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## ORIGINAL ARTICLE

# Characteristics of antibacterial molecular activities in *poplar* wood extractives



Wanxi Peng<sup>a,b,\*</sup>, Dongli Li<sup>a</sup>, Minglong Zhang<sup>a</sup>, Shengbo Ge<sup>a</sup>, Bo Mo<sup>a</sup>,  
Shasha Li<sup>a</sup>, Makoto Ohkoshi<sup>b,\*</sup>

<sup>a</sup> School of Materials Science and Engineering, Central South University of Forestry and Technology, Changsha, China

<sup>b</sup> Laboratory of Biomaterials Science, Kyoto Prefectural University, Kyoto, Japan

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## KEYWORDS

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**Abstract** As one of the dominant plantations in north and central China, poplar was considered as the uppermost wood raw materials, however, the chemical constituents of poplar wood weren't effectively used by high added value. Therefore, the molecules of wood extractives in *Populus lasiocarpa* and *Populus tomentosa* were extracted and studied to further utilize the bio-resources. The results showed that the LD-010, LD-021, LD-150, LD-174 wood extractives were identified as having 3, 24, 3, 27 components, respectively. *P. lasiocarpa* wood was fit to extract 2,4-hexadiyne, 1,3,3-trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene, and *P. tomentosa* wood was fit to extract 1,5-hexadien-3-yne, (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene. So the extractives of *poplar* wood contained rich and rare drug and biomedical activities.

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## 1. Introduction

Poplar tree, which was known as aspen and cottonwood, was a native of the Northern Hemisphere. It could grow from 15 to 50 m tall and had trunks up to 2.5 m (Heping et al., 1999). The leaves were oval to heart-shaped, and green in the spring and summer; the flowers bloomed from March to May; the fruits are small, thick-skinned capsules which contained dozens of tiny seeds covered with silky white hairs; the bark was smooth, rough, uneven, soft, wrinkled, cracked and range in color from

white to dark gray (Rennenberg et al., 2010; Ashraf et al., 2013a). According to the bark color, poplar was mainly divided into four categories. White poplar, which had a high drought tolerance and diamond-shaped marks in the bark, could easily grow in different types of soil and climate conditions; lombardy poplar was long and column-like while its branches extend upward rather than outward from the trunk; eastern poplar was known as the Eastern Cottonwood, big and feature serrated edges; balsam poplar was known as black cottonwood and thrived in swampy soil (Divya, 2011; Ashraf et al., 2013b). Poplar trees were extremely desirable for homeowners looking to infuse their yards with shade and beauty. However, poplar trees are not recommended to plant close to houses or other buildings because it's roots may damage buildings.

\* Corresponding authors at: School of Materials Science and Engineering, Central South University of Forestry and Technology, Changsha, China (W. Peng).

E-mail addresses: [pengwanxi@163.com](mailto:pengwanxi@163.com) (W. Peng), [mohkoshi@kpu.ac.jp](mailto:mohkoshi@kpu.ac.jp) (M. Ohkoshi).

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Poplar wood had a variety of different ways to contain snowboards, boats, boxes, pallets, paper, matches, electric guitars, harps, and violas (Daniel et al., 2012; Surhio et al., 2014). Poplar grown for 2–5 years till harvest is usually burned, as biofuel for energy instead of non-renewable energy sources such as coal reaching as high as 12 oven dry tones every year per tree (Poulomi et al., 2010; Batool et al., 2015). What's more, poplar wood could be used for cheap plywood, matches, pallets and camembert cheese boxes (Daniel et al., 2012). *Populus lasiocarpa* and *Populus tomentosa* were the dominant species of plantation in north and central China and more than 2 Mha were planted (Yoshinori and Eckhard, 1976; Ningxia et al., 2012; Naureen et al., 2014). However, the researches on the active ingredients of poplar wood were scant. Therefore, the molecular characteristics of wood extractives were investigated and analyzed by the optimized extracting techniques so as to further utilize *P. lasiocarpa* and *P. tomentosa* wood resource.

## 2. Materials and methods

### 2.1. Materials

*P. lasiocarpa* wood was collected from the Zhumadian Forest Zone, Henan Province, China. *P. tomentosa* wood was collected from the Linyi Forest Zone, Shandong Province, China. The fresh wood was shaved, powdered and kept in vacuum. Acetic ether, methanol, benzene, petroleum ether and ethanol were of chromatographic grade and prepared for the experiments. Cotton thread and cotton bag were both extracted by benzene/ethanol solution for 12 h. The ratio  $V_{\text{ethanol}}/V_{\text{benzene}}$  was 2 double.

### 2.2. Experiment methods

Weighed 54 powder of wood, which were about 20 g each (0.1 mg accuracy) were then parceled by the cotton bag and tied by cotton thread, and signed. Extraction was carried out in 350 ml solvents by the Foss method for 7 h. Solvents were ethanol/methanol ( $V_{\text{ethanol}}/V_{\text{methanol}} = 2$ ), petroleum ether/acetic ether ( $V_{\text{petroleum ether}}/V_{\text{acetic ether}} = 2$ ), and benzene/ethanol solution, 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 piece was taken out, dried in 105 °C to oven dry, and weighed. The extractives were obtained by evaporation in 60–70 °C.

Weighed 2 pieces of wood, which were 20 g each (1.0 mg accuracy), were finally parceled by cotton bag and tied by cotton thread, and signed. Ethanol/Methanol extraction and petroleum ether/acetic ether extraction (EMPA) were gradually carried out by large-caliber Soxhlet. After one step extraction, one piece was taken out, dried in 105 °C to oven dry, and weighed. The wood extractives were obtained by evaporation in 60–70 °C.

### 2.3. GC/MS condition

The ethanol/methanol extractives of *P. lasiocarpa* wood (LD-010), ethanol/methanol extractives of *P. tomentosa* wood

(LD-021), benzene/ethanol extractives of *P. tomentosa* wood (LD-150), EMPA extractives of *P. lasiocarpa* wood (LD-174) were analyzed, respectively. Each 0.5 mg extractive was analyzed by a GC/MS-QP2010 (Shimadzu Corp., Japan). The GC/MS analysis was the same as that in the documents (Wanxi et al., 2013, 2014; Yusoff et al., 2013).

### 2.4. Experiment analyses

The LD-010, LD-021, LD-150, LD-174 extractives were obtained respectively. The total ion chromatograms of four extractives by GC/MS were shown in Fig. 1. Relative content of each component was counted by area normalization. The results are listed in Tables 1–4, respectively. By analyzing the MS data, the NIST standard MS map by computer, open-published books and papers, the components and their contents were identified.

## 3. Results and discussion

### 3.1. Molecular properties of poplar wood extractives

According to GC/MS result, 3 components were identified from The LD-010 wood extractives of *P. lasiocarpa*. Its main components were 2,4-hexadiyne (97.046%), dibutyl phthalate (0.471%), benzo[h]quinoline, 2,4-dimethyl-(0.723%), carbonic acid, monoamide, N-(2-ethyl- phenyl)-, propyl ester (1.760%).

The 24 components were identified from The LD-021 wood extractives of *P. tomentosa*. Its main components were benzene, 2-[(tert-butyldimethylsilyl) oxy]-1-isopropyl-4-methyl- (20.61%), 2,4-cyclohexadien-1-one, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy- (12.28%), dibutyl phthalate (12.09%), 4-methylmannonic. delta.-lactone (11.42%), 1,2-benzenedicarboxylic acid, diisooctyl ester (5.92%), 5(1h)-azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8s-cis)- (5.72%), 1h-cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl-, [1ar-(1a.alpha.,4.beta.,4a.beta.,7.beta.,7a.beta.,7 b.alpha.)]- (5.67%), 2-pentanone, 1-(2,4,6-trihydroxyphenyl) (4.17%), 1,2,5-oxadiazol-3-amine, 4-(4-methoxyphenoxy)- (3.88%), 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)- (3.42%), 4-((1e)-3-hydroxy-1-propenyl)-2-methoxy-phenol (2.89%), n-hexadecanoic acid (2.11%), 3-phenylbicyclo(3.2.2)nona-3,6-dien-2-one (1.82%), benzo[b] naphtho[2,3-d]furan (1.32%), 9,12-octadecadienoic acid (z,z)- (1.20%), hexanedioic acid, bis(2-ethylhexyl) ester (1.10%), 1h-indole, 1-methyl-2-phenyl- (1.00%), oleic acid (0.70%), benzoic acid, 4-hydroxy- (0.63%), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (0.54%), thiazol-4(5h)-one, 2-(4-morpholy)-5-(2-pyridylmethylideno)- (0.43%), 9-octadecyne (0.42%), phthalic acid, hexadecyl propyl ester (0.35%), 3,5-dimethoxy-4-hydroxycinnamaldehyde (0.34%).

The 3 components were identified from the LD-150 wood extractives of *P. tomentosa*. Its main components were 1,5-hexadien-3-yne (94.04%), dibutyl phthalate (3.95%), 1H-indole, 1-methyl-2-phenyl- (2.01%).

The 27 components were identified from the LD-174 wood extractives of *P. lasiocarpa*. Its main components were; 1,3,3-trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene (20.10%), 5(1h)-azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8s-cis)- (10.32%), n-hexadecanoic acid (8.82%), 4-dehydroxy-n-(4,5-methylene

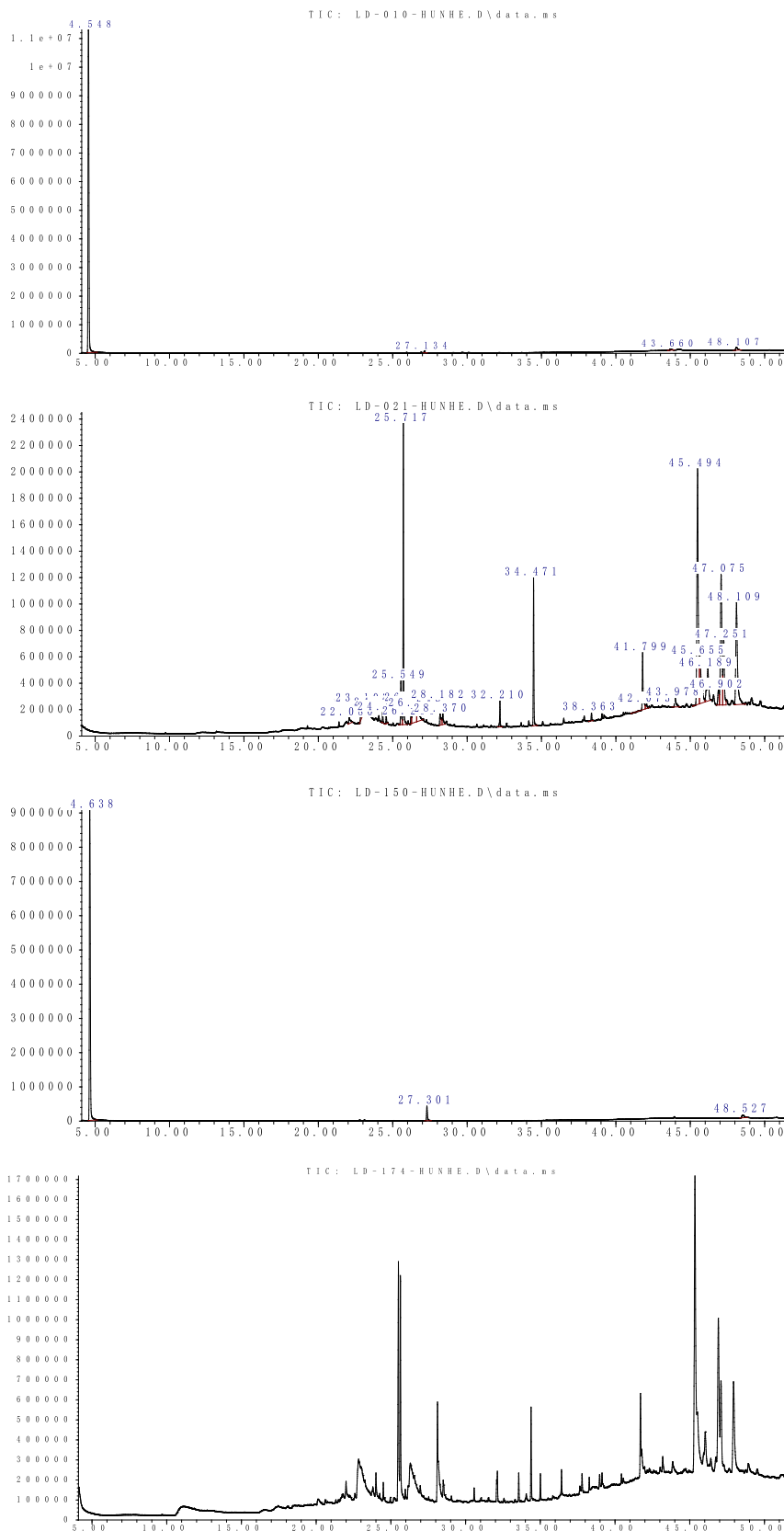


Figure 1 Total ion chromatograms of four extractives of *Populus* wood by GC/MS.

**Table 1** Compounds of LD-010 wood extractives of *Populus lasiocarpa* by GC/MS.

No.	R.T. (min)	P.A. (%)	Chemical compound
1	4.548	97.05	2,4-Hexadiyne
2	27.134	0.47	Dibutyl phthalate
3	43.66	0.72	Benzo[h]quinoline, 2,4-dimethyl-
4	48.107	1.76	Carbonic acid, monoamide, N-(2-ethylphenyl)-, propyl ester

dioxy-2-nitrobenzylidene)tyramine (7.70%), 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester (7.36%), benzo[h]quinoline, 2,4-dimethyl- (5.98%), 9,12-octadecadienoic acid (z,z)- (5.49%), 5-methyl-5,8-dihydro-1,4-naphthoquinone (5.30%), phenol, 2,6-dimethyl-4-nitro- (4.30%), eicosane (4.01%), benzo[b]naphtho[2,3-d]furan (3.43%), anthracene, 9,10-dihydro-9,9,10-trimethyl- (3.10%), 1,2-benzenedicarboxylic acid, diisooctyl ester (2.86%), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (2.02%), cyclotrisiloxane, hexamethyl- (1.44%), pentadecanoic acid (1.06%), 2-(acetoxymethyl)-3-(methoxycarbonyl)biphenylene (0.95%), hexanedioic acid, bis(2-ethylhexyl) ester (0.90%), 4-ethoxy-2,5-dimethoxybenzaldehyde (0.84%), benzoic acid, 3-ethoxy- (0.75%), octadecanoic acid (0.69%), nonadecane, 9-methyl- (0.54%), 2-ethylacridine (0.5026%), 9-octadecyne (0.49%), octadecane (0.41%), 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)- (0.33%), 3,5-dimethoxy-4-hydroxycinnamaldehyde (0.33%).

**Table 3** Compounds of LD-150 wood extractives of *Populus lasiocarpa* by GC/MS.

No.	R.T. (min)	P.A. (%)	Chemical compound
1	4.64	94.04	1,5-Hexadien-3-yne
2	27.30	3.95	Dibutyl phthalate
3	48.53	2.01	1H-Indole, 1-methyl-2-phenyl-

### 3.2. Resource properties of poplar wood extractives

There were many rare components in the extractives of poplar wood. Because of its officinal value, dibutyl phthalate was a pesticide to keep internal environment homeostasis (Wanxi et al., 2014). Hexanedioic acid, bis(2-ethylhexyl) ester, which was an industrial chemical, was used in Canada in various products such as cosmetics and so on ([www.chemicalsubstances.gc.ca](http://www.chemicalsubstances.gc.ca)). Phthalic acid derivatives were the main constituents of *Elaeagnaceae* plant which could cure chronic cardiovascular and cerebrovascular diseases and 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 whose binding energy was calculated by in silico method and compared with known inhibitors (Aparna et al., 2012; Qureshi et al., 2015). (Z,Z)-9,12-octadecadienoic acid had been identified as the main medical component of dried worms, and has diuretic, swelling and detoxification properties (Guo and Wei, 2006; Wanxi et al., 2013). 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-, which could protect

**Table 2** Compounds of LD-021 wood extractives of *Populus tomentosa* by GC/MS.

No.	R.T. (min)	P.A. (%)	Chemical compound
1	22.08	0.63	Benzoic acid, 4-hydroxy-
2	22.98	1.99	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
3	23.04	0.38	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
4	23.10	0.52	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
5	24.33	0.54	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
6	24.57	0.42	9-Octadecyne
7	25.55	2.11	<i>n</i> -Hexadecanoic acid
8	25.72	12.09	Dibutyl phthalate
9	26.24	0.34	3,5-Dimethoxy-4-hydroxycinnamaldehyde
10	26.39	4.17	2-Pentanone, 1-(2,4,6-trihydroxyphenyl)
11	26.65	1.82	3-Phenylbicyclo(3.2.2)nona-3,6-dien-2-one
12	28.18	1.20	9,12-Octadecadienoic acid (Z,Z)-
13	28.37	0.70	Oleic acid
14	32.21	1.10	Hexanedioic acid, bis(2-ethylhexyl) ester
15	34.47	5.92	1,2-Benzenedicarboxylic acid, diisooctyl ester
16	38.36	0.35	Phthalic acid, hexadecyl propyl ester
17	41.80	3.42	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
18	42.07	0.43	Thiazol-4(5H)-one, 2-(4-morpholyl)-5-(2-pyridylmethylideno)-
19	43.98	1.00	1H-Indole, 1-methyl-2-phenyl-
20	45.49	20.61	Benzene, 2-[(tert-butyl)dimethylsilyloxy]-1-isopropyl-4-methyl-
21	45.66	5.67	1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.beta.,4a.beta.,7.beta.,7a.beta.,7b.alpha.)]-
22	46.19	3.88	1,2,5-Oxadiazol-3-amine, 4-(4-methoxyphenoxy)-
23	46.90	1.32	Benzo[b]naphtho[2,3-d]furan
24	47.08	12.28	2,4-Cyclohexadien-1-one, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-
25	47.25	5.72	5(1H)-Azulene, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-
26	48.11	11.42	4-Methylmannonic.delta.-lactone

**Table 4** Compounds of LD-174 wood extractives of *Populus tomentosa* by GC/MS.

No.	R.T. (min)	P.A. (%)	Chemical compound
1	22.01	0.75	Benzoic acid, 3-ethoxy-
2	22.84	1.45	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
3	22.87	0.22	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
4	22.89	0.35	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
5	24.00	1.06	Pentadecanoic acid
6	24.49	0.49	9-Octadecyne
7	25.51	8.82	<i>n</i> -Hexadecanoic acid
8	25.64	7.36	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
9	26.17	0.33	3,5-Dimethoxy-4-hydroxycinnamaldehyde
10	26.31	4.29	Phenol, 2,6-dimethyl-4-nitro-
11	26.57	0.84	4-Ethoxy-2,5-dimethoxybenzaldehyde
12	28.12	3.62	9,12-Octadecadienoic acid ( <i>Z,Z</i> -)
13	28.18	1.86	9,12-Octadecadienoic acid ( <i>Z,Z</i> -)
14	28.51	0.69	Octadecanoic acid
15	30.58	0.41	Octadecane
16	32.08	0.54	Nonadecane, 9-methyl-
17	32.13	0.89	Hexanedioic acid, bis(2-ethylhexyl) ester
18	33.56	0.85	Eicosane
19	34.38	2.86	1,2-Benzenedicarboxylic acid, diisooctyl ester
20	35.01	0.83	Eicosane
21	36.42	1.02	Eicosane
22	37.80	0.65	Eicosane
23	38.28	0.33	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
24	38.97	0.50	2-Ethylacridine
25	39.13	0.67	Eicosane
26	41.71	3.10	Anthracene, 9,10-dihydro-9,9,10-trimethyl-
27	41.79	0.95	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene
28	43.19	0.71	Cyclotrisiloxane, hexamethyl-
29	45.35	20.10	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene
30	45.52	5.98	Benzo[h]quinoline, 2,4-dimethyl-
31	46.04	3.43	Benzo[b]naphtho[2,3-d]furan
32	46.76	0.72	Cyclotrisiloxane, hexamethyl-
33	46.92	10.32	5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8 <i>S</i> -cis)-
34	47.09	5.30	5-Methyl-5,8-dihydro-1,4-naphthoquinone
35	47.94	7.70	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine

liver, resist fatigue and strengthen the body's resistance, and improve human immunity, was considered as important substances in practical and clinical uses with a huge potential in nutraceutical and pharmaceutical industries (Kim and Karadeniz, 2012). And the extractives of poplar wood were rich in drug and biomedical activities. Based on the relative content, *P. lasiocarpa* wood was fit to extract 2,4-hexadiyne, 1,3,3-trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene, and *P. tomentosa* wood was fit to do (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, 1,5-hexadien-3-yne.

#### 4. Conclusion

The ethanol/methanol extractives of *P. lasiocarpa* wood (LD-010), ethanol/methanol extractives of *P. tomentosa* wood (LD-021), benzene/ethanol extractives of *P. tomentosa* wood (LD-150), EMPA extractives of *P. lasiocarpa* wood (LD-174) were identified as having 3, 24, 3 27 components, respectively. *P. lasiocarpa* wood was fit to extract 2,4-hexadiyne, 1,3,3-trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl)-cyclohexene, and *P. tomentosa* wood was fit to extract 1,5-hexadien-3-yne, (all-E)-2,6,10,15,19,23-hexamethyl-

ethyl-2,6,10,14,18,22-tetracosahexaene. And the extractives of poplar wood contained rich and rare drug and biomedical activities.

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