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ORIGINAL ARTICLE

A Study on the Chemical Compositions of the Yinqiaosan (Lonicerae and Forsythiae Powder) at Different Time of Later-decoction by Gas Chromatography Mass Spectrometry

Yachun Shu[1,2,†], Yajun Chen[1,†], Kunming Qin[2,3], Xiao Liu[2], Baochang Cai[2]

[1]Department of Pharmacy, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210029, [2]Engineering Center of State Ministry of Education for Standardization of Chinese Medicine Processing, Nanjing University of Chinese Medicine, Nanjing 210046, [3]Research and Development Center, Nanjing Haichang Chinese Medicine Group Corporation, Nanjing 210061, China

†These authors contributed equally to this work

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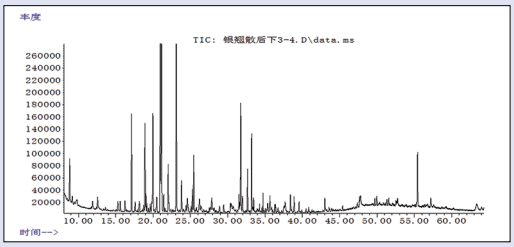
ABSTRACT

Background: Yinqiaosan (Lonicerae and Forsythiae Powder), as a famous prescription of Dr. Wu Jutong in Qing dynasty of China, has the effects of diaphoresis cooling, fire-purging, and detoxicaton. It is mainly used in the treatment of influenza, hand-foot-mouth disease, esophagitis, pneumonia, acute tonsillitis, mumps, and other viral infections. It is one of the widely used traditional Chinese medicine prescriptions with proven curative effects in clinical use. **Objective:** To research the material basis of Yinqiaosan decoction when decocting mint, herba schizonepetae in different length of later-decoction time, to find the influence on volatile components of Yinqiaosan decoction decocted later in different length of time, to lay the foundation to further clarify the after-decoction mechanism of Yinqiaosan, and the specification of Yinqiaosan decoction process. **Materials and Methods:** Gas chromatography mass spectrometry method is used to analyze the volatile components of Yinqiaosan decoction samples decocted for 0, 3, 5, 8, and 10 min. **Results:** Later-decocting mint and herba schizonepetae at different time when decocting Yinqiaosan had a significant influence on the volatile components of the solution. 54 different chemical components were identified: 25 were identified when later-decocting the sample for 3 min; 13 were identified when later-decocting the sample for 5 min; 11 were identified when later-decocting the sample for 8 min; 7 were identified when later-decocting the sample for 10 min; and 26 were identified when later-decocting the sample for 0 min. There were more volatile components in the sample after-decocted for 3 min. A total of 54 different chemical components were identified in different later-decocting solution samples. These components form the basis of the Yinqiaosan drug effect. **Conclusions:** The length of later-decoction time of mint and herba schizonepetae was confirmed to be 3 min when decocting Yinqiaosan.

Key words: Gas chromatography mass spectrometry, later-decoction, later-decoction time, volatile components, Yinqiaosan decoction

SUMMARY

- Later-decocting mint and herba schizonepetae at different time had a significant influence on the volatile components of the solution
- Fifty-four different chemical components were identified in different later-decocting solution samples
- There were more volatile components in the sample after-decocted for 3 min
- The volatile components content was high. These components form the important basis of the Yinqiaosan drug effect.
- Total ion flow diagram of volatile oils in the Yinqiaosan sample with mint, herba schizonepetae after 3 min decoction.



Abbreviations used: GC-MS: Gas chromatography mass spectrometry, TCM: Traditional Chinese medicine.

Correspondence:

Prof. Baochang Cai,
No. 12, Yongjin Road,
Nanjing High-tech Industry Zone,
Jiangsu 210061, China.
E-mail: bccai@126.com

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INTRODUCTION

The Yinqiaosan (Lonicerae and Forsythiae Powder), as a famous prescription of Dr. Wu Jutong in Qing dynasty of China, is believed to have the effects of diaphoresis cooling, fire-purging, and detoxicaton. It is mainly used in the treatment of influenza,[1] hand-foot-mouth disease,[2] esophagitis, pneumonia, acute tonsillitis, mumps, and other viral infections. It is one of the widely used traditional Chinese medicine (TCM) prescriptions with proven curative effects in clinical use.

Later-decoction is a special and common method for decocting herbs in TCM. Volatile materials, such as mint and amomum cardamomum, are added later during the decoction, usually 5–10 min before the end of a decoction since they are easy to volatilize or destroy when decocting due to their heavy volatile oil content. Later-decoction helps prevent losing the volatile components of the medicine and the effective constituent from being destroyed and decomposed, and thus plays an important role

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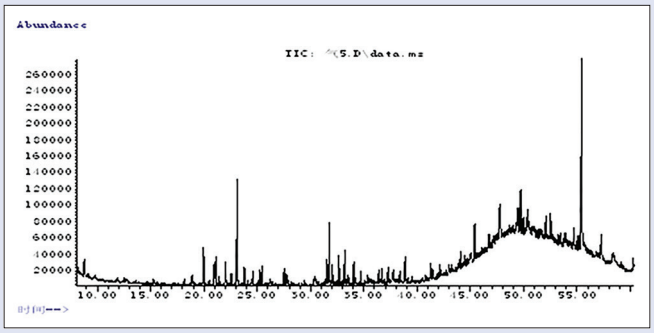

Figure 1: Total ion flow diagram of volatile oils in the Yinqiaosan sample with Mint, Herba schizonepetae after 0 min decoction

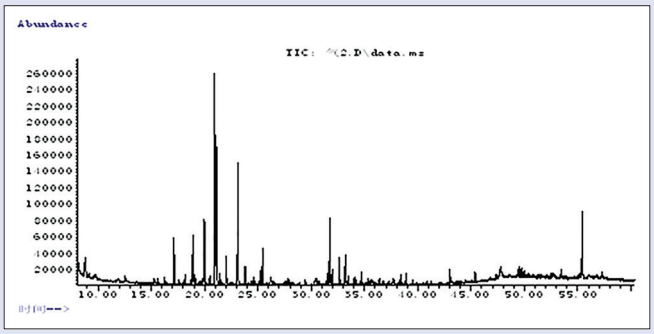

Figure 2: Total ion flow diagram of volatile oils in the Yinqiaosan sample with Mint, Herba schizonepetae after 3 min decoction

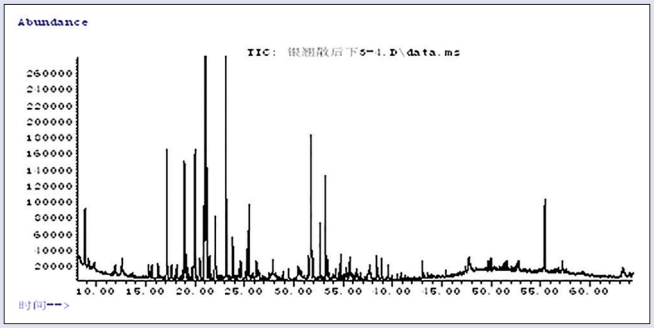

Figure 3: Total ion flow diagram of volatile oils in the Yinqiaosan sample with Mint, Herba schizonepetae after 5 min decoction

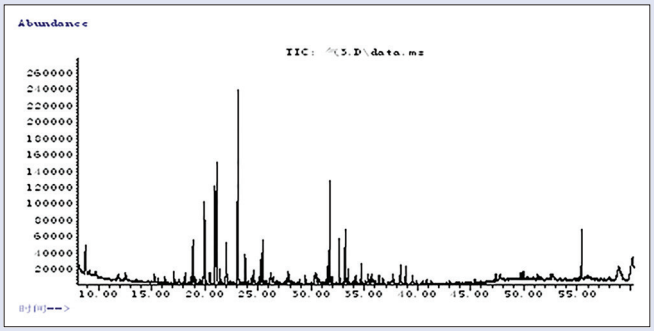

Figure 4: Total ion flow diagram of volatile oils in the Yinqiaosan sample with Mint, Herba schizonepetae after 8 min decoction

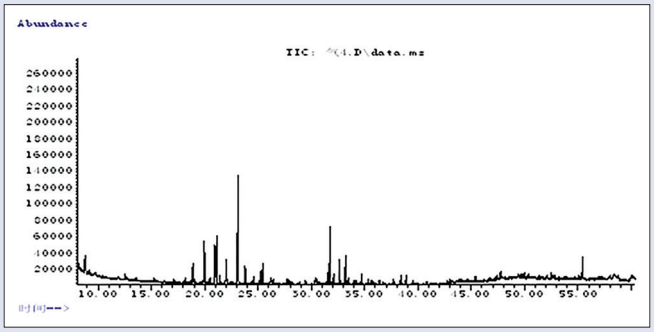

Figure 5: Total ion flow diagram of volatile oils in the Yinqiaosan sample with Mint, Herba schizonepetae after 10 min decoction

in ensuring the decocting quality.[3] Yinqiaosan is composed of 9 prepared Chinese crude drugs including *Lonicerae Japonicae* Flos, Forsythiae Fructus, Menthae Haplocalycis Herba, Schizonepetae Herba, Platycodonis Radix, Arctii Fructus, Sojae Semen Praeparatum, Glycyrrhizae Radix Et Rhizoma, and Lophatheri Herba, wherein Menthae Haplocalyx Herba and Schizonepetae Herba are rich in volatile materials and later-decoction becomes necessary. The experiment described in this study was to study the material basis of Yinqiaosan decoction when decocting mint, herba schizonepetae at different time length of later-decoction, and to find its effects on volatile components of the Yinqiaosan decoction by gas chromatography-mass spectrometry (GC-MS). And also to lay a foundation for further clarification of the later-decoction mechanism of Yinqiaosan, and the specifications of the decoction process.

MATERIALS AND METHODS

Reagents and materials

Agilent 6890/5975B GC-MS made in American and G1701DAD.03.00.611 workstation; NIST05 standard mass spectrometry database.

The Yinqiaosan medicinal pills were purchased from Nanjing Haichang Chinese Medicine Group Corporation with source herbs identified as follows: *L. japonicae* Flos (the dried buds of *L. Japonica* Thunb.), Forsythiae fructus (the dried fruits of *Forsythia suspense* (Thunb. Vahl), Menthae *Haplocalycis*Herba (the dried aerial parts of Mentha haplocalyx Briq.), Schizonepetae Herba (the dried aerial parts of Schizonepeta tenuisfolia Briq.), Arctii fructus (the dried ripe fruits of Arctium lappa L.), Platycodonis Radix (the dried roots of *Platycodon grandiflorum* (Jacq.) A. DC.), Sojae Semen Praeparatum (the fermentation products of mature seeds of Glycine max (L.) Merr.), Glycyrrhizae Radix Et Rhizoma (the dried roots and rhizomes of *Glycyrrhiza uralensis* Fisch.), and Lophatheri Herba (the dried stems and leaves of Lophatherum gracile Brongn.).

Gas chromatography and mass spectrometry conditions

Chromatographic column: HP-5MS quartz capillary column (30.0 mm × 0.25 mm × 0.25 μm); temperature of injection port: 250°C; temperature programming: Initial temperature at 60°C, rising to 150°C at 3°C/min, to 180°C at 10°C/min, and to 250°C at 3°C/min and then kept for 5 min; sample amount: 2 μL; carrier gas: Helium (purity >99.99%); flow velocity: 1.0 mL/min; splitless.

EIion source; ion source temperature: 230°C; temperature of the quadrupole: 150°C; temperature of the interface port: 220°C; electron

multiplier voltage: 1717.6 V; electron energy: 70 eV; mass scanned area: 35–500 m/z.

Sample preparation

Measuring with precision, the following samples were collected respectively: *L. japonicae* Flos (9 g), Forsythiae Fructus (9 g), Arctii Fructus (9 g), Platycodonis Radix (6 g), Sojae Semen Praeparatum (5 g), Glycyrrhizae Radix Et Rhizoma (5 g), Lophatheri Herba (4 g), Menthae Haplocalycis Herba (6 g), and Schizonepetae Herba (5 g). They were mixed in 464 mL of distilled water (8 times of prescription total dosage, V/W) and soaked for 30 min.

Later-decocting the sample for 0 min: Decocted to boiling with strong heating fire using traditional marmite, after that, decocted for 15 min with gentle heating fire. The decocted solution was then immediately filtered while it is hot, and its volume was measured, and then purified water was added to the decocted solution until a certain volume is reached.

Later-decocting the sample for 3 min: Decocted to boiling by traditional marmite with strong heating fire, after that, decocted for 15 min with gentle heating fire (later-decocting them for 12 min, add Menthae Haplocalycis Herba and Schizonepetae Herba in and decocting them for 3 min). The decocted solution then immediately filtered while it is hot, and its volume is measured, and then purified water was added to the decocted solution until a certain volume is reached.

Later-decocting the sample for 5 min: Decocted to boiling by traditional marmite with strong heating fire, after that, decocted for 15 min with gentle heating fire (later-decocting them for 10 min, add Menthae Haplocalycis Herba and Schizonepetae Herba in and decocting them for 5 min). The decocted solution then immediately filtered while it is hot, and its volume is measured, and then purified water was added to the decocted solution until a certain volume is reached.

Later-decocting the sample for 8 min: Decocted to boiling by traditional marmite with strong heating fire, after that, decocted for 15 min with gentle heating fire (later-decocting them for 7 min, add Menthae Haplocalycis Herba and Schizonepetae Herba in and decocting them for 8 min). The decocted solution then immediately filtered while it is hot, and its volume is measured, and then purified water was added to the decocted solution until a certain volume is reached.

Later-decocting the sample for 10 min: Decocted to boiling by traditional marmite with strong heating fire, after that, decocted for 15 min with gentle heating fire (later-decocting them for 5 min, add Menthae Haplocalycis Herba and Schizonepetae Herba in and decocting them for 10 min). The decocted solution then immediately filtered while it is hot, and its volume is measured, and then purified water was added to the decocted solution until a certain volume is reached

Purified water was added to each sample till it reached a certain volume. About 50 mL of decoction was taken from each sample, extracted with 50 mL of chloroform, added with proper amount of anhydrous sodium sulfate, and then kept untouched for 24 h. The chloroform layer was taken and filtered through 0.45 μm filter membrane and thus, the test solution was obtained.

Analyzing method

The HP-5MS chromatographic column was used to optimize chromatographic separation condition and determine the optimum separation condition. Samples were analyzed according to the selected analysis conditions by GC-MS. NIST 05 Standard Mass Spectral Search Library was used for searching and the peak area normalization method for measuring the relative proportion of each component.

RESULTS AND DISCUSSION

Based on the above experimental method and conditions, the volatile components of the Yinqiaosan decoction later-decocted for 0, 3, 5, 8, and 10 min were analyzed. Total ion flow diagram of each sample is shown in Figures 1-5. Samples were analyzed according to the selected analysis conditions by GC-MS. 54 components were identified through NIST 05 Standard Mass Spectral Search Library and a computer retrieval and artificial map analysis. With peak area normalization method, relative proportion of each component is shown in Table 1.

The chemical compositions of the Yinqiaosan decoction were very complicated. The volatile compounds included mostly monoterpenes and its oxygen-containing derivatives, some nonterpenoid aromatics, and aliphatic series. Search results from the mass spectrum database demonstrated the following chemical compositions: Alcohols, ketones, alkenes, esters, and alkanes.[4] GC-MS method was used to measure the volatile components of the Yinqiaosan decoction, and 54 kinds of chemical compositions were identified. 25 different chemical compositions were identified when later-decocting the sample for 3 min; 13 were identified when later-decocting the sample for 5 min; 11 were identified when later-decocting the sample for 8 min; 7 were identified when later-decocting the sample for 10 min; 26 were identified when later-decocting the sample for 0 min (i.e., mint, herbal schizonepetae were decocted with other medicines at the same time).[5]

The common composition of these 5 samples was found to be furan menthone. However, the sample later-decocted for 0 min contained the lowest amount of it. Except the sample later-decocted for 0 min, the common compositions of the other 4 samples were mostly mint and herba schizonepetae. Relative proportion of the samples later-decocted for 0, 3, 5, and 8 min were 11.98%, 14.88%, 12.84%, and 12.93%, respectively. Relative proportion of 2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, 4-methyl-1-(1-methylethenyl)-cyclohexanol in the sample later-decocted for 5 min was the highest. The sample later-decocted for 3 min also contained (R)-(+)-3-methylcyclohexanone (existed in herba schizonepetae), phenylethyl alcohol (existed in lophatherum gracile),[6] (+)-isomenthone (existed in mint), cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-, 4-terpineol, 2-methyl-5-(1-methylethenyl)-cyclohexanol, and other chemical compositions, such as vanillin, 2,5-bis (1,1-dimethylethyl)-phenol, and 3-Homoadamantanol. The sample later-decocted for 5 min contained (R)-(+)-3-methylcyclohexanone, menthone, pulegone, 2-octynal diethyl acetal, 1,2-cyclohexanediol, 2,5-bis (1,1-dimethylethyl) -phenol, n-hexadecanoic acid, 2-Cyclohexen-1-one, etc., which were mostly found in mint and herba schizonepetae.[7,8] The sample later-decocted for 8 min contained 3-methylcyclohexanone, cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2S-trans)-, and 5-methyl-2-(1-methylethylidene) cyclohexanone, which existed in mint, and 2-methylene bornane was detected in the sample later-decocted for 10 min; later-decocted for 0 min, the following compounds were detected apart from the original 26: Sulfurous acid, butyl nonyl ester, 1-chlorooctadecane, 2,4-bis (1,1-dimethylethyl)-phenol, hexadecane, 10-methylnonadecane, tetratetracontane, octadecane, 1-chlorooctadecane, tetrapentacontane, beta-iso-methyl ionone, bromoacetic acid, octadecyl ester, pregnane, sulfurous acid, octadecyl-2-propyl ester, 5-butyl-6-hexyloctahydro-, sulfurous acid, eicosane, and so on. There were only trace amounts of original volatile components of mint, herba schizonepetae, lophatherum gracile in the decoction left. Only a small amount of 2-cyclohexen-1-one, 3-methylcyclohexanone, 2,6,6-trimethyl-2,4-cycloheptadien-1-one, etc., was left. It can be concluded that since mint and herba schizonepetae

Table 1: Analysis of main volatile components in the Yinqiaosan decoction samples with different time by gas chromatography-mass spectrometer

Retention time (min)	Compound name	Molecular formula	Relative molecular mass	Relative percentage				
				Later-decocting for 3 min	Later-decocting for 5 min	Later-decocting for 8 min	Later-decocting for 10 min	Later-decocting for 0 min
8.793	3-methylcyclohexanone	$C_7H_{12}O$	112.16	-	-	4.03	-	1.86
8.83	(R)-(+)-3-methylcyclohexanone	$C_7H_{12}O$	112.17	2.29	3.27	-	-	-
15.281	Phenylethyl alcohol	$C_8H_{10}O$	122.16	0.74	-	-	-	-
17.105	Cyclohexanone, 5-methyl-2-(1- methylethyl)-, (2S-trans)-	$C_{10}H_{18}O$	154.25	-	-	0.96	-	-
17.121	Menthone	$C_{10}H_{18}O$	154.25	-	4.41	-	-	-
17.126	Cyclohexanone, 5-methyl- 2-(1-methylethyl)-, trans-	$C_{10}H_{18}O$	154.24	4.59	-	-	-	-
17.597	(+)-isomenthone	$C_{10}H_{18}O$	154.24	0.68	-	-	-	-
18.185	4-terpineol	$C_{10}H_{18}O$	154.25	0.57	-	-	-	-
18.907	2-methyl-5-(1-methylethenyl)-cyclohexanol	$C_{10}H_{18}O$	154.25	4.26	-	-	-	-
19.052	2-methyl-5-(1-methylethenyl)-cyclohexanone	$C_{10}H_{16}O$	152.23	19.05	-	-	-	-
19.816	4-methyl-1-(1-methylethenyl)-cyclohexanol	$C_{10}H_{16}$	136.00	0.4	4.46	3.43	3.26	-
20.95	Pulegone	$C_{10}H_{16}O$	152.23	-	19.34	-	-	-
20.956	5-methyl-2-(1-methylethylidene)-cyclohexanone	$C_{10}H_{16}O$	152.23	17.93	-	7.92	-	1.24
21.116	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{14}O$	150.22	-	-	-	-	2.06
21.132	2-Cyclohexen-1-one, 2-methyl -5-(1-methylethenyl)-, (S)-	$C_{10}H_{14}O$	150.22	11.98	14.88	12.84	12.93	-
22.571	Sulfurous acid, butyl nonyl ester	$C_{13}H_{28}O_3S$	264.17	-	-	-	-	0.66
23.806	2,4 (1H,3H)-Pyrimidinedione, 5-(1,1-dimethylethyl)-	$C_8H_{12}N_2O_2$	168.08	1.70	-	-	-	-
23.111	2-octynal diethyl acetal	$C_{12}H_{22}O_2$	198.30	-	11.20	-	-	-
24.582	1-chlorooctadecane	$C_{18}H_{37}Cl$	288.94	-	-	-	-	0.97
25.267	1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	$C_{10}H_{18}O_2$	170.24	1.14	1.31	2.09	2.48	-
25.432	2-Methylene Bornane	$C_{11}H_{18}$	150.00	-	-	3.93	-	-
25.433	3-methyl-6-(1-methylethylidene)-2-cyclohexen-1-one	$C_{10}H_{14}O$	150.21	-	-	-	4.46	1.03
25.443	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-	$C_{10}H_{14}O$	150.22	2.98	3.5	-	-	1.35
26.197	Thichloroacetic acid nonyl ester	$C_{11}H_{19}Cl_3O_2$	289.62	1.03	-	-	-	-
27.855	Vanillin	$C_8H_8O_3$	152.14	0.75	-	-	-	-
31.744	Mint furanone	$C_{10}H_{14}O_2$	166.00	5.57	6.73	9.08	12.34	4.31
31.99	2,4-Bis (1,1-dimethylethyl)-phenol	$C_{14}H_{22}O$	206.33	-	-	-	-	0.93
31.968	Phenol, 2,5-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	206.32	1.06	1.55	-	-	-
32.648	3-Homoadamantanol	$C_{11}H_{18}O$	166.26	1.64	-	-	-	-
33.199	Octahydro-1H-pyrido (1,2-c) pyrimidine	$C_8H_{16}N_2$	140.20	2.49	-	-	-	-
33.477	2,4 (1H,3H)-Pyrimidinedione, 5-amino-	$C_4H_5N_3O_2$	127.10	0.54	-	-	-	-
33.477	4-Amino-2,6-dihydroxypyrimidine	$C_4H_5N_3O_2$	127.10	-	-	0.90	-	-
34.049	Hexadecane	$C_{16}H_{34}$	226.44	-	-	-	-	1.00
35.333	(6R,7E,9R)-9-Hydroxy-4,7-megastigmadien-3-one	$C_{14}H_{17}NO_2$	231.29	0.47	-	-	-	-
36.317	2-fluorobenzoic acid, undec-2-enylester			0.22	-	-	-	-
36.408	10-methylnonadecane	$C_{20}H_{42}$	282.55	-	-	-	-	0.56
36.707	Tetratetracontane	$C_{44}H_{90}$	619.19	-	-	-	-	0.80
38.868	Octadecane	$C_{18}H_{38}$	254.49	-	-	-	-	1.88
38.89	2-cyclohexen-1-one,4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222.30	0.86	-	1.52	-	-
38.89	2-cyclohexen-1-one,4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222.28	-	1.15	-	-	-
43.003	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	0.80	1.48	-	-	-
44.062	1-chlorohexadecane	$C_{16}H_{33}Cl$	260.89	-	-	-	-	1.02
44.452	Tricyclo[4.2.2.2 (2,5)]dodecan-3-one	$C_{12}H_{18}O$	178.30	-	-	-	-	1.21
46.736	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	914.68	-	-	-	-	1.34
47.143	Bicyclo[3.1.1]heptan-3-one, 2-(but-3-enyl)-6,6-dimethyl-	$C_{10}H_{14}O$	150.21	-	-	-	-	1.02
47.383	E, E, Z-1,3,12-nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294.47	-	-	-	-	0.80
47.742	Beta-iso-methyl ionone	$C_{14}H_{22}O$	206.33	-	-	-	-	3.88
49.705	Bromoacetic acid, octadecyl ester	$C_{20}H_{39}BrO_2$	390.21	-	-	-	-	3.09
50.352	Pregnane	$C_{21}H_{36}$	288.51	-	-	-	-	1.39
52.074	Sulfurous acid, octadecyl 2-propyl ester	$C_{21}H_{44}O_3S$	376.64	-	-	-	-	1.62
53.454	1H-Indene, 5-butyl-6-hexyloctahydro-	$C_{19}H_{36}$	264.28	-	-	-	-	0.87
54.679	Sulfurous acid, pentadecyl 2-propyl ester	$C_{18}H_{38}O_3S$	334.50	-	-	-	-	1.11
55.422	Phenol, 2,2'-methylenebis[6- (1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340.50	2.7	6.27	4.2	4.65	24.12
57.257	Eicosane	$C_{20}H_{42}$	282.55	-	-	-	-	2.32

were not later-decocted with other 7 medicines, chemical compositions of these medicines interacted with each other under high heat decoction, triggering chemical changes, and yielding new compounds; and the longer decocting time is responsible for the heavy loss of volatile components in mint and herba schizonepetae.

CONCLUSIONS

The experiment found that different time patterns of later-decoction of mint and herba schizonepetae had a significant influence on their volatile components. The sample in which mint and herba

schizonepetae were later-decocted for 3 min contained more volatile components with high concentration, which are also the important pharmaceutical basis of the Yinqiaosan. Therefore, when decocting Yinqiaosan, the best length of later-decoction time for mint and herba schizonepetae should be 3 min.

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Conflicts of interest

There are no conflicts of interest.

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Baochang Cai

ABOUT AUTHOR

Baochang Cai, is a professor of Nanjing University of Chinese Medicine. Mainly research field: the quality control of Traditional Chinese Medicine, and the mechanism of TCM processing.