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Molecular Characteristics of Anti-inflammatory Activities in Wood Extractives of *Quercus aliena*

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As one of economic species of wood plants in the Orient, *Quercus aliena* was also considered as the important bioresource. The molecular characteristics of extractives from *Quercus aliena* wood was analyzed to better utilize this bioresource. Fresh *Quercus aliena* wood were pound to pieces and extracted by single extraction and three-step extraction. And then the extractives were obtained and analyzed by GC/MS. The leaching rule of wood extractives from *Quercus aliena* was obvious, and 21 compounds were identified from wood extractives. What's more, the wood extractives contained the rich and rare drug constituents, such as 3,3,7,11- tetramethyltricyclo [5.4.0.0 (4,11)] undecan-1-ol, 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)]undecan-1-ol, 2,6,10,14,18, 22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, all-E)-, etc. so the wood extractives of *Quercus aliena* could be suitable for the extraction of bio-drugs.

Key words: Anti-inflammatory Activities, *Quercus aliena,* Wood Extractives, Bio-drug Molecular, GC/MS.

Quercus aliena, a long-lived and slow-growing tree, was a species of oak in the family Fagaceae, and was also known as the Oriental white oak[1,2]. *Q. aliena* could grow to 30 m tall with tree diameter up to 1 m. The leaves, which had 9 to 15 lobes on each side and a 10~13 mm petiole, repelled slugs and grubs, were obovate to oblong, glabrous above, glabrous to densely grey-white hairy below, mostly 10~20 cm long and 5~14 cm wide[2]. The flowers were monoecious and pollinated by wind, its individual flowers were either male or female, but both sexes could be found on the same plant, the acorns were 17~25 mm long, 13~18 mm wide, solitary or 2~3 together, and mature in about six months after pollination[1,2]. *Q. aliena* was in flower from April to May, and its seed ripened from September to October. The seed, which was about 25mm long, was buried in boggy ground overwinter and dug up in the spring when it would have lost most of its astringency[3]. Its seed could be dried, ground into a powder and used not only as a thickening in stews etc or mixed with cereals for making bread, but only as a coffee substitute. So *Q. aliena* was also considered as an economic and medicinal plant.

Q. aliena, which was native to Korea, Japan and China, had straight trunk, beautiful patterns, large diameter at breast height and high hardness. The wood of *Q. aliena* was very fine and beautiful, then often used for boat building and wood flooring in house for a long time. At present, the utilization of *Q. aliena* biomass was synthetically studied more and more. The seed was firstly crushed into a powder, and then used as soup thickener, cereals, breads, substitute for coffee, and so on. The bark, which had a rich source of tannin, was used as a powerful astringent[1,2,4]. Yue-Ken *et al* found that the ethanol extracts of leaf, bark and xylem of *Q. aliena* Blume had

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antimicrobial effect against Gram-positive and Gram-negative bacteria, and the ethyl acetate was the strongest antimicrobial activity[4]. Zhu-Ping *et al* studied the synthetic drugs[5-8]. Especially, the researchers in our university had analyzed many wooden products, pyrolysis products[9-15], and woody extractives[12-23], and some researchers also explored a variety of biological active ingredients and wood biomass[16-30]. Therefore, the wood extractives of *Q. aliena* was obtained by kinds of extraction methods, and the molecular characteristics were investigated in order to better utilize this wood resource.

MATERIALS AND METHODS

Fresh wood of *Q. aliena* was collected from the Tongbai Mountain, Henan Province, China. The fresh wood was dried at room temperature, pound to pieces, and then kept in vacuum. Acetic ether, methanol, benzene, petroleum ether and ethanol were chromatographic grade for the subsequent experiments. Cotton thread and cotton bag were both extracted in benzene/ethanol solution for 12 h.

Experiment methods

Single extraction

Weighed 54 pieces of wood, each was about 5g (0.1mg accuracy) and finally parceled by cotton bag tied by cotton thread. Extraction was carried out in 350ml solvents by the Foss method for 1, 3, 4, 5, 6, 7 hours, respectively. Solvents were ethanol/methanol ($V_{ethanol}/V_{methanol}$ =2), petroleum ether/acetic ether ($V_{petroleum\ ether}/V_{acetic\ ether}$ =2), and benzene/ethanol solution ($V_{ethanol}/V_{benzene}$ =2). Ethanol/methanol extraction, petroleum ether/ acetic ether extraction, and benzene/ethanol extraction were carried out at temperature of 75°C, 90°C and 95°C, respectively. After extraction, one piece was took out, dried in 105°C to oven dry, and weighed. The extractives was obtained by evaporation at the temperature of 60~70°C.

Three-step extraction

Weighed 27 pieces of wood, each was 20g (1.0mg accuracy), and finally parceled by cotton bag tied by cotton thread. Three-step extraction was carried out by large-caliber Soxhlet according to different orders combined by EM-PA-BE (ethanol/methanol '! petroleum ether/acetic ether '!benzene/ ethanol), PA-BE-EM (petroleum ether/ acetic ether '! benzene/ethanol '! ethanol/ methanol), BE-EM-PA (benzene/ethanol '! ethanol/ methanol '! petroleum ether/acetic ether), respectively. After each step extraction, one piece was took out, dried in 105°C to oven dry, and weighed. The extractives was obtained by evaporation at the temperature of 60~70°C.

GC/MS condition

Among the above extractives, the BE extractives, EM extractives, EM extractives in EM-PA-BE method, empaBE extractives in EM-PA-BE extraction, BE extractives in BE-EM-PA method, beEM extractives in BE-EM-PA method, paBE extractives in PA-BE-EM method, pabeEM extractives in PA-BE-EM method 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[16-23].

RESULTS

The leaching rates of single extractions and three-step extractions were listed in Table-1 and Table-2. The EB, ME, EM, empaBE, BE, beEM, paBE, pabeEM wood extractives were obtained, respectively. The total ion chromatograms of 8 extractives by GC/MS were shown in Figure-1. Based on the MS data, NIST standard MS map, open-published books and papers, the components and their contents were identified[16-29].

DISCUSSION

Leaching rule of wood extractives of *Q. aliena*

The leaching rate trend of *Q. aliena* wood extractives in different solvents was described in Table-1. It was observed that during ethanol/ methanol extraction, the leaching rate of stem extractives fluctuated, and reached the maximum (3.91%) when extraction time was 6h. During petroleum ether/acetic ether extraction, the leaching rate of stem extractives first increased and then decreased, and reached the maximum (2.94.70%) when extraction time was 7h. During benzene/ alcohol extraction, the leaching rate of stem extractives fluctuated, and reached the maximum (4.20%) when extraction time was 7h. The optimal extraction time of ethanol/methanol extraction,

petroleum ether/acetic ether extraction, and benzene/alcohol extraction were 6h, 7h, and 7h, respectively.

During three-step extraction, the ethanol/ methanol extraction, petroleum ether/acetic ether extraction, and benzene/alcohol extraction were done for 6h, 7h, and 7h, respectively. The statistical results showed that the leaching rates of *Q. aliena* wood extractives by EM-PA-BE method were 8.17%, 5.74% by BE-EM-PA method, and 10.61% by PA-BE-EM method, resprectively. And it was observed that the leaching rate of each single extraction was less than that of three-step extractions. Table-2 also showed that the three-step extractions gradually increased leaching rates of wood extractives, which were larger than that of

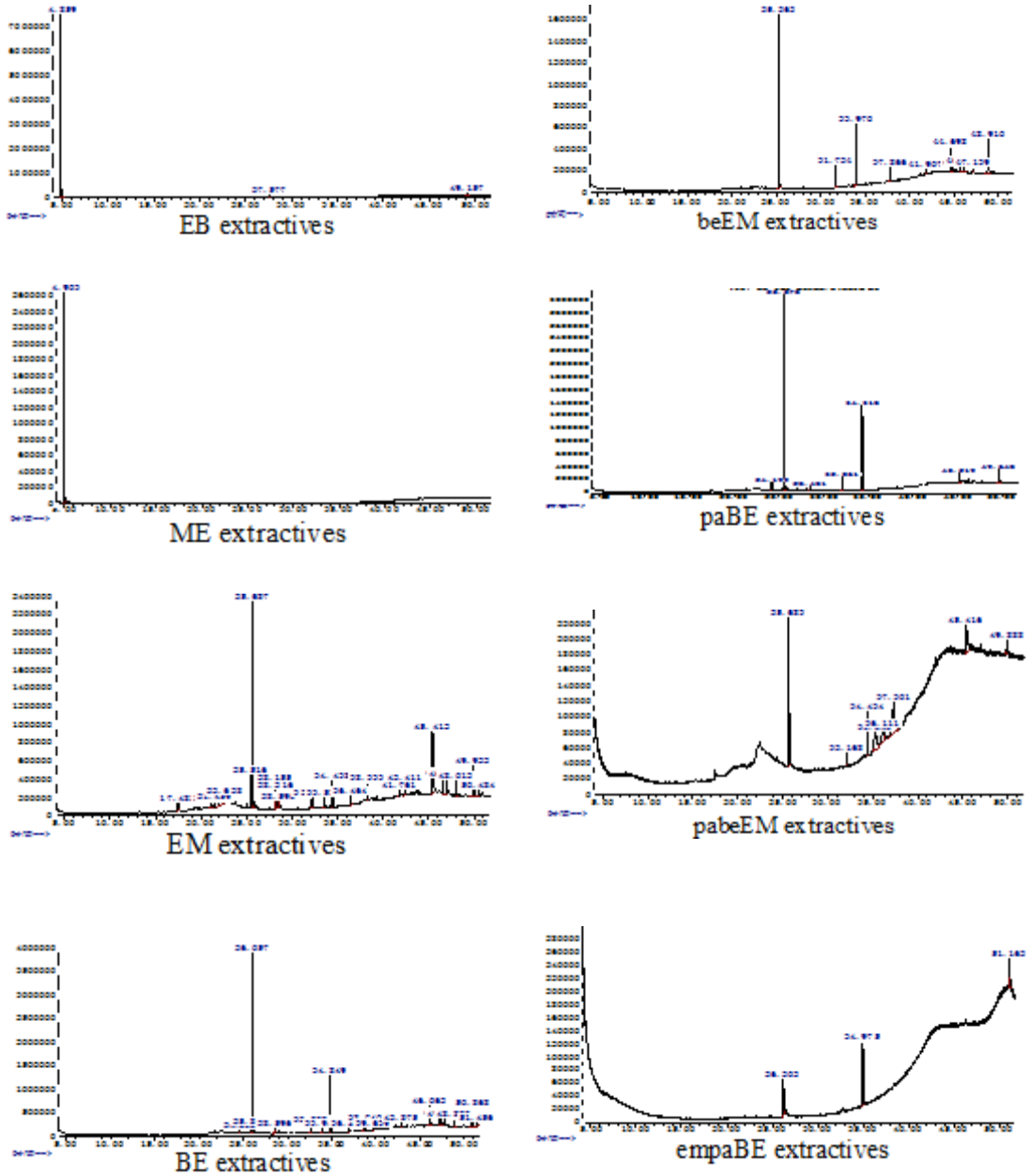

Fig. 1. Total ion chromatogram of 8 wood extractives of *Q. aliena* by GC/MS

any single extraction. During three-step extraction, PA-BE-EM method was the optimum extraction mode for the leaching rate was 10.61%.

Molecular Properties of wood Extractives of *Q. aliena*

According to GC/MS result, 3 components were identified from EB extractives of *Q. aliena* wood in single extraction as: 1,5-hexadien-3-yne (97.95%), phthalic acid, butyl hexyl ester (0.61%), 2-p- nitrophenyl-oxadiazol-1,3,4-one-5 (1.43%).

Only 1 component was identified from ME extractives of *Q. aliena* wood in single extraction. The result showed that the 1 component was 1,5-hexadien-3-yne.

21 components were identified from EM extractives of *Q. aliena* wood. The result showed that the main components were phthalic acid, butyl hexyl ester (22.86%), 4-cyclohexene-1,2-dicarboximide, N-butyl-, cis- (15.79%), myo- Inositol (9.49%), 9,10-methanoanthracen- 11-ol, 9,10-dihydro-9,10,11- trimethyl- (8.94%), oleic acid (4.52%), n-hexadecanoic acid (4.47%), 9,12-octadecadienoic acid (Z,Z)- (3.79%), indolizine, 2-(4-methylphenyl)- (3.69%), [1,2,4]triazolo [1,5-a] pyrimidine-6- carboxylic acid, 4,7-dihydro- 7-imino-, ethyl ester (3.62%), benzo[h]quinoline, 2,4-dimethyl- (3.41%), inositol, 1-deoxy- (3.33%), 1-heptacosanol (3.05%), phthalic acid, dodecyl octyl ester (2.97%), 1H-indole, 1-methyl-2-phenyl- (2.10%), (all-E)-2,6,10,15,19,23- hexamethyl- 2,6,10,14,18,22-tetracosahexaene (1.84%), 6,13-diazadispiro[4.1.5.2]tetradecan-14-one (1.63%), hexanedioic acid, bis(2-ethylhexyl) ester (1.28%), 3(2H)-furanone, 4-methoxy-2,5-dimethyl- (1.22%), 2-naphthalenemethanol,1,2,3,4,4a,5,6,7- octahydro-±,±,4a,8-tetramethyl-, (2R-cis)- (0.73%), agarospirol (0.70%), octadecanoic acid (0.54%).

Table 1. Leaching rate trend of each single extraction [%]

Extraction time [h]	ethanol /methanol	petroleum ether /acetic ether	benzene /ethanol
1	0.43	2.53	2.79
3	3.33	2.16	2.17
4	2.82	1.58	4.13
5	3.51	2.03	2.16
6	3.91	2.69	3.83
7	2.33	2.94	4.20

16 components were identified from BE extractives of *Q. aliena* wood. The result showed that the main components were dibutyl phthalate (40.40%), phthalic acid, 2-ethylhexyl hexyl ester (13.26%), 7-heptadecyne, 17-chloro- (11.44%), 3,3,7,11- tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol(9.47%), [1,2,4]triazolo[1,5-a] pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester (3.56%), 9,12-octadecadienoic acid (Z,Z)- (3.48%), benz[b]-1,4-oxazepine- 4(5H)-thione, 2,3-dihydro- 2,8-dimethyl- (3.07%), 1,2,5- oxadiazol-3-amine, 4-(4-methoxy - phenoxy)- (2.95%), 1H-indole, 1-methyl-2-phenyl- (2.30%), n-hexadecanoic acid (2.25%), hexanedioic acid, bis(2-ethylhexyl) ester (2.03%), 19,23-hexamethyl-, (all-E)- (1.87%), cyclopentadecane (1.32%), 1- eicosene (1.30%), phthalic acid, butyl tetradecyl ester (0.64%), benzo [h]quinoline, 2,4-dimethyl- (0.64%).

10 components were identified from beEM extractives of *Q. aliena* wood. The result showed that the main components were phthalic acid, butyl hexyl ester (42.85%), hexanedioic acid, bis(2-ethylhexyl) ester (5.22%), phthalic acid, 2-ethylhexyl hexyl ester (13.96%), 2,6,10,14, 18,22-tetracosahexaene, 2,6,10, 15, 19,23-hexamethyl-, (all-E)- (3.13%), 1H-indole, 1-methyl-2- phenyl- (1.07%), 2-amino-4- hydroxy- 6,8- dimethyl-7 (8H)-pteridinone (11.69%), benzo[h] quinoline, 2,4-dimethyl- (1.43%), 2,4,6- cyclo -heptatrien-1-one, 3,5-bis-trimethylsilyl- (2.50%), 3,3,7,11-tetramethyltricyclo [5.4.0.0(4,11)]undecan- 1-ol (1.43%), cyclohexane, 1,1,2-trimethyl-3,5- bis(1-methylethenyl)-, (2±,3²,5²)- (16.73%).

7 components were identified from bcJY extractives of *Q. aliena* wood. The result showed

Table 2. Leaching rate of three-step extraction [%]

Extraction	EM-PA-BE			BE-EM-PA			PA-BE-EM		
Time[h]	6	7	7	7	6	7	7	7	6
Step	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Leaching rate	3.33	0.83	4.01	1.97	0.89	2.88	4.47	4.72	1.42

that the main components were phthalic acid, butyl isohexyl ester (2.06%), dibutyl phthalate (53.84%), 9,17- octadecadienal, (Z)- (0.73%), hexanedioic acid, bis(2-ethylhexyl) ester (3.92%), phthalic acid, 2-ethylhexyl isobutyl ester (24.35%), 1H-indole, 1-methyl-2-phenyl- (6.12%), 1,2,5-oxadiazol-3- amine, 4-(4-methoxyphenoxy)- (8.99%).

8 components were identified from pabeEM extractives of *Q. aliena* wood. The result showed that the main components were dibutyl phthalate (25.15%), hexanedioic acid, bis(2-ethylhexyl) ester (1.43%), phthalic acid, neopentyl 2-propyl ester (5.55%), 2H-bisoxireno[2,3:8,8a]azuleno [4,5-b] furan-7(3aH)-one, octahydro-3a, 8c-dimethyl-6- methylene- (15.96%), 1,2,5-oxadiazol-3-amine, 4-(4-methoxyphenoxy)-(11.50%), 1H-pyrrole-2,5- dione, 1-(4-chlorophenyl)- (25.04%), 2,4,6-cycloheptatrien- 1-one, 3,5-bis- trimethylsilyl- (12.08%), 2,4-cyclo- hexadien- 1-one, 3,5-bis(1,1-dimethylethyl)-4- hydroxy- (3.29%).

3 components were identified from empaBE extractives of *Q. aliena wood*. The result showed that the main components were dibutyl phthalate (50.84%), phthalic acid, 2-methoxyethyl undecyl ester (49.16%), 3,3,7,11-tetramethyltricyclo[5.4. 0.0 (4,11)]undecan-1-ol.

Resource Properties of wood extractives of *Q. aliena*

There were many biomedical components in the wood extractives of *Q. aliena*. Because of its officinal value, 3,3,7,11- tetramethyltricyclo [5.4.0.0(4,11)] undecan- 1-ol was the one volatile alcohol of *Picea crassifolia* needle and branch which could lure Ips typographus Linnaeus[24]. Phthalic acid, isobutyl nonyl ester could potentially cure chronic cardiovascular and cerebrovascular diseases and had anti-tumor, anti-inflammatory, antibacterial functions[25]. In 1996, Okugawa *et al* found that agarospirol could be considered to be neuroleptic. (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, was considered as important substances in practical and clinical uses with a huge potential in nutraceutical and pharmaceutical industries[15-23]. 2,4,6-cycloheptatrien- 1-one, 3,5-bis-trimethylsilyl- was one of the bioactive components of *Microcosmus exasperatus* which might heal some diseases[20-25]. 9,12-octadecadienoic acid, methyl ester, and 9,12-octadecadienoic acid (Z,Z)- had been identified as the main medical component of dried worms, and has diuretic, swelling and detoxification properties[2-24]. According to the relative content , the extractives was suitable to extract phthalic acid derivatives, dibutyl phthalate, and (all-E)-2,6,10,15, 19,23- hexamethyl-2,6,10,14, 18,22-tetracosahexaene. And there were some drug activities in wood extractives of *Q. aliena*.

CONCLUSION

The leaching rule of wood extractives from *Q. aliena* was obvious. The optimal extraction time of ethanol/methanol extraction, petroleum ether/ acetic ether extraction, and benzene/alcohol extraction were 6h, 7h, and 7h, respectively. The leaching rate of each single extraction was less than that of three-step extractions among which PA-BE-EM method was the optimum extraction mode for the leaching rate was 10.61%. What's more, wood extractives of *Q. aliena* was rich in drug activities, such as 3,3,7,11- tetramethyltricyclo [5.4.0.0(4,11)]undecan- 1-ol, 2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23- hexamethyl-, (all-E)-. And the wood extractives of *Q. aliena* contained many rare drug activities.

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