CHEMICAL COMPONENTS FROM DIFFERENT PARTS OF *FORSYTHIA SUSPENSA* VAHL WITH DIFFERENT EXTRACTION METHODS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

by

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With different extraction methods, chemical components from the branch, root and bark of Forsythia suspensa (Thunb.) Vahl were analyzed by gas chromatography-mass spectrometry (GC-MS). The result showed that there were a large number of chemical components in Forsythia suspense, in which there were also different components with different extraction solvent ethanol and benzene/ethanol, respectively. Exacted by ethanol vs. benzene/ethanol, the branch had 79 peaks and 55 chemical components in ethanol, and 31 peaks and 17 components in benzene/ethanol, respectively. The root had 32 peaks and 23 chemical components in ethanol, and 38 peaks and 25 components in benzene/ethanol. As to bark, there were 54 peaks and 39 components with ethanol, 25 peaks and 14 components with ethanol. There were also some common components in different parts with different solvent. It could be found that there were 3 and 7 common extracts in the branch, root and bark Forsythia suspense exacted by ethanol and benzene/ethanol, respectively. It was also found that the same part of Forsythia suspense could have common with different extracted methods. Extracted by ethanol and benzene/ethanol, the branch, root and bark of Forsythia suspense had 5, 7 and 12 common components, respectively.

Key words: *Forsythia suspense, GC-MS, extractives, branch, root, bark*

Introduction

Forsythia suspense (Thunb.) Vahl is a perennial deciduous shrub of the family Oleaceae. It is widely distributed in Henan, Hebei, Shandong, Shanxi, Hubei, and Sichuan Province, at an elevation of about 250-2200 m in the hillside shrubs or under forest. Branches of *Forsythia suspense* are clustered and drooping with hollow internode and solid pith. Leaves are opposite, single or trilobate, ovate or ovate-elliptic. Corollas are yellow with 1-3 flowers in leaf axils flowering from March to September. The fruits are ovoid, ovate-elliptic, sparsely lentic surface with a fruiting period from July to September.

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Forsythia suspense is a Chinese herbal medicine with a long history in China. It is found that main medicinal parts of *Forsythia suspense* are its dry fruits with the effects of clearing away heat, detoxifying, dispersing and swelling. There are some studies focusing on chemical composition of its fruits [1-4], and some others focus on leaves [5, 6], flowers [7-9], and seeds [10]. In recent years, due to the widespread use of GC-MS, the composition and content of volatile components in different plants has gradually become an important indicator for evaluating the quality of plants. Branches and roots account for the most biomass of *Forsythia suspense*, whereas, studies on its branches and roots are rarely reported. By using different methods, this experiment intends to analyze the difference of volatile components in different parts of *Forsythia suspense*, and provide a certain guiding role for the rational use of *Forsythia suspense*.

Material and methods

Experimental materials

The branch, root and bark of *Forsythia suspense* were collected from Luanchuan forest area in Henan Province, China. The chemical constituents of two extracts of *Forsythia suspensa* were determined by GC-MS qualitative analysis technique [11-13]. We named two kinds of extractives as B1, and B2 samples, which were extracted by ethanol, ethanol/benzene (1:2), respectively.

Experimental methods

The GC-MS determination: GC condition: quartz capillary column was 30 mm × 0.25 mm × 0.25 um, starting at 50 ºC, without retention, followed by a rate of 8 ºC/min up to 250 ºC without retention, and then finally at a rate of 5 ºC/min to 300 ºC without retention. The temperature of the inlet was 250 ºC, column flow was 1.0 ml/min, split ratio was 20:1, and the carrier gas was high helium.

The MS condition: ionization mode was EI, the electron energy was 70 Ev, the temperature of ion source was 230 ºC, the temperature of quadrupole was 150 ºC, scan the starting point was 30-600, the wiley7n.1 standard spectrum and computer search qualitative were used [14, 15].

Results and discussion

Comparison of main chemical composition of the same part with different treatments

With different extraction solvent in GC-MS analysis, different parts as branch, root and bark of *Forsythia suspensa* had different peaks and chemical components, fig. 1 and tab. 1. Exacted by ethanol, the branch of *Forsythia suspensa* had 79 peaks and 55 chemical components, its root had 32 peaks and 23 chemical components, and its bark had 54 peaks and 39 chemical components, respectively. When exacted byethanol/benzene (1:2), its branch had 31 peaks and 17 chemical components, its root had 38 peaks and 25 chemical components, and its bark had 25 peaks and 14chemical components, respectively.

With different extraction solvent, chemical component from different parts of *Forsythia suspense* had different proportion, too. By using ethanol, the highest proportion component was 5-Undecanol, 2-methyl- (19.96%) in brach, Phenol, 4,4'-(tetrahydro-1H,3H-furo[3,4-c] furan-1,4-diyl)bis[2-methoxy- (53.79%) in root, and 2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]-, (3R-trans)- (28.49%).When extracted with benzene/ethanol, the highest proportion was Phenol, 4,4'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis[2-methoxy- (53.94%) in branch, 2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl) methyl]-, (3R-trans)- (40.91%) in root, Phenol, 4,4'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis[2-methoxy- (43.54%) in bark.

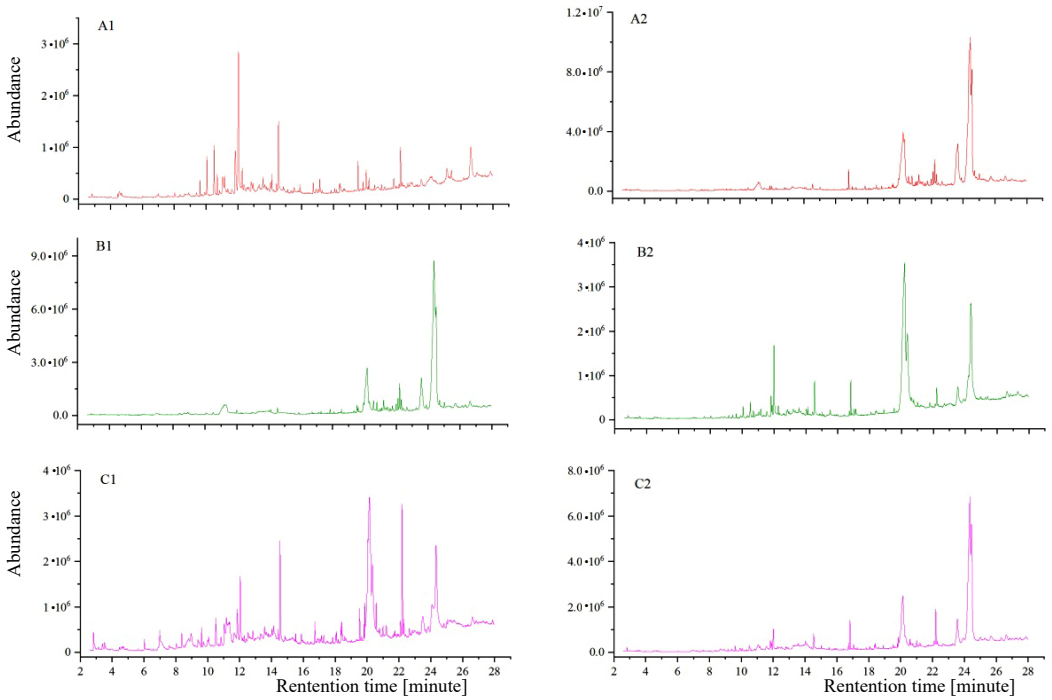

Figure 1. Total ion chromatograms of *Forsythia suspensa* which was extracted by ethanol and benzene/ethanol; *Notes: A, B, C refers to the branch, root, and bark of Forsythia suspense, respectively. 1, 2 refers to ethanol and benzene/ethanol, respectively*

From fig. 1 and tab. 1, it can be seen that the identified compounds can be classified into acids, alcohols, sugars, phenols, pyrimidines and the like. Albuterol, also known as salbutamol, wattene, bismuthidine, chuanle, and hydroxybutyrate, is a clinically used anti-asthmatic drug, a selective β2 receptor agonist. The 9,12-Octadecadienoic acid (Z, Z)- can be used to make lipid-lowering drugs for the treatment and prevention of atherosclerosis. The 10-Undecenoic acid, ethyl ester can be used as a raw material intermediate for the spinal contrast agent iodophenyl ester. Some other compounds can be used as pharmaceutical intermediates, such as Alpha-l-rhamnopyranose, D-Galactose, Benzofuran, 2,3-dihydro-, Thiophene, tetrahydro-2-methyl-, Thymine, [1,2,4] Triazolo [1,5-a] pyrimidin-7-ol, 5-methyl-.

Table 1. The GC-MS analysis of branch, root and bark sample of *Forsythia suspensa* with different extraction methods

No.	Retention time [minute]	Component	Branch		Root		Bark	
			B1	B2	B1	B2	B1	B2
1	2.83	N, N-Dimethyl-O-(1-methyl-butyl)-hydroxylamine					1.12	
2	2.84	Undec-10-ynoic acid, propyl ester	0.45			0.32		
3	3.39	Thiophene, tetrahydro-2-methyl-					0.35	
4	3.53	Thietane, 2,4-dimethyl-					0.54	
5	4.55	Ethanethiol, 2-(diethylboryloxy)-	0.68					
6	6.02	Thymine			0.25		0.48	
7	6.89	Alpha-l-rhamnopyranose	0.28					

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Table 1. (Continuation)

No.	Retention time [minute]	Component	Branch		Root		Bark	
			B1	B2	B1	B2	B1	B2
8	6.99	D-Galactose			0.41		2.66	1.79
9	6.99	.beta.-D-Glucopyranose, 1-thio-,1-[N-hydroxy-5-(methylthio) pentanimidate]	1.10					
10	7.58	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	0.26					
11	8.02	Benzofuran, 2,3-dihydro-	0.45				0.31	
12	8.37	Decanoic acid, 3-hydroxy-, methyl ester					0.67	
13	8.89	Lactose	0.79	0.36			2.62	1.08
14	8.96	.alpha.-d-Mannofuranoside, isopropyl-					1.76	
15	9.39	Phenol, 2-methyl-5-(1-methylethyl)-	0.37					
16	9.63	.epsilon.-N-Formyl-L-lysine					0.50	
17	9.63	5-Ethoxy-2-ethoxymethyl-3,4-dihydro-2H-pyrrole	0.87					
18	10.05	9-Oxa-bicyclo[3.3.1]nonane-1,4-diol	2.33					
19	10.06	Melezitose	3.37		0.49	0.64	12.11	0.56
20	10.50	cis-1,2-Cyclododecanediol	3.64					
21	10.51	9-Decenoic acid				0.81	1.49	0.54
22	10.69	2-Hydroxy-6-methyl-3-cyclohexen-1-carboxylic acid	1.18					
23	10.83	Benzeneethanol, 4-hydroxy-	0.33				0.48	
24	11.03	9-[2-Deoxy-.beta.-d-ribohexopyranosyl] purin-6(1H)-one	2.86			0.50	1.54	
25	11.16	trans-Isoeugenol	1.85			0.41		
26	11.16	D-Fructose, 1,3,6-trideoxy-3,6-epithio-			2.78			
27	11.16	d-Mannose	0.22			0.28	3.60	0.40
28	11.29	Sucrose		2.92	2.95			
29	11.36	Cyclohexanepropanoic acid, 3,4-dihydroxy-	0.52					
30	11.83	1,3-Dioxolane, 4-[[(2-methoxy-4-octadecenyl)oxy]methyl]-2,2-dimethyl-	6.28					
31	11.88	Pyrazol-4-amine, 1-(4-fluorobenzyl)-				0.54		
32	12.03	5-Undecanol, 2-methyl-	19.96			4.33		
33	12.04	1-[2-Methyl-3-(methylthio)allyl]cyclohex-2-enol				1.10	4.04	1.57
34	12.26	9-Oxabicyclo[3.3.1]nonane-2,6-diol	1.97			0.71		
35	12.53	2-Oxabicyclo[3.3.0]oct-7-en-3-one, 7-(1-hydroxypentyl)-					0.56	
36	12.61	trans-2-Dodecenoic acid	0.47			0.24		
37	12.83	(Hexahydropyrrolizin-3-ylidene)-acetaldehyde					0.36	
38	12.83	[1,2,4]Triazolo[1,5-a]pyrimidin-7-ol, 5-methyl-	1.37			0.56		
39	12.94	Albuterol	0.78					
40	13.56	Methyl 4,5-tetradecadienoate	0.78					
41	14.04	10-Undecenoic acid, ethyl ester	1.18			0.29		
42	14.12	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	0.92			0.25		
43	14.42	.beta.-(4-Hydroxy-3-methoxyphenyl) propionic acid	0.59					
44	14.54	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	5.76	0.35	0.47	1.89	3.56	1.08
45	14.86	1-Methylverbenol, methyl ether	0.23					

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Table 1. (Continuation)

No.	Retention time [minute]	Component	Branch		Root		Bark	
			B1	B2	B1	B2	B1	B2
46	15.33	Undec-10-ynoic acid, tetradecyl ester	0.22					
47	15.52	Undec-10-ynoic acid, butyl ester	0.90					
48	15.88	9-Amino-1-methyl-3,6-diazahomoadamantane					0.31	
49	15.88	Phthalic acid, butyl oct-3-yl ester	0.39					
50	16.72	n-Hexadecanoic acid	0.70				0.62	
51	16.82	Dibutyl phthalate		0.71		1.04		1.27
52	16.90	3-(1-Acetyl-2,2-dimethyl-5-oxocyclopentyl)-propionic acid, methyl ester	0.41					
53	17.06	Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1. alpha.,2.alpha.,3.beta.,6.alpha.)]-	0.33			0.54		
54	17.13	trans-Sinapyl alcohol	1.14			0.32		
55	17.28	2-Propen-1-ol, 3-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	0.21					
56	18.08	1-(2,4-Dihydroxybenzoyl)-3-ethyl-5-trifluoromethyl-5-hydroxy-2-pyrazoline	0.33					
57	18.08	3-Penten-2-one,4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (E)-					0.38	
58	18.20	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	0.20					
59	18.36	9,12-Octadecadienoic acid (Z,Z)-	0.73				0.49	
60	18.41	9,12-Octadecadienoyl chloride, (Z,Z)-	0.98					0.42
61	18.41	Oleic Acid					1.16	
62	19.54	Tributylacetylcitrate	1.65		0.47		0.92	
63	19.80	Geranylisovalerate					0.42	
64	19.87	Junenol	0.66					
65	19.87	Selin-6-en-4.alpha.-ol					1.33	0.59
66	20.05	2,4,4-Trimethyl-3-(3-methylbutyl) cyclohex-2-enone	1.99					
67	20.13	2(3H)-Furanone, dihydro-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]-, (3R-trans)-		21.56	13.76	40.91	28.49	18.34
68	20.23	Ferruginol	0.83					
69	20.33	Dihydrofuran-2-one, 4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)-		11.51	13.35	33.20	14.60	20.78
70	20.57	3.beta.-(Methoxymethoxy)androst-5-en-17-one		0.25	0.85		0.91	
71	20.57	3.beta.-Benzoyloxyandrost-5-en-17-one	0.33					
72	21.00	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	0.33				0.32	
73	21.21	Phenol, 2-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl)-			0.90			
74	21.21	2-Hydroxyfluorenone, trimethylsilyl ether		0.76			0.47	
75	21.45	3-Benzofuranmethanol, 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-5-(3-hydroxy-1-propenyl)-7-methoxy-		0.32		0.51		
76	21.78	2.alpha.-Methyl-5.alpha.-androstan-3.alpha.-ol-17-one	1.01					

→

Table 1. (Continuation)

No.	Retention time [minute]	Component	Branch		Root		Bark	
			B1	B2	B1	B2	B1	B2
77	22.10	Salsoline		0.74			0.52	
78	22.11	3-(1-Methyl-1-silacyclobutyl)benzoic acid	0.00		0.80			
79	22.11	Ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-						0.44
80	22.20	13-Isopropylpodocarpen-12-ol-20-al	3.09	1.88	2.25	1.15	4.58	2.45
81	22.31	Androsta-5,7-diene, 4,4-dimethyl-		0.61	0.71		0.57	
82	22.31	Retinal	0.96					
83	22.66	Pisiferol	0.33	0.31	0.28			
84	23.51	2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-(3. alpha.,5a.alpha.,9.alpha.,9a.alpha.)]-		1.44	1.32	3.36	3.90	3.87
85	23.93	2-(2,6,6-Trimethylcyclohex-1-enyl) cyclopropanecarboxylic acid, methyl ester						0.88
86	24.08	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol					1.25	
87	24.37	Phenol, 4,4'-(tetrahydro-1H,3H-furo[3,4-c] furan-1,4-diyl)bis[2-methoxy-		53.94	53.79	6.10		43.54
88	24.71	(4aR,5S)-1-Hydroxy-4a,5-dimethyl-3-(propan-2-ylidene)-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one			0.80			
89	25.01	9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-, (4aS-trans)-			0.35			
90	25.12	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	12.09					
91	25.72	(+)-Lariciresinol		1.01	1.02			
92	26.61	.gamma.-Sitosterol	8.05					
93	26.64	.beta.-Sitosterol			1.17			
		other	0.30	1.32	0.84			0.39

Notes: B1, ethanol, B2, ethanol/benzene (1:2).

Comparison of common components in the different part

By using ethanol as a solvent, the component of extracts from branch, root and bark were very different. Three common components were found as (E)-4-(3-Hydroxyprop-1-en-1-yl)-2 -methoxyphenol, 13-Isopropylpodocarpen-12-ol-20-al and Tributylacetylcitrate, which accounted for 10.49%, 3.19%, 9.06% of the total volatile components of branch, root and bark, respectively. From different parts branch, root and bark, (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol accounted for 5.76%, 0.47%, 3.56%,13-Isopropylpodocarpen -12-ol-20-al accounted for 3.09%, 2.25% and 4.58%, and Tributylacetylcitrate accounted for 1.65%, 0.47% and 0.92%, respectively.

When using benzene/ethanol as a solvent, there were seven common components in the extracts of branch, root and bark, which accounted for 91.40%, 87.65%, and 91.33% of the total volatile components of the three different parts, respectively. Among the seven components, there were three components accounting for a large proportion. Phenol, 4,4'-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis[2-methoxy- had high proportion with 53.94%, 6.10% and 43.54% of

total products of branch, root and bark, respectively. The 2(3H)-Furanone, dihydro-3, 4-bis[(4-hydroxy-3-methoxyphenyl)methyl]- accounted for 21.56%, 40.91% and 18.34% of the of branch, root and bark, respectively. The (3R-trans)-Dihydrofuran-2-one, 4-(3,4-dimethoxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)- accounted for 11.51%, 33.20% and 20.78%, respectively. The other four common ingredients are 13-Isopropylpodocarpen-12-ol-20-al, 2H-3,9a-Methano-1-benzoxepin, octahydro-2,2,5a,9-tetramethyl-, [3R-, Dibutyl phthalate, and (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol, and the sum of these four substances had only small proportion with 4.38%, 7.44% and 8.67% inthe total volatile components of branch, root and bark, respectively. This result was similar with other study that different parts as fruit, leave, flower, and seed of *Forsythia susoensa* had different chemical composition and active ingredients content [16-18].

Comparison of common components in the same part

There were 15 common components from *Forsythia suspense*, and there were 5, 7, and 12 common components in the extracts of branch, root and bark, when ethanol and benzene/ethanol were used as solvents, respectively. The five same substance from branch were found as (E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol, 13-Isopropylpodocarpen-12-ol-20-al, Melezitose, Tributylacetylcitrate and Pisiferol, which accounted for 13.86% and 3.68% of the branch with the ethanol and benzene/ethanol, respectively. The correlation between the two treatment results was very poor ($r = 0.14$, $P = 0.819$). Processed with the two different solvent, there were seven common chemical components from roots with high proportion (85.42% and 87.25%) and poor correlation ($r = 0.141$, $P = 0.763$), and there were also 12 common extractions from bark with high percentage (83% and 53.05%) and significant correlation ($r = 0.82$, $P= 0.001$).On the whole, there are more identical components from bark than branch and root of *Forsythia suspense* when extracted with ethanol and benzene/ethanol, respectively.

Conclusions

Ethanol and benzene/ethanol are two important chemical solvents used to extract compounds from plants. When *Forsythia suspensa* were extracted with ethanol and benzene/ethanol, the obtained chemical substances from the branches, roots and bark were different, and the substances from ethanol extract were more than from benzene/ethanol. Exacted with ethanol, there were more acid compound (such as 10-Undecenoic acid, ethyl ester, trans-2-Dodecenoic acid, 9,12-Octadecadienoic acid (Z, Z)-, n-Hexadecanoic acid), carbohydrate (such as Sucrose, d-Mannose, Alpha-l-rhamnopyranose, Lactose), lipid (10-Undecenoic acid, ethyl ester, beta.-(4-Hydroxy-3-methoxyphenyl)propionic acid, Tributylacetylcitrate).

On the other hand, there were also some common components in different parts with different solvent. Exacted by ethanol, there were 3 common components in branch, root and bark, whereas there were 7 common components in the three parts with benzene/ethanol. It was also found that the same part of *Forsythia suspense* could have common with different extracted methods. Disposed by ethanol and benzene/ethanol, the branch, root and bark of *Forsythia suspense* had 5, 7, and 12 common extracts, respectively. With different solvent ethanol and benzene/ethanol, components exacted from different part branch, root and bark of *Forsythia suspense* were different. At the same time, there were also some common extracts in the same parts. This gives a clue that we could select some kind solvent with different needs when extracted components from different part of *Forsythia suspense*.

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