FOURIER TRANSFORM INFRARED AND GAS CHROMATOGRAPHY-MASS SPECTROMETER EXTRACTS FROM *EUSCAPHIS JAPONICA* BARK

by

Zihan WEI[a], Zhiyong SUN[b], Yaoming WANG[c], Shuaiwei DONG[b], Chunxia LV[b], Ting WANG[a], Shuangxi YAN[a*], and Chao WANG[a*]

[a] School of Forestry, Henan Agricultural University, Zhengzhou, China
[b] Scientific Research Institution,
Henan Xiaoqinling National Nature Reserve Administration Bureau, Sanmenxia, China
[c] Science and Technology Department, Luanchuan Laojunshan Forest Farm, Luoyang, China

Original scientific paper
https://doi.org/10.2298/TSCI190611038W

Due to the lack of studies on the chemical constituents of Euscaphis japonica bark, infrared spectroscopy and gas chromatography-mass spectrometer (GC-MS) techniques were used to analyze the ethanol, phenyl alcohol and methanol extracts from Euscaphis japonica bark, laying a foundation for the efficient utilization of Euscaphis japonica bark. Through experimental verification, different extracts of Euscaphis japonica bark can yield different chemical substances: Stigmast-4-en-3-one, (1R,8a alpha)-1,4a beta-dimethyl -7 beta -(1-hydroxy-1-methylethyl)decalin-1 alpha-ol, lactose, Vitamin E, 9,12-octadecadienoic acid, n-hexadecanoic acid, Arsenous acid Tris(trimethylsilyl)ester 4-hydroxy-3-methoxycianamylic alcohol, etc. It was determined that most of the chemicals in Euscaphis japonica bark are soluble in ethanol reagents. According to the relevant mass spectrometry data, Euscaphis japonica bark contains useful pharmaceutical ingredients and chemical raw materials and has broad development prospects.

Key words: *Euscaphis japonica extract, FT-IR, GC-MS*

Introduction

Euscaphis japonica is a deciduous small tree belonging to the genus *Piton* in the oil-family of the province, also known as piton, alba, and chicken eye [1]. *Euscaphis japonica* is primarily distributed in the warm climates of China, South Korea, North Korea, and Japan [2]. *Euscaphis japonica* is a medicinal plant with great potential. Its roots, bark, flowers, and fruits are all used in medicine [3]. Among them, the root has the effect of detoxification and clearing heat, and is mainly used to treat diseases such as colds and enteritis. Fruits dispel wind-cold, qi analgesic effect and can be used to treat irregular menstruation, hernia pain, and stomach aches. Its seed oil can be used to make soap, while the bark can be extracted from baking gum and used as an economic forest. Presently, global studies on *Euscaphis japonica* primarily focus on tissue culture, seed germination and cultivation technology, while there is littleresearchon the chemical composition analysis and pharmacological effects of *Euscaphis japonica* [4]. Therefore, this study investigated the chemical composition of *Euscaphis japonica* bark extract by FT-IR, GC-MS and other technologies, laying a foundation for the efficient utilization of *Euscaphis japonica* [5].

* Corresponding author, e-mail: 987243272@qq.com; 131459@qq.com

Experimental materials and methods

Experimental materials

Euscaphis japonica trees were collected from the Laojunshan in Luoyang, Henan province, China and the collected *Euscaphis japonica* bark was scraped with a knife. Ethanol, phenyl alcohol (volume ratio 1:1), and methanol were pure chromatography grade [6].

Experimental method

Take 10 grams of *Euscaphis japonica* bark powder and bottle them separately, add ethanol, phenyl alcohol, methanol 300 ml for extraction. Then take out the 10 ml liquid and bottle it and set aside [7].

The FT-IR method

First, KBr was ground and pressed into the infrared spectrum recorder to collect the background [8]. Then a drop of the extracted solution was added to the mortar and the KBr grinding tablet in the infrared spectrum recorder to record the infrared data of ethanol, benzoic alcohol, and methanol, and corrected to remove the background [9].

The GC-MS method

A 10 mL extracted sample was removed and injected into the GC-MS (GC: gas spectrum column hp-5 ms (30 m × 250 μs × 0.25 μs) for measurement. Collect data using helium. The heating process of the gas spectrum column consisted of: initial temperature at 50 °C, rising to 250 °C with the speed of 10 °C/min, and then increasing to 280 °C at a rate of 5 °C/min. The mass spectrometer parameters consisted of: electron ionization mode EI, voltage 70 eV, current 150 A, helium gas flow rate 1 mL/min, separation ratio 50:1, scanning mass range 30-600 amu, ion source temperature 230 °C, quadrupole temperature 150 °C [10, 11].

Results and analysis

The FT-IR analysis

Infrared analysis of ethanol, phenyl alcohol and methanol extracts from *Euscaphis japonica* bark were performed, as shown in fig. 1 [12]. The peak heights of the three extracts were similar and primarily distributed in 3400-3500 cm^{-1}, 2900-3000 cm^{-1}, 2840-2890 cm^{-1}, 1680-1750 cm^{-1}, 1600-1670 cm^{-1}, 1490-1500 cm^{-1}, 1350-1390 cm^{-1}, 1170-1250 cm^{-1}, 1000-1050 cm^{-1}, *etc.*, possible. It is caused by the stretching vibration of their bonds, and it is presumed to contain ethers, phenols, aromatic hydrocarbons, olefins, alkanes, alcohols, *etc*. [13-18].

The GC-MS analysis

Ethanol, phenyl alcohol and methanol extracts from *Euscaphis japonica* bark were analyzed by GC-MS, and mass spectrometry data were obtained, tabs. 1-3. On this basis, the relative content of each component was obtained. Finally, it chemical composition extract was knowed by referring to the relevant mass spectrometry data [19-26].

The 31 compounds were identified by the GC-MS detection of *Euscaphis japonica* bark ethanol extract, among which the most prominent were: Stigmast-4-en-3-one (16.70%), n-hexadecanoic acid (3.05%), Vitamin E (1.50%), Melezitose (8.31%), 9,12-octadecadienoic (1.07%), lactose (3.21%), and (3beta,24S)-stigmast-5-en-3-ol (7.36%).

A total of 15 substances were identified by GC-MS detection of the *Euscaphis japonica* bark phenyl alcohol extract, among the most prominent were: Stigmast-4-en-3-one

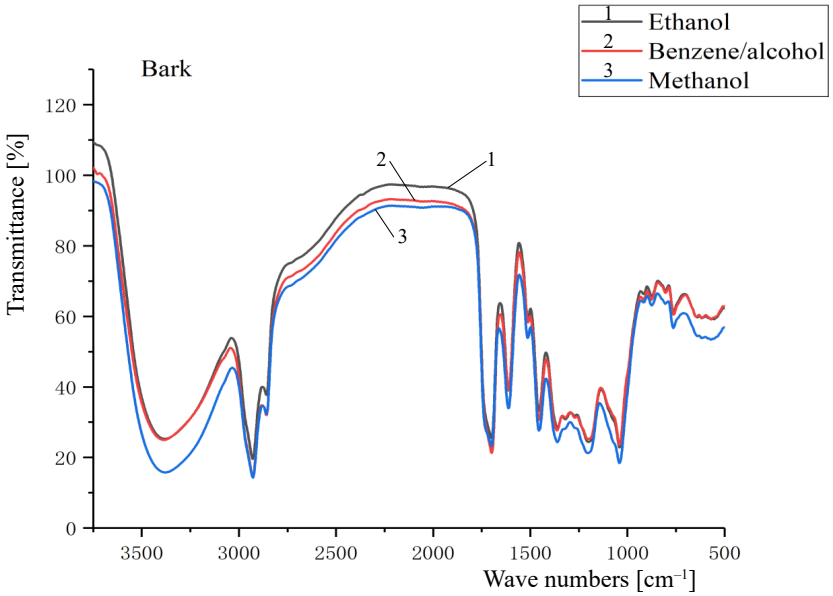

Figure 1. The FT-IR extracts from *Euscaphis japonica* bark

(40.10%), (1R,8a alpha)-1,4a beta-dimethyl -7 beta- (1-hydroxy-1-methylethyl)decalin-1 alpha-ol (1.63%), industrene4516 (1.67%), lactose (2.11%), and Arsenou S acid tris(trimethylsilyl) ester (4.17%). Its extract detection identified a total of 8 substances among which the main ones were: 4, 22-Stigmastadiene-3-one (3.04%), 1-(3-Methoxy-4-hydroxyphenyl)propene-3-ol (2.84%), n- hexadecanoic Acid (3.08%), and Melezitose (3.02%), and so on.

Its result analysis showed that the three extracts of *Euscaphis japonica* bark contained many useful components, among which n-hexadecanoic acid was mainly used for chemical reagents in the soap and food additives industry [27-29]. Vitamin E is used medically for nutritional anemia in infants and abortion in women [30]. The 9,12-octadecadienoic can be used not only to produce chemical products such as paint and ink but also to reduce blood lipids in medical treatment [31]. Lactose is used for food additives [32].

Table 1. The GC-MS results of the ethanol extract of *Euscaphis japonica* bark

No	Retention time [minute]	Compounds	Relative content [%]
1	5.05	Propanamide, N-(2,6-dimethylphenyl)-3-(4-morpholyl)-	0.62
2	6.21	Undec-10-ynoic acid	0.53
3	7.34	Lactose	1.31
4	8.62	Melezitose	1.75
5	9.45	3,5-Heptadienal, 2-ethylidene-6-methyl-	0.47
6	10.91	Melezitose	2.94
7	11.19	3-Allyl-6-methoxyphenol	0.65
8	11.25	6-Nonyl-5,6-dihydro-2H-pyran-2-one	0.84
9	11.42	Cyclohexanone, 5-(1-hydroxy-2-propenyl)-2,2-dimethyl-, (R*,S*)-(.+-.)-	0.77

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Table 1. (Continuation)

No	Retention time [minute]	Compounds	Relative content [%]
10	11.54	Melezitose	2.85
11	11.90	Phenol, 3,5-bis(1,1-dimethylethyl)-	0.53
12	12.21	Cyclohexanone, 5-(1-hydroxy-2-propenyl)-2,2-dimethyl-, (R*,S*)-(.+-.)-	0.73
13	13.18	Lactose	1.27
14	13.30	Lactose	0.89
15	13.42	Melezitose	0.78
16	13.58	Melezitose	0.65
17	13.69	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	4.65
18	13.75	1,6(2H,7H)-Naphthalenedione, 3,4,8,8a-tetrahydro-8a-methyl-	1.30
19	14.04	Triisobutyl(3-phenylpropoxy)silane	1.19
20	14.60	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-pentenyl)-	2.48
21	14.69	2,5-Dihydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one	1.08
22	14.77	Aromadendrene oxide-(1)	0.61
23	14.85	Menthol, 1'-(butyn-3-one-1-yl)-, (1S,2S,5R)-	0.38
24	14.94	3,9-Dimethyltricyclo[4.2.1.1(2,5)]decan-9-ol	0.74
25	15.52	Undec-10-ynoic acid, nonyl ester	0.68
26	15.83	1-Cyclohexene-1-propanol, 2,6,6-trimethyl-	0.49
27	15.96	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.53
28	16.74	n-Hexadecanoic acid	3.30
29	16.86	5-hydroxy-4-nitro-1-decalinone	0.72
30	16.93	1-Cyclohexene-1-propanol, 2,6,6-trimethyl-	0.40
31	17.06	Undec-10-ynoic acid, nonyl ester	0.69
32	17.18	n-Nonenylsuccinic anhydride	0.83
33	17.72	Undec-10-ynoic acid, butyl ester	0.59
34	18.38	9,12-Octadecadienoic acid (Z,Z)-	1.16
35	18.42	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	3.13
36	18.62	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	1.26
37	19.55	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	0.41
38	19.72	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	0.39
39	20.22	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	0.91
40	21.01	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	0.76
41	21.32	Vitamin E	1.62
42	21.64	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	0.65

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Table 1. (Continuation)

No	Retention time [minute]	Compounds	Relative content [%]
43	22.42	Benzoic acid, 4-methyl-2-trimethylsilyl-, trimethylsilyl ester	0.36
44	23.06	Stigmast-4-en-3-one	18.05
45	23.59	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	3.41
46	24.24	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	1.98
47	25.18	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	2.28
48	25.41	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	1.87
49	25.55	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	0.75
50	26.66	.gamma.-Sitosterol	7.95
51	27.10	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	0.83
52	27.54	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	9.14
53	27.88	Benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester	4.82

Table 2. The GC-MS results of benzyl alcohol extract from *Euscaphis japonica* bark

No	Retention time [minute]	Compounds	Relative content [%]
1	2.61	5,6-Azulenedimethanol, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-, (3a.alpha.,8.beta.,8a.alpha.)-	2.35
2	7.34	Lactose	1.38
3	8.63	Melezitose	1.69
4	10.89	2-tert-Butyl-1,2,4-triaza-spiro[4.6]undecane-3-thione	2.00
5	11.53	Melezitose	47.26
6	13.17	Melezitose	1.01
7	13.69	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	1.87
8	14.60	9-Ethoxy-10-oxatricyclo[7.2.1.0(1,6)]dodecan-11-one	1.92
9	14.70	9-Ethoxy-10-oxatricyclo[7.2.1.0(1,6)]dodecan-11-one	1.16
10	15.52	2H-Benzocyclohepten-2-one, decahydro-9a-methyl-, trans-	1.30
11	16.74	n-Hexadecanoic acid	4.92
12	17.18	n-Nonenylsuccinic anhydride	1.96
13	18.43	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)]-	1.10
14	19.56	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	1.48
15	21.31	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	2.32
16	23.07	Stigmast-4-en-3-one	2.12
17	23.55	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	5.19

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Table 2. (Continuation)

No	Retention time [minute]	Compounds	Relative content [%]
18	25.19	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	2.93
19	25.41	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	2.48
21	26.67	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	0.98
22	27.10	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3.alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-	1.73
23	27.55	Arsenous acid, tris(trimethylsilyl) ester	2.01
24	27.90	2,6-Dihydroxyacetophenone, 2TMS derivative	8.81

Table 3. The GC-MS results of methanol extract from *Euscaphis japonica* bark

No	Retention time [minute]	Compound name	Relative content [%]
1	10.98	Melezitose	1.52
2	11.23	Methyl 3,4-tetradecadienoate	1.54
3	11.41	Melezitose	1.50
4	13.69	(1R,4aR,7R,8aR)-7-(2-Hydroxypropan-2-yl)-1,4a-dimethyldecahydronaphthalen-1-ol	2.80
5	14.59	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	2.84
6	16.74	n-Hexadecanoic acid	3.08
7	18.43	4,22-Stigmastadiene-3-one	3.04
8	23.16	.gamma.-Sitostenone	74.35
9	25.40	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	1.92
10	26.67	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	3.46
11	27.07	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	3.97

Conclusion

Euscaphis japonica bark extracts different substances in organic solvents, among which the ethanol extract showed the most. It was speculated that most substances in *Euscaphis japonica* bark were soluble in ethanol. All three organic solvents can extract n-hexadecanoic acid, while vitamin E exists only in ethanol extracts, indicating that n-hexadecanoic acid is soluble in three organic solvents, while vitamin E is insoluble in benzyl alcohol and methanol. *Euscaphis japonica* bark contains many chemical raw materials and medical ingredients, so it can be used as a raw material for pharmaceuticals and chemical production. *Euscaphis japonica* bark study provides a scientific theoretical basis for the efficient utilization.

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Paper submitted: June 11, 2019
Paper revised: August 17, 2019
Paper accepted: September 11, 2019