus nigra* has been introduced and largely planted in China, and the waste wood was still abandoned. Therefore, the wood extractives of *Populus nigra* were studied to further utilize the bio-resources. The result shown that the optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 1h, 7h and 3h, respectively. Among sequential extractions, EPB extraction was the optimum extraction mode for the LR was 17.32%. The wood extractives included hexanedioic acid, bis(2-ethylhexyl) ester, phthalic acid derivatives, squalene, 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)]undecan-1-ol, other rare drug and biomedical activities. The wood extractives of *Populus nigra* was fit to extract rare dibutyl phthalate and squalene.

Keywords: Antiinflammatory molecules, woody bioactivities, *Populus nigra*, GC-MS.

INTRODUCTION

Populus nigra was native to Europe, Asia and Africa, and had a wide distribution area (Zsuffa, 1974; François *et al*, 1998). It was a species of cottonwood poplar and a medium-sized to large deciduous tree which could reach 30 m tall and have a trunk up to 1.5 m diameter. The leaves were 5~8 cm long and 6~8 cm broad, diamond-shaped to triangular, green on both surfaces. The species grown in low-lying areas of moist ground, with flowers in catkins and pollination by wind. It was widely planted in Turkey for domestic use for industry (Tunçtaner, 1998; Fikret *et al*, 2004). Since the 1960s, it was widely selected and became apparent that the Poplar's very invasive roots destroyed land drainage systems. And *Populus nigra* had been introduced and largely planted in China at the beginning of the 80th century (Özel *et al*, 2010; Fikret *et al*, 2004; Hu *et al*, 2001). *Populus* acreage had be more than 20 Mha in China, were also the most important wood raw material forest.

Populus nigra was of economic interest as a pure species. *Populus nigra* was a potential feedstock for cellulosic ethanol production (Guerra *et al*, 2013). Asghar *et al* (2011) found the surface temperature rose progressively from the initial value to the dry-bulb temperature but the core temperature remained at an almost constant value as the wet-bulb temperature even at the end of drying. Maedeh *et al* (2013) discovered that steaming at temperature of 120°C for 1 h as the best treatment was recommended to modify the water vapor diffusion coefficient. Bilal *et al* (2013) resulted that The maximum and minimum dimensions of fusiform initials and ray initials were in winter and summer seasons respectively while the horizontal and vertical diameters of ray initials

were maximum and minimum in summer and winter seasons respectively (Fossati *et al*, 2003). Overall, the researches on *Populus nigra* timber processing focused on solid wood. However, the small molecules of *Populus nigra* wood were still a lack of research. Therefore, the molecular characteristics of wood extractives were investigated and analyzed by some optimized extracting techniques so as to further utilize *Populus nigra* wood resources.

MATERIALS AND METHODS

Materials

Fresh *Populus nigra* wood were collected from the Dongtinghu Forest Zone, Hunnan Province, China. The fresh wood was powdered and kept in vacuum. Acetic ether, methanol, benzene, petroleum ether and ethanol, which were prepared for the subsequent experiments, were all chromatographic grade. Cotton bag and thread were extracted in benzene/ethanol solution for 12 h. The ratio ($V_{\text{ethanol}}/V_{\text{benzene}}$) was 2 double.

Experiment methods

Single extraction: Weighed 54 powder of wood, each was about 20g (0.1mg accuracy) and then parceled into the cotton bag and tied by cotton thread, and signed. Extraction was carried out in 350ml solvents by the Foss method for 1, 3, 4, 5, 6, 7 hours. Solvents were ethanol/methanol solution ($V_{\text{ethanol}}/V_{\text{methanol}} = 2$), petroleum ether/acetic ether solution ($V_{\text{petroleum ether}}/V_{\text{acetic ether}} = 2$), and benzene/ethanol solution ($V_{\text{ethanol}}/V_{\text{benzene}} = 2$), respectively. Ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/ethanol extraction were done at the temperature of 75°C, 90°C and 95°C, respectively. After extraction, one was took out,

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dried in 105°C to oven dry, and weighed.

Sequential extraction: Weighed 27 pieces of wood, each was 20g (1.0mg accuracy), and finally parceled into cotton bag and tied by cotton thread, and signed. Three-steps extraction was carried out by large-caliber Soxhletor according to the different solvent orders of benzene/ethanol extraction → ethanol/methanol extraction → petroleum ether/acetic ether extraction (BEP), ethanol/methanol extraction → petroleum ether/acetic ether extraction → benzene/ethanol extraction (EPB), petroleum ether/acetic ether extraction → benzene/ethanol extraction → ethanol/methanol extraction (PBE), respectively. After every step extraction, one was took out, dried in 105°C to oven dry, and weighed. The wood extractives was obtained by evaporation in 60~70°C.

GC/MS condition: The PApbe (LD-181), BEbep (LD-182), BEpbe (LD-185), BEepb (LD-187), EMPbe (LD-189) wood extractives were analyzed, respectively. Each 0.5 mg extractives was analyzed by online linked GC/MS (gas chromatograph/ mass spectrometer), respectively. The GC/MS analysis was done as the same as the documents (Wanxi *et al*, 2013; 2014).

RESULTS

LR was shorter form leaching rate. The LRs of single extractions were listed in Table 1, and The LRs of sequential extractions were listed in Table 2. The PApbe, BEbep, BEpbe, BEepb, EMPbe wood extractives were obtained, respectively. The total ion chromatograms of four extractives by GC/MS were shown in fig. 1.

Analyses

Leaching rule of wood extractives of *Populus nigra*

The LR trend of *Populus nigra* wood extractives in different solvents was described in Table 1. It was observed that during ethanol/methanol extraction, the LR of wood extractives deduced, and reached the maximum (4.13%) when extraction time was 1h. During petroleum ether/acetic ether extraction, the LR of wood extractives also fluctuated, and reached the maximum (3.01%) when extraction time was 7h. During benzene/alcohol extraction, the leaching rate of stem extractives fluctuated, and reached the maximum (4.79%) when extraction time was 3h. And the optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 1h, 7h, and 3h, respectively.

During the sequential extraction, the ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were done for 1h, 7h, and 3h, respectively. The statistical results showed that the LRs of *Populus nigra* wood extractives by BEP extraction was

16.31%, 17.32% by EPB extraction, and 13.30% by PBE extraction. Comparing with Table 1 and Table 2, it was showed that the LRs of single extractions was less than that of sequential extractions. Table 2 also shown that the LRs of ethanol/methanol extraction were higher than others in the sequential extractions, and their LRs were all larger than that of single extractions. During the sequential extractions, EPB extraction was the optimum extraction mode for the leaching rate was 17.32%.

Molecular properties of *Populus nigra* wood extractives

Relative content of each component was counted by area normalization. Analyzing the MS data, the NIST standard MS map by computer, open-published papers and books, then components and their contents were identified.

According to GC/MS result, 6 components were identified from wood PApbe extractives of *Populus nigra*. The result showed that the components were 2,4-hexadiyne (64.404%), hexadecane, 2,6,10,14-tetramethyl- (1.54%), dibutyl phthalate (11.486%), 2-piperidinone, N-[4-bromo-n-butyl]- (2.555%), hexadecane, 2,6,10,14-tetramethyl- (2.129%), 4-dehydroxy- N-(4,5-methylenedioxy- 2-nitrobenzylidene) tyramine (17.885%).

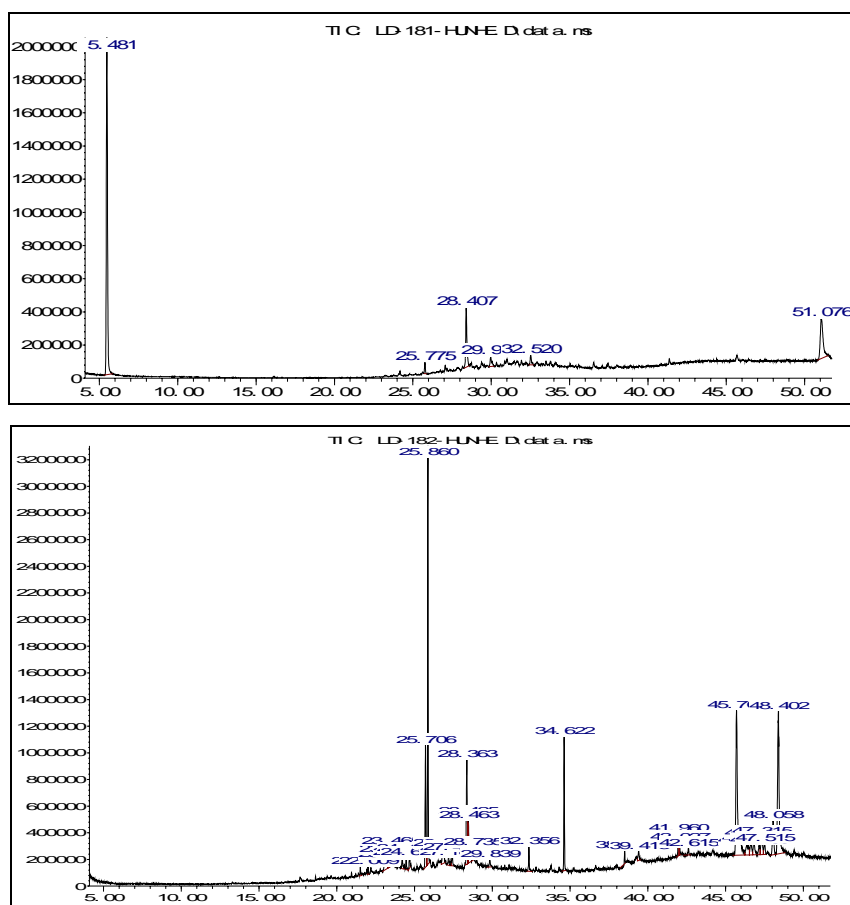
The 33 components were identified from wood BEbep extractives. The result showed that the components were 2-naphthalenemethanol,1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha., 4a.alpha., 8a.beta.)]- (0.444%), pentadecane, 2,6,10,14-tetramethyl- (0.356%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (1.945%), octadecane (0.837%), hexadecane, 2,6,10,14-tetramethyl- (1.22%), pentadecanoic acid (0.758%), 7-methylthieno[3,2-b]pyridine (0.711%), cyclodecene (0.639%), n-hexadecanoic acid (5.97%), dibutyl phthalate (16.881%), 1,11-tridecadiene (1.569%), 2-methyl-Z-4-tetradecene (0.537%), cyclic octaatomic sulfur (0.843%), 9,12-octadecadienoic acid (Z,Z)- (7.799%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (2.731%), 9,12-octadecadien-1-ol, (Z,Z)- (0.784%), hexadecane, 2,6,10,14-tetramethyl- (0.626%), hexanedioic acid, bis(2-ethylhexyl) ester (1.325%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (5.626%), squalene (0.214%), 1-heptacosanol (0.77%), N-methyl-1-adamantaneacetamide (1.181%), 2-methyl-7-phenylindole (0.755%), 6H-purin-6-one, 1,7-dihydro-2-(methylamino)- (0.385%), 1,3,3-trimethyl-2-hydroxymethyl- 3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene (17.655%), cyclotrisiloxane, hexamethyl- (1.907%), 1,2-benzenediol, 3,5-bis(1,1-dimethylethyl)- (1.393%), 1,2-benzisothiazol-3-amine tbdms (1.609%), 5-methyl-2-phenylindolizine (0.866%), benzo[h]quinoline, 2,4-dimethyl- (2.079%), benz[b]-1,4-oxazepine-4(5H)-thione, 2,3-dihydro-2,8-dimethyl- (0.999%), 1,2,5-oxadiazol-3-amine, 4-(4-

Table 1: LRs of each single extraction [%]

Extraction time [h]	Ethanol/methanol	Petroleum ether/acetic ether	Benzene/ethanol
1	4.13	2.43	2.91
3	3.13	1.94	4.79
4	2.99	1.88	4.06
5	2.91	2.57	3.78
6	2.27	2.03	3.61
7	2.55	3.01	4.06

Table 2: LRs of sequential extraction

Extraction	PBE			BEP			EPB		
	7	3	1	3	1	7	1	7	3
Time [h]	7	3	1	3	1	7	1	7	3
Step	1 st	2 nd	3 rd	1 st	2 nd	3 rd	1 st	2 nd	3 rd
LR [%]	4.03	4.29	4.78	5.39	5.52	5.40	5.75	5.71	5.86
Total LR [%]	13.10			16.31			17.32		

**Fig. 1:** Total ion chromatograms of five wood extractives by GC/MS (continued)

methoxyphenoxy)- (3.167%), testosterone (15.418%).

The 4 components were identified from wood BEpbe extractives. The result showed that the components were phthalic acid, isobutyl non-5-yn-3-yl ester (1.718%), dibutyl phthalate (52.929%), hexanedioic acid, bis(2-ethylhexyl) ester (5.14%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (40.213%).

The 1 component was identified from wood BEpbe extractives. The result showed that the component was phthalic acid, isopropyl propyl ester.

The 3 components were identified from wood EMpbe extractives. The result showed that the components were 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (5.305%), anthracene, 9,10-dihydro-9,9,10-trimethyl-

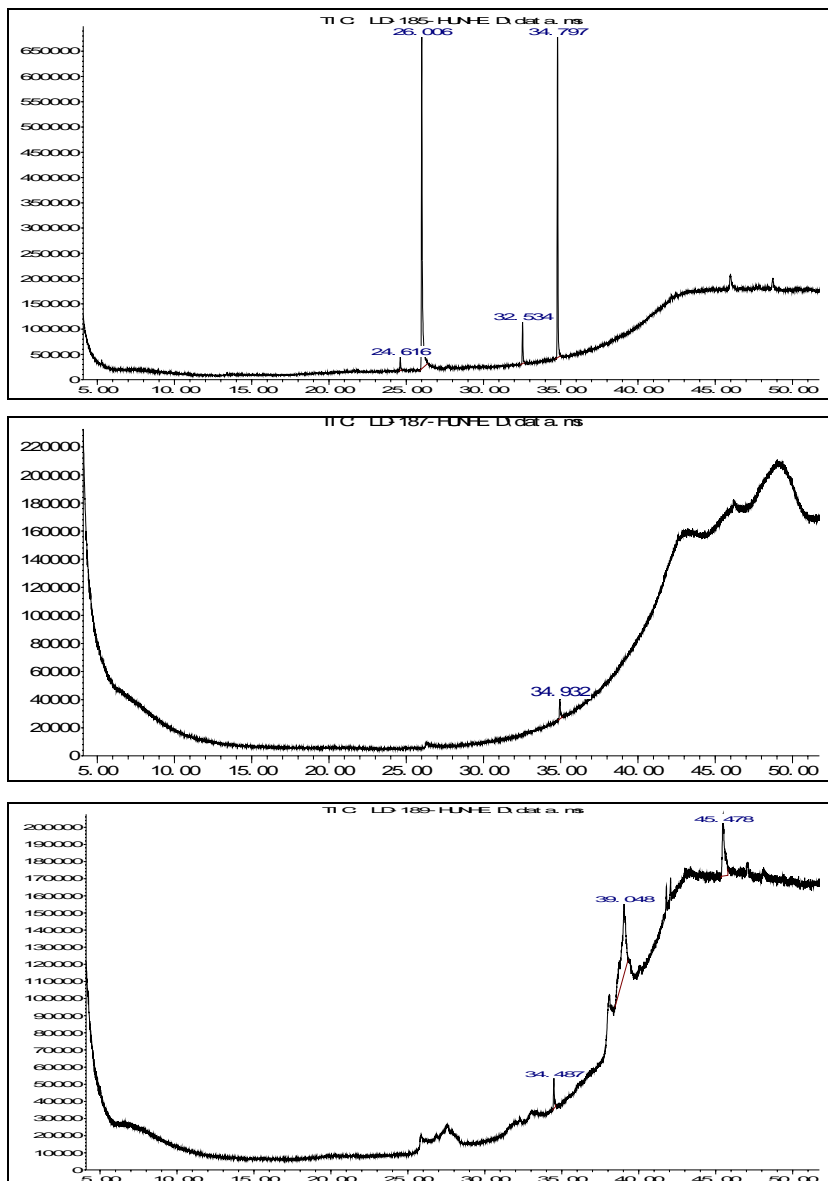


Fig. 1(continued): Total ion chromatograms of five wood extractives by GC/MS.

(64.498%), 1,2-benzenediol, 3,5-bis(1,1-dimethylethyl)- (30.196%).

Resource properties of wood extractives of *Populus nigra*

There were many anti-inflammatory components in the wood extractives of *Populus nigra*. Because of its inner officinal value, dibutyl phthalate was a pesticide to protect the environment homeostasis (Wanxi *et al*, 2014). Hexanedioic acid, bis(2-ethylhexyl) ester was used in cosmetics, auto interior protectant products and so on (www.chemicalsubstances.gc.ca).

3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)] undecan-1-ol was the one alcohol of volatile compositions in needle and branch of *Picea crassifolia* which could lure *Ips typographus* Linnaeus (Ruijie *et al*, 2011). Phthalic acid

derivatives were the main active constituents of *Elaeagnaceae* plant which had anti-tumor, anti-inflammatory, antibacterial functions (Hao *et al*, 2006). The n-hexadecanoic acid might help in designing of specific inhibitors of phospholipase A(2) as anti-inflammatory agents (Aparna *et al*, 2012). (Z,Z)-9,12-octadecadienoic acid, (Z,Z,Z)- 9,12,15-octadecatrienoic acid, (Z,Z)-9,12-octadecadien-1-ol had been identified as the main medical components of dried worms, and has diuretic, swelling and detoxification properties (Guo *et al*, 2006; Wanxi *et al*, 2013; Vimalavady *et al*, 2013). Squalene was considered as important substances in practical and clinical uses with a huge potential in nutraceutical and pharmaceutical industries, and could protect liver, resist fatigue and strengthen the body's resistance, and improve human

immunity (Kim *et al*, 2012). According to the relative content, *Populus nigra* wood was fit to extract the expensive dibutyl phthalate and squalene. And there were many drug and biomedical activities in the *Populus nigra* wood extractives.

CONCLUSION

The leaching rule of extractives from *Populus nigra* wood was obvious. The optimal extraction time of petroleum ether/ acetic ether extraction, ethanol/methanol extraction, and benzene/alcohol extraction were 7h, 1h, and 3h, respectively. The LR of single extractions were less than those of sequential extractions among which EPB extraction was the optimum extraction mode for the LR was 17.32%. What's more, *Populus nigra* wood extractives, which was drug and medical activities, including hexanedioic acid, bis(2-ethylhexyl) ester, phthalic acid derivatives, squalene, 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)] undecan-1-ol, and so on. According to the relative content, *Populus nigra* wood was fit to extract the expensive dibutyl phthalate and squalene. And the wood extractives of *Populus nigra* were rich in rare drug and biomedical activities.

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