

## REPORT

# Molecular basis of antibacterial activities in extracts of *Eucommia ulmoides* wood

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**Abstract:** As one of the famous medicinal plants in China, the bark of *Eucommia ulmoides* is considered as the important traditional Chinese medicine. However, the constituents of *Eucommia ulmoides* wood isn't used effectively. Therefore, the molecules of wood extracts in *Eucommia ulmoides* are studied to further utilize the resources. The results show that the optimal extraction time period of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction are 3h, 5h, and 4h, respectively. Among sequential extractions, PABEEM extraction is the optimum extraction mode for the LRs is 22.95%. Its wood extracts included hexanedioic acid, bis(2-ethylhexyl) ester, 3,3,7,11-tetramethyltricyclo [5.4.0.0 (4,11)]undecan-1-ol, (all-E)- 2,6,10,15,19,23-hexamethyl- 2,6,10,14,18,22-tetracosahexaene, other rare drug and biomedical activities. And the wood extracts of *Eucommia ulmoides* is fit to extract 1,5-hexadien-3-yne, (all-E)- 2,6,10,15,19,23-hexamethyl- 2,6,10,14,18,22-tetracosahexaene.

**Keywords:** Antibacterial Molecules, Wood Activities, *Eucommia ulmoides*, GC-MS

## INTRODUCTION

*Eucommia ulmoides* is a species of small tree native to China, grows up to about 15 m length. It is widely planted on hills and mountains of China, Europe, North America and elsewhere since 10-35 million year (Call *et al.*, 1997). The leaves are arranged alternately, 8~16 cm long, deciduous, simple ovate with an acuminate tip and serrated margin; flowers are inconspicuous, small and greenish from March to May; fruit is a winged samara of 2~3 cm length and 1~2 cm width (Zheng *et al.*, 2012; Dai *et al.*, 2013; Zhang *et al.*, 2013). It is near threatened in the wild, but is highly valued in herbology such as Traditional Chinese medicine and widely cultivated in China for its bark. *Eucommia ulmoides* bark is most commonly used for medicinal purposes, and it can also be dried and packed in capsule form (Liu *et al.*, 2012; Shi *et al.*, 2012). And *Eucommia ulmoides* is rare, useful and valuable.

*Eucommia ulmoides* has been used in Traditional Chinese Medicine for thousands of years and in modern medicine to treat a wide range of ailments (Yang *et al.*, 2003; Victor *et al.*, 2007; Kwon *et al.*, 2012). The advanced equipments and instruments are largely used for the research on *Eucommia ulmoides* biomass. The bark has been largely studied and known to include geniposidic acid, (+)-pinosresinol-4,4'-di-o- $\beta$ -d-glucopyranoside, syringaresinol diglycoside, baicalein, wogonin, caprylic acid, triterpenoids, chlorogenic acid (Bamba *et al.*, 2010; Chai

*et al.*, 2012; Liu *et al.*, 2012; Ren *et al.*, 2012; Shi *et al.*, 2012; Nakazawa *et al.*, 2013; Shi *et al.*, 2013). The leaves includes geniposidic acid, aucubin, asperuloside, quercetin, kaempferol, astragaloside, baicalein, wogonin, chlorogenic acid, ferulic and caffeic acid (Kim *et al.*, 2004; Li *et al.*, 2005; Kim *et al.*, 2009; Fujikawa *et al.*, 2010; Zheng *et al.*, 2012; Dai *et al.*, 2013; Zhang *et al.*, 2013; Hong *et al.*, 2013). However, the active ingredients in *Eucommia ulmoides* wood are less expanded. Therefore, the molecular characteristics of the wood extracts are investigated and analyzed by some optimized extracting techniques in order to further utilize *Eucommia ulmoides* resources.

## MATERIALS AND METHODS

### Materials

Fresh *Eucommia ulmoides* wood are collected from the Zhangjiajie Forest Zone, Hunnan Province, China. The fresh wood is powdered and kept in vacuum. Acetic ether, methanol, benzene, petroleum ether and ethanol (chromatographic grade) are prepared for the subsequent experiments. Cotton thread and cotton bag are extracted in benzene/ethanol solution for 12h.

### Experiment methods

#### Single extraction

Weighed 54 powder of wood, each is about 20g (0.1mg accuracy) and then parceled by the cotton bag and tied by cotton thread, and signed. Extraction is carried out in 350ml solvents by large-caliber Soxhlet for 1, 3, 4, 5, 6, 7

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hours. The solvents are 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 are done at the temperature of 75°C, 90°C and 95°C, respectively. After extraction, one is taken out, dried at 105°C, and weighed. The extracts are obtained by evaporation in 60~70°C.

### Sequential extraction

Weighed 27 pieces of wood, each is 20g (1.0mg accuracy), and finally parceled by cotton bag and tied by cotton thread and signed. Three-step extraction is gradually carried out in benzene/ethanol → ethanol/methanol → petroleum ether/acetic ether (BEEMPA), ethanol/methanol → petroleum ether/acetic ether → benzene/ethanol (EMPABE), petroleum ether/ acetic ether → benzene/ethanol → ethanol/methanol (PABEEM), respectively (table 1). After every step extraction, one is taken out, dried at 105°C, and weighed. The extracts are obtained by evaporation in 60~70°C.

### GC/MS conditions

Among the above extracts, EM extracts of single extraction (LD-007), EB extracts of single extraction (LD-009), PA extracts of PABEEM extraction (LD-155), BE extracts of BEEMPA extraction (LD-156), EM extracts of EMPABE extraction (LD-157), paBE extracts of PABEEM extraction (LD-159), and emPA extracts of EMPABE extraction (LD-160) are analyzed, respectively. Each one of 0.5mg extracts is analyzed by online linked GC/MS (gas chromatograph/ mass spectrometer), respectively. The GC/MS analysis is done per the documents (Wanxi *et al.*, 2013; 2014).

## RESULTS

The leaching rates (LRs) of single extractions and extractions are listed in table 2 and table 3. The LD-007, LD-009, LD-155, LD-156, LD-157, LD-159 and LD-160 extracts are obtained respectively. The total ion chromatograms of four extracts by GC/MS are shown in fig. 1.

### Analysis of results

#### Leaching of wood extracts of *Eucommia ulmoides*

Table 2 show the LRs trend of *Eucommia ulmoides* wood extracts. It is observed that during ethanol/methanol extraction, the LRs of wood extracts fluctuate, and reach the maximum (7.22%) after the extraction time of 3h. During petroleum ether/acetic ether extraction, the LRs of wood extracts also fluctuate, and reach the maximum (2.86%) after the extraction time of 5h. During benzene/alcohol extraction, the leaching rate of stem extracts fluctuate, and reach the maximum (5.32%) after

the extraction time of 4h. While the optimal extraction time of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction are 3h, 5h and 4h, respectively.

During the sequential extraction, the ethanol/methanol extraction, petroleum ether/acetic ether extraction and benzene/alcohol extraction are done for 3h, 5h, and 4h, respectively. The statistical results show that the LRs of *Eucommia ulmoides* wood extracts by BEEMPA extraction is 12.87%, 19.69% by EMPABE extraction, and 22.95% by PABEEM extraction. Comparing with table 2 and table 3, it is observed that the LRs of single extractions are less than ones of sequential extractions. table 3 also show that the LRs of sequential extractions gradually increase except for EMPABE extraction, which are larger than ones of single extractions. During the sequential extractions, PABEEM extraction is the optimum extraction mode, which the LR is 22.95%.

### Molecular properties of *Eucommia ulmoides* wood extracts

Relative content of each component is counted by area normalization. While analyzing the MS data, open-published books and papers, the components and their contents are identified and obtained.

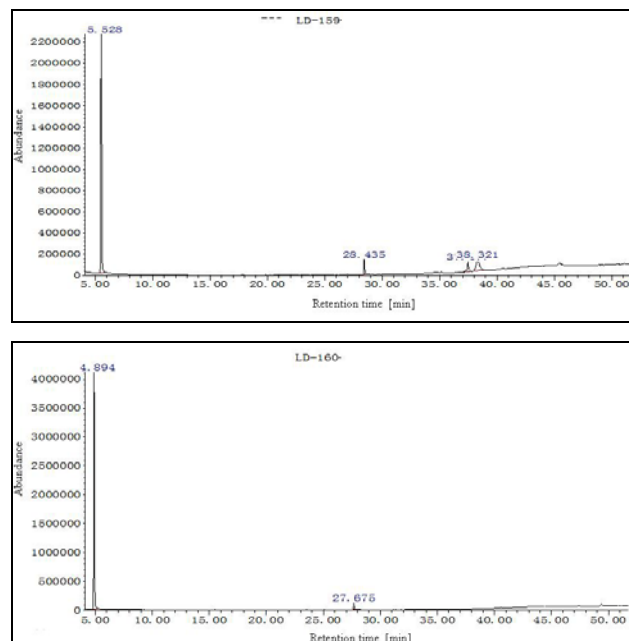
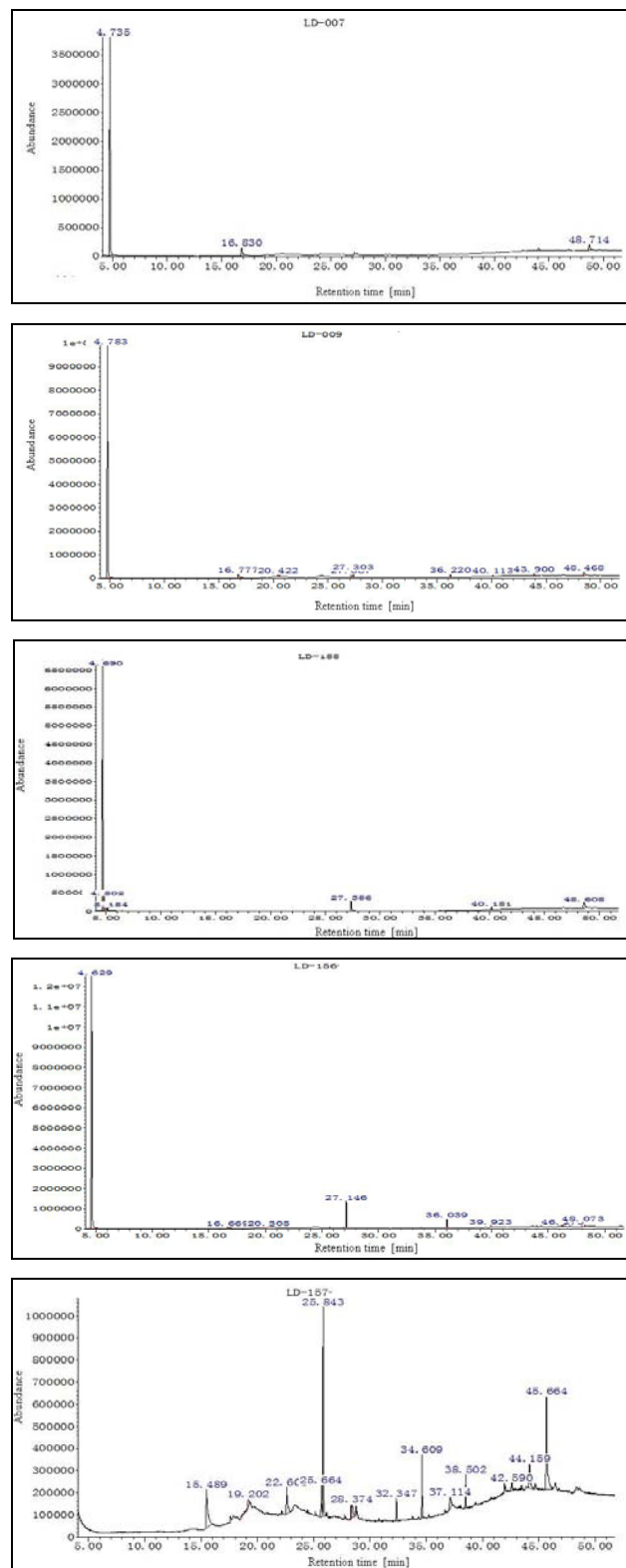
According to GC/MS results, 3 components are identified from wood EM extracts of single extraction of *Eucommia ulmoides*. The result shows that the components are 1,5-hexadien-3-yne (89.525%), tricyclo [3.3.2.0(3,7)] decan-9-one (6.146%), 3,3,7,11-tetramethyl -tricyclo [5.4.0.0(4,11)] undecan-1-ol (4.328%).

The 8 components are identified from wood EB extracts of single extraction. The result show that the components are 1,5-hexadien-3-yne (88.625%), cyclopentanone, 2-cyclopentylidene- (2.906%), beta.-D-glucopyranose, 1,6-anhydro- (1.458%), n- hexadecanoic acid (0.775%), dibutyl phthalate (1.759%), phthalic acid, ethyl pentadecyl ester (0.93%), (all-E)-2,6,10,15,19,23-hexamethyl- 2,6,10,14,18,22-tetracosahexaene (0.348%), 1H-indole, 1-methyl-2-phenyl- (3.199%).

The 6 components are identified from wood PA extracts of PABEEM extraction. The result show that the components are 1,5-hexadien-3-yne (84.722%), 1,3-hexadien- 5-yne (4.246%), heptane(0.986%), dibutyl phthalate(4.155%), (all-E)-2,6,10,15,19,23- hexamethyl- 2,6,10,14,18,22-tetracosahexaene(0.852%), 1H-indole, 1-methyl-2-phenyl- (5.038%).

The 8 components are identified from wood BE extracts of BEEMPA extraction. The result show that the components are 1,5-hexadien-3-yne (84.148%), 3-octen-5-yne, (E)- (1.54%). beta.-D-glucopyranose, 1,6-anhydro- (0.318%), dibutyl phthalate (6.736%), phthalic acid, 2-

ethylhexyl hexyl ester (2.949%), 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- (0.526%), 1H-pyrrole-2,5-dione, 1-(4-chlorophenyl)- (0.699%), 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)] undecan-1-ol (3.085%).



**Fig. 1:** Total ion chromatograms of five wood extracts by GC/MS

The 14 components are identified from wood EM extracts of EMPABE extraction. The result show that the components are 3-octen-5-yne, (E)- (13.231%), hexanoic acid, 6- bromo- (1.511%), tetracyclo[3.3.1.0.1(3,9)]decan-10-one (6.428%), n-hexadecanoic acid (4.129%), dibutyl phthalate (21.811%), 9,12-octadecadienoic acid (Z,Z)- (1.166%), 9- octadecenoic acid, (E)- (3.604%), hexanedioic acid, bis(2-ethylhexyl) ester (2.368%), phthalic acid, cycloheptyl isohexyl ester (6.57%), benzofuran-2-one, 2,3-dihydro-3,3-dimethyl-4-nitro- (3.884%), (all-E)-2,6,10,15, 19,23- hexamethyl-2,6,10,14, 18,22-tetracosahexaene(3.222%), 1H-indole, 1-methyl-2-phenyl- (1.338%), benzo[h] quinoline, 2,4-dimethyl- (6.012%), 4-dehydroxy-N-(4,5- methylenedioxy-2-nitrobenzylidene) tyramine (24.726%).

The 4 components are identified from wood paBE extracts of PABEEM extraction. The result show that the components are 1,5-hexadien-3-yne (77.912%), phthalic acid, butyl hexyl ester (4.696%), phthalic acid, ethyl isopropyl ester (4.405%), testosterone (12.986%).

The 2 components are identified from wood emPA extracts of EMPABE extraction. The result shows that the components are 1,5-hexadien-3-yne (97.251%), phthalic acid, butyl hexyl ester (2.749%).

Based on the above molecular properties, the wood extracts of *Eucommia ulmoides* are different in varied solvents and extraction order, suggesting that the molecules of wood extracts leached out at the different time. And there are the vast differences in the different three-steps extraction method. According to

**Table 1:** Three-step extraction methods

Extraction	Extraction solution		
	1 <sup>st</sup> step	2 <sup>nd</sup> step	3 <sup>rd</sup> step
1	Petroleum ether/acetic ether	Benzene/ethanol	Ethanol/methanol
2	Benzene/ethanol	Ethanol/methanol	Petroleum ether/acetic ether
3	Ethanol/methanol	Petroleum ether/acetic ether	Benzene/ethanol

**Table 2:** LR of each single extraction [%]

Extraction time [h]	Ethanol/methanol	Petroleum ether/acetic ether	Benzene/ethanol
1	6.84	1.10	4.27
3	7.22	1.51	5.11
4	6.68	1.39	5.32
5	6.94	2.86	4.47
6	5.22	0.76	4.28
7	5.83	1.44	4.76

**Table 3:** LR of sequential extraction

Extraction	BEEMPA			EMPABE			PABEEM		
	3	5	4	3	5	4	3	5	4
Time [h]	3	5	4	3	5	4	3	5	4
Step	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
LR [%]	3.64	4.25	4.98	6.28	7.41	6.00	8.37	7.39	7.19

the relative content, *Eucommia ulmoides* wood is fit to extract and separate 1,5-hexadien-3-yne, (all-E)-2,6,10,15, 19,23-hexamethyl- 2,6,10,14,18,22- tetracosahexaene.

#### Resource properties of extracts of *eucommia ulmoides* wood

There are many biomedical components in the extracts of *Eucommia ulmoides* wood. Because of its officinal value, dibutyl phthalate is a pesticide to keep internal environment homeostasis (Wanxi *et al*, 2014). Hexanedioic acid, bis (2-ethylhexyl) ester, an industrial chemical, is used in Canada in various products such as cosmetics, heavy-duty hand cleanser and lubricant (www.chemical substances.gc.ca). 3,3,7,11-tetramethyltricyclo [5.4.0.0 (4,11)] undecan-1-ol is the main alcohol of volatiles of needle and branch from *Picea crassifolia* which could lure *Ips typographus* Linnaeus (Ruijie, 2011). Phthalic acid derivatives are the main constituents of *Elaeagnaceae* plant, which has anti-tumor, anti-inflammatory, antibacterial activities (Hao *et al*, 2006). N-hexadecanoic acid might help in designing of specific inhibitors of phospholipase A (2) as anti-inflammatory agents whose binding energy is calculated by in silico method and compared with known inhibitors (Aparna *et al*, 2012). 9,12- octadecadienoic acid, methyl ester, and 9,12- octadecadienoic acid (Z,Z)- are identified as the main medical component of dried worms, and has diuretic, oncotic and detoxification properties (Guo *et al*, 2006; Wanxi *et al*, 2013). (all-E)-2,6,10,15, 19,23-hexamethyl-2,6,10, 14,18,22- tetracosahexaene, which

could protect liver, resist fatigue and strengthen the body's resistance, and improve human immunity, is considered as an important substance in general and clinical practices (Kim *et al*, 2012). There are many drugs and biomedical activities in the wood extracts of *Eucommia ulmoides*.

#### CONCLUSION

The leaching of wood extracts from *Eucommia ulmoides* is obvious. The optimal extraction time period of ethanol/methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction are 3h, 5h and 4h, respectively. The LR of single extractions are less than those of sequential extractions among which PABEEM extraction is the optimum extraction mode with the LR of 22.95%. Further, the wood extracts of *Eucommia ulmoides* is rich in bio-drugs and biomedical activities including hexanedioic acid, bis(2-ethylhexyl) ester, 3,3,7,11- tetramethyltricyclo [5.4.0.0(4,11)] undecan- 1-ol, (all-E)-2,6,10,15,19,23- hexamethyl-2,6,10, 14, 18,22 -tetracosahexaene, phthalic acid derivatives, and so on. According to the relative content, *Eucommia ulmoides* wood is fit to separate 1,5- hexadien-3- yne, (all-E)- 2,6,10,15,19,23- hex amethyl- 2,6,10,14, 18, 22 -tetracosahexaene.

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