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The study on the chromatographic fingerprint of *Fructus xanthii* by microwave assisted extraction coupled with GC–MS

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Abstract

The chromatographic fingerprint of *Fructus xanthii*, a kind of Traditional Chinese Medicines (TCMs), was studied by microwave assisted extraction (MAE) coupled with gas chromatography-mass spectrometry (GC–MS). The optimized conditions of MAE were examined. The method of MAE was evaluated in contrast to heat reflux extraction (HRE) method and by the validation tests of precision and repeatability. The relative standard deviations (RSDs) of retention time and peak area of each component were less than 0.2% and 6%, respectively. Twenty-five different batches of samples collected from different producing areas and the toasting process of *F. xanthii* were studied. The characteristic differences in the producing areas and the chemical variances in the toasting process were obtained and studied by principal components analysis (PCA) and similarity analysis. The trends of main varying components were attempted to be described in order to specify the related pharmacology and toxicology in crude and toasted samples. The results suggest that the chromatographic fingerprint developed by MAE coupled with GC–MS provides useful information to reveal the quality of *F. xanthii* and evaluate the quality changes in the producing process.

Keywords: Microwave assisted extraction; GC-MS; *Fructus xanthii*; Chromatographic fingerprint; Traditional Chinese Medicine

1. Introduction

Fructus xanthii, the fruit of *Xanthium strumarium,* commonly known as Cang'erzi in China, is used for the treatment of cramping and numbness of the limbs, ulcer, sinusitis, catarrhs and pruritus [1]. The chemical components of *F. xanthii* are complex, including volatile components in essential oils and medium extraction components such as amino acid, organic acid and glycosides [2]. In the previous studies on *X. strumarium*, some compounds such as atractyloside (Atr) [3], carboxyatractyloside (carboxyAtr) [3], thiazinedione [4], sesquiterpene lactones e.g. xanthatin [5] and xanthinin [6] were isolated and identified from the different parts of *X. strumarium*, such as leaf, stem and fruit. Despite its healing/curing effects, *F. xanthii* is toxic at high dose and has been shown to cause fatal nephrosis and hypohepatia [2]. The Atr and carboxyAtr are suspected to be the main toxic components of *F. xanthii* [7,8]. In clinical application, *F. xanthii* is always toasted in order to decrease the toxicity [1,2]. However, the toasting procedure affects the chemical components and efficacy of *F. xanthii*. Therefore, it is highly desirable to investigate the chemical composition of *F. xanthii* and the effects of the toasting procedure.

A chromatographic fingerprint is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and/or chemically characteristics in Traditional Chinese Medicines (TCM) sample [9]. In the TCM research, the drugs are complex mixtures, usually containing hundreds of chemical constituents. However, only a few separate markers or pharmacologically active constituents can demonstrate to stand for the complex medicine or their preparations. Therefore, fingerprint technology has been introduced to achieve quality control of herbal medicines in recent years [9–14].

Soxhlet and normal boiling-water extraction method are the common methods in TCM extraction [1], but these methods are time consuming and require large amounts of the organic solvent. Therefore, some efficient and selective methods such as microwave assisted extraction (MAE), supercritical fluid extraction (SFE) [15] and accelerated solvent extraction (ASE) [16] have been adopted for the component study in recent years. Among these methods, MAE is an effective extraction method

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for many kinds of herbs especially when the target compounds for extraction are polar. MAE utilizes the energy of microwaves to arouse molecular movement and rotation of liquids with a permanent dipole leading to a very fast heating of the solvent and sample [17]. Some applications of MAE for biologically active compounds have been reported, such as the extraction of taxanes from *Taxus* biomass [18], tanshinones from the root of *Salvia miltiorrhiza* [19], glycyrrhizic acid from licorice root [20], quercetin and its glycosides from guava leaves [21], and ginsenosides from ginseng root [22], etc.

Due to the powerful separation efficiency and the sensitive detection, GC–MS has become a popular and useful analytical tool in the research field of herbal medicines [23–26]. The method of MAE coupled with GC–MS has been used for some desirable components analysis in TCM studies, such as camphor and borneol in *Flos Chrysanthemi Indici* [27],and paeonol in *Cynanchum paniculatum* and *Paeonia suffruticosa* [28]. In this study, we developed a method for investigating the chromatographic fingerprint of *F. xanthii* by MAE coupled with GC–MS. Chromatographic fingerprint based on this method was established for the characteristic analysis of samples from different producing areas and for the diversity of toasted samples. The fingerprints obtained from this method can provide useful information for the evaluation of the difference in *F. xanthii* grown in the various areas of China and for further interpretation of the quality changes in toasting process.

2. Experimental

2.1. Samples

Twenty-five batches of *F. xanthii* crude samples were purchased from different pharmacies in three producing areas of China (4 batches in Guangxi, 8 batches in Henan and 13 batches in Guangdong). All the samples are in clinical use and have been identified in the pharmacies. Among them, one sample collected from Guangdong was used for the MAE parameters optimization experiment and for the analysis of toasting process. The toasted samples were prepared in an oven by controlling the oven temperature at 210 °C and different toasting time of 1, 2, 3, 4, 5, 6, 7, 9, 11 and 13 min. And then, samples were ground and treated in the same procedures in which the crude samples were done. All the samples were ground to fine powder with particle size of 0.38 mm (40 mesh).

2.2. Sample extraction procedure and preparation

An MDS-6 microwave dissolving/extracting apparatus obtained from Shanghai Xinyi Company (Shanghai, China) was used. Duplicate measurements of each sample were performed for the optimization of MAE conditions. The same batch of *F. xanthii* crude sample from Guangdong, China, was used. Due to the complexity of the components of *F. xanthii*, optimization could only provide the reasonably optimized conditions. The integrality of the chromatogram and the intensity of components were used to evaluate the final operating conditions. Three types of solvents, ethanol, methanol (Analytical grade, Guangzhou Reagent Factory, Guangdong, China) and water, were studied. 2.00 g sample and 30 ml solvent were placed in 200 ml Teflon extraction vessel. The extraction temperature was in the range of the solvents' boiling temperature, and the extraction time was 30 min.

Liquid/solid ratio is another important factor. In the MAE procedure, the liquid/solid ratio (ml/g) of 10:1, 15:1, 25:1 and 30:1 in 200 ml vessel, and 25:1 and 30:1 in 70 ml vessel were investigated. Two grams of sample was placed in a 200 ml vessel or 1.00 g in a 70 ml vessel; ethanol was used as the extraction solvent with an appropriate volume. The extraction time was 30 min.

Extraction temperature and time were also considered in the MAE system. Temperatures of 75, 80 and 85 °C and extraction times of 10, 15, 20, 25 and 30 min were investigated. Two grams of sample was placed in 200 ml vessel, and 30 ml ethanol was used.

The conventional extraction method of heat reflux extraction (HRE) was performed as a comparison method to MAE. The same experimental conditions, 2.00 g *F. xanthii* crude sample from Henan, 30 ml ethanol and extraction temperature of 80 °C, were used in the comparison experiments. In order to compare with MAE, the extraction times of HRE were set at 30 and 120 min.

After the samples were extracted by MAE or HRE, the extract was filtrated and then condensed to 10 ml in a rotor evaporator (Shenke Instrument, Shanghai, China) at 55 °C. Fifty microlitre of the solution taken from the condensed extract was dried with nitrogen gas at 25 °C in a 1.5 ml tube. Then, 50 μl N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA)(Sigma, USA) was added to make trimethylsilyl (TMS) derivatives at 80 °C for 1 h.

2.3. GC–MS analysis

The chromatographic separation was carried out in a Shimadzu GC–MS-QP2010 system with a DB-5 coated fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA). One microlitre of the derivative sample was injected into GC–MS using split mode (50:1). The purge flow was 3.0 ml/min. The injector temperature was 280 °C. The column temperature was programmed as follows: initial temperature at 120 °C for 3 min, 5 °C/min to 240 °C, 240 °C for 5 min, 10 °C/min to 300 °C and 300 °C for 10 min. Mass conditions were as follows: electron impact ionization (EI); interface temperature, 250 °C; ion source temperature, 200 °C; the detector voltage, 1 kV; solvent delay, 4 min. Helium was used as the carrier gas at a flow rate of 1.5 ml/min. All data were obtained by collecting the full-scan mass spectra within the scan range of 50–600 amu.

Compounds were tentatively identified by comparing their mass spectra with those of the National Institute of Standards and Technology (NIST) library. Some selected compounds were further verified by comparing their mass spectra and retention time with those of authentic reference compounds such as butanedioic acid, hexadecanoic acid, octadecanoic acid, trans-9-octadecenoic acid (the 3rd Regent Factory of Shenyang, China)

and inositol (Shanghai Bio Life Science & Technology Co. Ltd., China).

2.4. Statistical data processing

Data analysis was performed by an original software 'chromatographic data processing system' based on the Matlab 6.5 (Mathworks, Natick, MA, USA) [29]. The software was specially coded for analyzing a series of chromatographic data and evaluating the similarities of different chromatograms by principal component analysis (PCA), the correlative coefficient and included angle cosine.

3. Results and discussion

3.1. MAE procedure and comparison of MAE with HRE

3.1.1. The optimization of MAE conditions

The ability of microwave-absorption of extracting solvents and the microwave-heat transformation obviously affects the extraction efficiency. The energy transfer between the polar molecules and the solvents, which is coupled with microwave and nonpolar solvents, is rapid and provides an effective mechanism for increasing cell rupture [30]. Because the major components of *F. xanthii* are polar molecules such as organic acids, alcohols, glucosides, the corresponding derivatives, ethanol, methanol and water, are suitable for the extraction. However, water as the extraction solvent would cause the extraction of unwanted amylum and other water-soluble proteins, which would hinder the subsequent work-up. The toxic methanol has the same effect as ethanol. Thus, ethanol was selected as the optimizing solvent.

The liquid/solid ratio and extraction vessel volume influence the MAE efficiency. The smaller solvent volume results in incomplete components dissolution. With the same liquid/solid ratio, the smaller vessel volume leads to a higher inner pressure, which can accelerate the extraction speed. On the other hand, some unstable compounds are decomposed or changed to another compounds when the inner pressure is too high. Therefore, a suitable liquid/solid ratio and vessel volume should be considered. Fig. 1 shows that with a high ratio of liquid/solid, the intensities of the main components in the retention time of about 30 min become less in the 70 ml vessel than in the 200 ml vessel. It is tentatively suggested that the components are decomposed or changed in structure in the higher pressure environment because the conditions are the same except that the liquid/solid ratio is changed. In the condition of liquid/solid ratio 15:1 and vessel volume of 200 ml, chemical components were dissolved and did not undergo degradation compared with the conditions of liquid/solid ratio 10:1 and 25:1. Therefore, liquid/solid ratio 15:1 and vessel volume of 200 ml were selected as the optimizing conditions.

The higher temperature and longer extraction time can enhance the extraction efficiency because of the increasing diffusivity of the solvent into cells and an enhanced desorption of the components from cells [30]. On the other hand, a relatively high extraction temperature results in thermal degradation of some

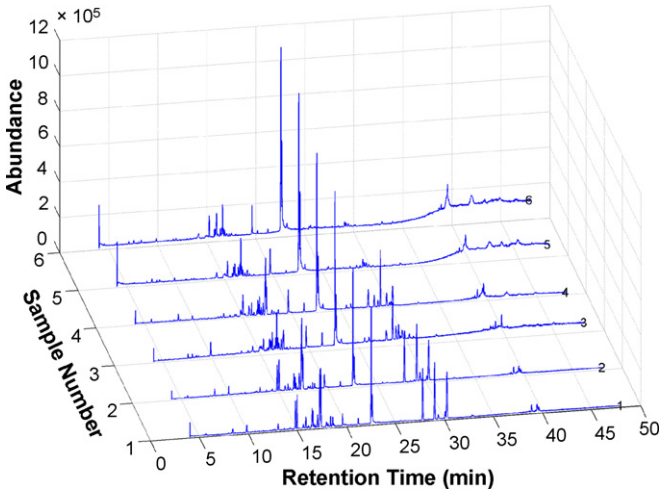

Fig. 1. Effect of liquid/solid ratio on the extraction of chemical components in *Fructus xanthii*: liquid/solid ratio (ml/g)/vessel volume (ml): 1, 10:1/200; 2, 15:1/200; 3, 25:1/200; 4, 30:1/200; 5, 25:1/70; 6, 30:1/70; solvent: ethanol; extraction temperature: 80 °C; extraction time: 30 min; sample source: Guangdong.

components [31]. The chromatograms show that the effect of temperature is not obvious (Fig. 2). Thus, extraction temperature of 80 °C was preferred. Fig. 3 shows that the signal intensity of components increased with the prolonging extraction time. However, longer extraction time may have no further or negative effects resulting in degradation or conversion of the components [31]. Thirty minutes of MAE was enough for the extraction of components of *F. xanthii*.

3.1.2. Comparison of MAE with HRE

In order to validate that the MAE method is suitable for the components extraction of *F. xanthii*, a conventional HRE method was performed as the comparison method. As is known, HRE method is the common extraction method in TCMs analysis. Fig. 4 shows that the extraction efficiency of MAE of 30 min is higher than that of HRE of 30 min, and close to that of HRE of

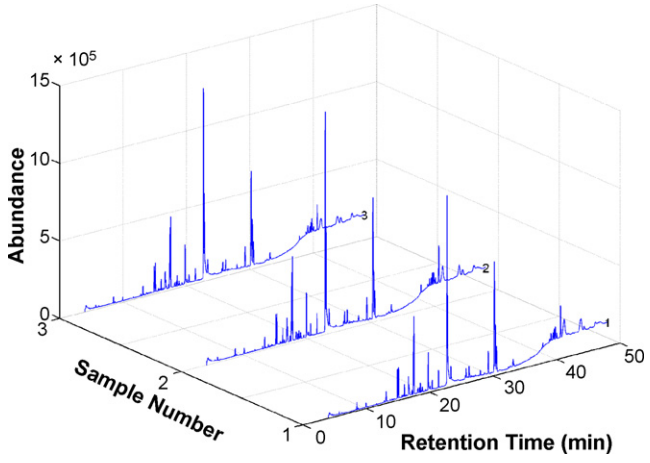

Fig. 2. Effect of temperature on the extraction of chemical components in *Fructus xanthii*: 1. 75 °C; 2. 80 °C; 3. 85 °C; solvent: ethanol; liquid/solid ratio (ml/g)/vessel volume (ml): 15:1/200; extraction time: 30 min; sample source: Guangdong.

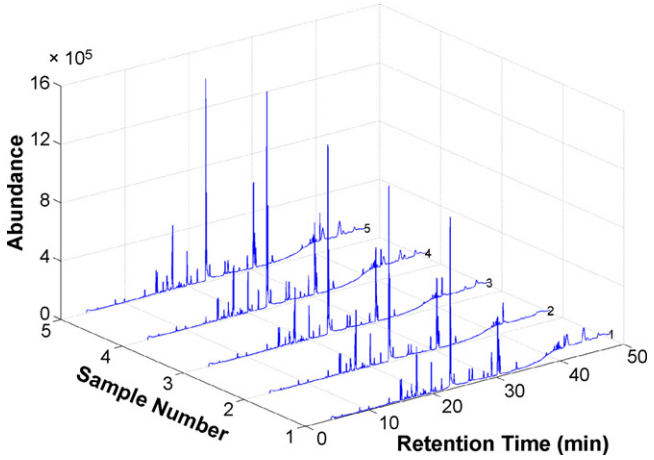

Fig. 3. Effect of time on the extraction of chemical components in *Fructus xanthii*: 1, 15 min; 2, 20 min; 3, 25 min; 4, 30 min; 5, 35 min; solvent: ethanol; liquid/solid ratio (ml/g)/vessel volume (ml): 15:1/200; extraction temperature: 80 °C; sample source: Guangdong.

120 min. The main reason why HRE required a long time to reach a relatively high extraction efficiency of components may be that the samples were not agitated in HRE compared with the MAE method. Agitation of samples could accelerate desorption and/or dissolution of molecules bound to the cellular matrix and reduce the extraction time. In MAE method, the direct interaction of microwave with ethanol molecules and free water molecules present in the cells resulted in the subsequent rupture of the cells and release of intracellular products into the solvent. Thus, MAE could greatly shorten the extraction time and give the same stable chromatograms both in the components' coincidence and retention times.

3.2. Standardization of chromatographic fingerprint and validation of the method

To standardize the fingerprint characteristic, 25 batches of *F. xanthii* samples from Guangdong, Guangxi, Henan, were

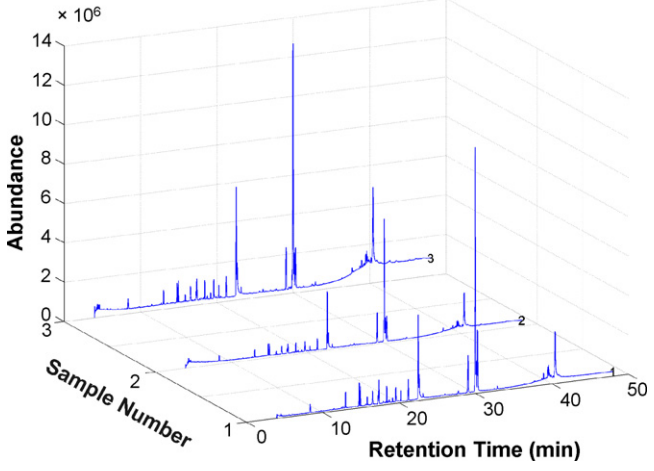

Fig. 4. The chromatograms of *Fructus xanthii* in different extraction methods: 1, MAE for 30 min; 2, HRE for 30 min; 3, HRE for 120 min; sample source: Henan.

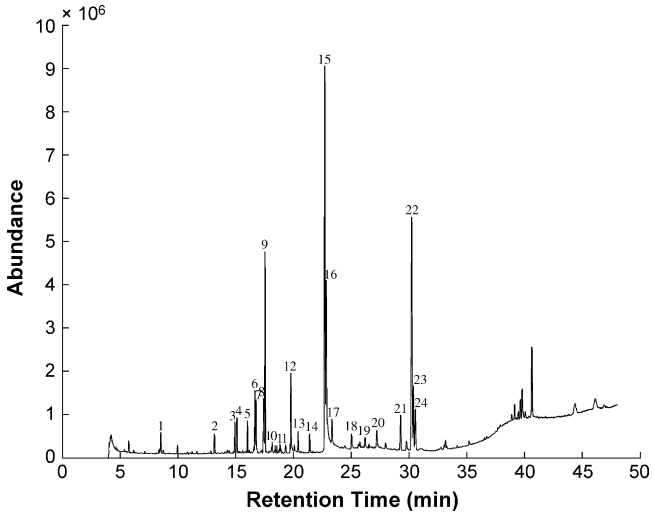

Fig. 5. Typical chromatographic fingerprint of *Fructus Xanthii*.

analyzed by MAE coupled with GC–MS. With the optimized conditions, a typical fingerprint chromatogram of *F. Xanthii* sample is shown in Fig. 5. More than 50 components could be found in all batches of samples. Peaks that existed in all 25 batches of samples were considered as "common peaks" for *F. xanthii*. Twenty-four common chemical components were found and marked in Fig. 5. Among these compounds, 14 were tentatively identified. These tentatively identified compounds are listed in Table 1. Because the *F. xanthii* extract was trimethylsilyl (TMS) derivatized, some compounds extracted convert to two or more tautomeric forms that might appear very close in the chromatogram, e.g., 2-keto-d-gluconic acids (peak 3, 4) and glucosides (peak 23, 24) in Fig. 5. Components such as butanedioic acid and cinnamate have proven to possess antifungal or antibacterial activity [32,33]. β-D-Fructofuranosyl-α-D-glucopyranoside was a potential bioactive

Table 1
Fourteen identified common components present in *F. xanthii* extract[a]

Peak no.	Fit value	Retention time (min)	Compound
[b]1	93	8.500	Butanedioic acid
2	92	13.14	Arabinitol
3	88	14.92	2-Keto-D-gluconic acid
4	87	15.10	2-Keto-D-gluconic acid
6	92	16.63	Altronic acid
7	91	16.73	Gluconic acid
9	95	17.52	Glucitol
[b]12	94	19.76	Hexadecanoic acid
[b]13	88	20.38	Inositol
14	90	21.40	Cinnamate
15	94	22.71	(Z,Z)-9,12-Octadecadienoic acid
[b]16	94	22.83	trans-9-Octadecenoic acid
[b]17	92	23.33	Octadecanoic acid
22	92	30.21	β-D-fructofuranosyl-α-D-Glucopyranoside

[a] Peak No. is corresponding to the peak number in Fig. 5.
[b] Compounds were further identified by using reference substances.

compound. Previous work had proposed that these types of compounds such as tripterygium wilfordii multiglucoside, total glucosides of astragalus, showed anti-inflammatory activity [34]. Also, it was suspected to be gastrointestinal or liver toxicant or neurotoxicant [35]. In the fingerprint chromatogram of *F. xanthii*, peaks 21, 23 and 24 have similar ionic fragment in mass spectra as β-D-Fructofuranosyl-α-D-glucopyranoside, they probably have some common structures of molecule or some similar kernels. Therefore, if these compounds could be separated and studied in detail, it would benefit the bioactivity/toxicity study and dose control in prescription of *F. xanthii*.

To validate this method, the tests of precision and repeatability were preformed based on the relative retention time (the ratio of peak retention time of sample constituents to the reference component) and the relative peak area (the ratio of peak area of sample constituents to the reference component). Among 24 common components, (Z, Z)-9, 12-octadecadienoic acid was a stable content with a high fraction. Therefore, it was chosen as the reference substance. Relative retention time of all common peaks and relative peak areas were obtained based on this component. All the validations were measured by calculating their relative standard deviations (RSDs) of the relative retention time and relative peak area of each target peak. Five reduplicated analyses of the same sample solution were performed to evaluate the precision of the proposed method. RSDs of relative retention times and relative peak areas of all common peaks were less than 0.1% and 6%, respectively. The repeatability of the method was confirmed by five different work solutions extracted from the same batch of *F. xanthii*. The RSDs of relative retention times and the relative peak areas were less than 0.2% and 6%, respectively. The stability test was performed with sample work solutions within 2 days. The RSDs of the relative retention times and relative peak areas were less than 0.2% and 3%, respectively. These results indicate that the method is reliable and applicable to the analysis of chromatographic fingerprint of *F. xanthii*.

3.3. Characteristic analysis of the chromatographic fingerprint of F. xanthii

The chromatographic fingerprint could effectively demonstrate the "integrity" and "fuzziness" or "sameness" and "differences" between various samples [9,36]. Twenty-five samples collected from different producing areas were analyzed. The chromatograms are shown in Fig. 6. All the normal samples have high similarity values in retention time while their peak abundances are different. The 24 common peaks of 25 samples were assigned as "common peaks" to indicate the similarity among various samples. RSD values of the relative retention times of 24 common peaks were less than 0.2%, which means the retention time of each component was comparatively stable. The various abundances of the components may be attributed to the growing environment, the climate of different areas and the different storage times. Besides the common peaks, there are about 25 noncommon peaks in different chromatograms, especially in the samples collected from Guangdong. But the noncommon peak area is about 8.2%, less than the national standard of 10%.

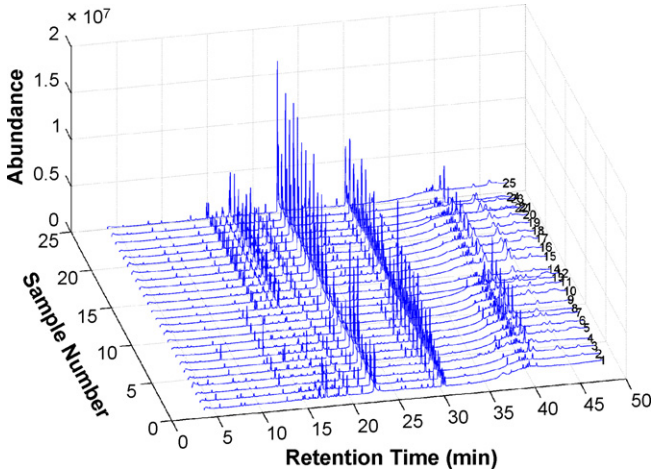

Fig. 6. The chromatograms of 25 samples collected from different producing areas: 1–4 samples produced from Guangxi; 5–12 samples produced from Henan; 13–25 samples produced from Guangdong.

The differences of the samples were analyzed by PCA [9–12,37,38], as a potential tool in their binary chromatograms. PCA describes the variation in data with minimum latent variables. The score values for the first two PCs (PC1 and PC2) are often used to represent the characteristics of the samples. Therefore, grouping or classification of the fingerprints is more easily discovered [11]. Fig. 7 shows the score plots derived from the first two PCs (in Fig. 7, each sample is represented as a marker). It was noticeable that the samples were clustered in different domains, which represented the "similarities" and "differences" of different batches of samples.

Meanwhile, the similarities among the 25 batches of samples were evaluated by calculating their correlation coefficient and the included angle cosine. The similarity analysis was conducted based on the common model of 25 batches of samples. The results are shown in Table 2. The similarity degrees of most

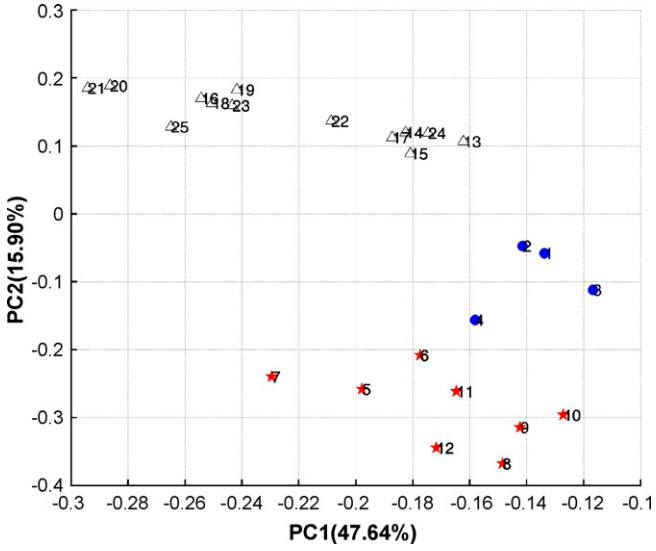

Fig. 7. Distributions of samples on the PCA: (●) samples produced from Guangxi; (⋆) samples produced from Henan; (△) samples produced from Guangdong.

Table 2
The similarity analysis of *F. xanthii* samples

Sample number	The included angle cosine	Correlation coefficient	Sample number	The included angle cosine	Correlation coefficient
1	0.966	0.959	14	0.927	0.935
2	0.978	0.973	15	0.983	0.979
3	0.974	0.968	16	0.971	0.974
4	0.969	0.966	17	0.987	0.983
5	0.956	0.963	18	0.976	0.979
6	0.964	0.967	19	0.978	0.978
7	0.971	0.979	20	0.973	0.976
8	0.914	0.928	21	0.973	0.977
9	0.940	0.945	22	0.927	0.935
10	0.941	0.942	23	0.927	0.935
11	0.961	0.965	24	0.770	0.788
12	0.933	0.940	25	0.904	0.914
13	0.927	0.935			

samples are more than 0.93, which indicate that the proportion and distribution of the components in most extracts of *F. xanthii* possess a high level of consistency.

3.4. Chemical variance in the toasting process of F. xanthii

F. xanthii always needs to be toasted to decrease the toxicity before clinical use in China. In TCMs, toasting degree is hardly controlled because it always depends on the experience of apothecary. In Pharmacopoeia of People's Republic of China, there is only a brief description concerning the toasting of *F. xanthii*. Zhang et al. [39] suggested that *F. xanthii* should be toasted at 210 °C for 16 min according to the contents of fatty oils. But the contents of fatty oils are not the ideal guidelines for the quality assessment of *F. xanthii* because fatty oils contain so many compounds that may have no curative effect. Therefore, it is highly desirable to conduct scientific study for the toasting technique of *F. xanthii*. In this research, the chromatograms of toasted samples at different toasting times were compared to find out the components variability by MAE coupled with GC–MS.

In the toasting process, color shade of the sample changed from brown to dark brown, black, and interiors dark brown (charred). The amounts of some components greatly changed with the heating time prolonged. The chromatograms of crude sample and 10 toasted samples are simultaneously shown in Fig. 8. The chromatographic fingerprints of 11 samples were very similar. The similarity between crude *F. xanthii* and other toasted samples can be explained by their correlation coefficients and the included angle cosine, shown in Table 3. With the continuation of the processing, the included angle cosine and correlation coefficient of crude *F. xanthii* decreases. Especially, when the toasted time was lengthened to 6 min, the similarity degree declined obviously, which was also conformed by PCA analysis (Fig. 9). The contribution percentage of the common peaks and the new components only appeared in the toasted samples compared with crude *F. xanthii* are listed in Table 4. Compared with the chromatographic fingerprint of crude sample, the intensities of organic acids at retention times 8.500, 19.76, 22.71, 22.83 and 23.33 min were relatively stable but their contribution percentage continuously increased. These organic

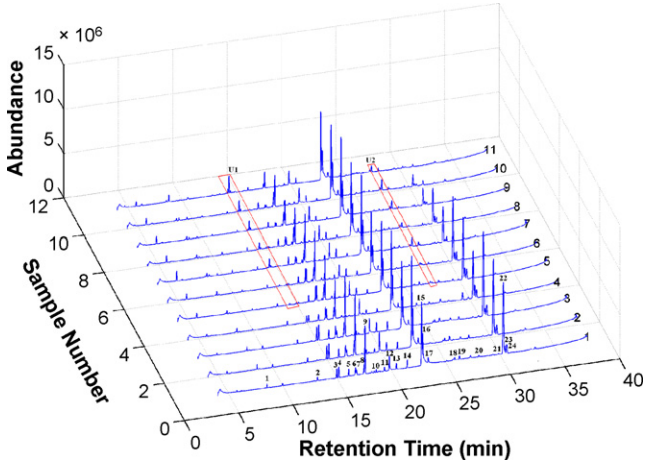

Fig. 8. The enlarged GC–MS chromatograms of crude *F. xanthii* and the processed samples: 1, the crude sample; 2–11, toasted samples corresponding to 1, 2, 3, 4, 5, 6, 7, 9, 11 and 13 min, respectively; U1 and U2: new compounds appeared in the toasting process.

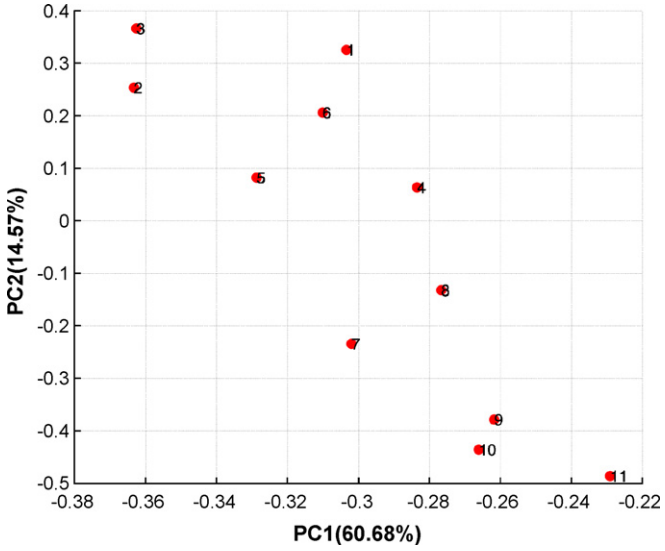

Fig. 9. Distributions of crude and toasted samples on the PCA: 1, the crude sample; 2–11, toasted samples corresponding to 1, 2, 3, 4, 5, 6, 7, 9, 11, 13 min, respectively.

Table 3
The similarity between *F. xanthii* and its toasted samples

Similarity[a]	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	11#
Correlation coefficient	1.000	0.981	0.977	0.978	0.964	0.989	0.927	0.921	0.922	0.912	0.896
The included angle cosine	1.000	0.968	0.959	0.961	0.938	0.982	0.873	0.874	0.864	0.846	0.819

[a] The similarity degree was calculated using crude sample as the reference sample.

Table 4
The contribution percentage of main components of toasted *F. xanthii*

Peak No.	R.T. (min)	Sample number										
		1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	11#
1	8.508	0.396	0.444	0.423	0.629	1.093	1.317	2.392	2.011	2.383	2.169	2.210
2	13.16	0.946	0.898	0.812	1.097	0.805	0.961	0.837	1.173	0.847	0.775	0.490
3	14.93	2.372	2.877	2.882	1.734	–	–	–	–	–	–	–
4	15.10	3.328	3.911	3.782	2.356	–	–	–	–	–	–	–
5	16.01	1.916	1.882	2.056	1.778	1.762	1.917	2.240	2.386	1.983	2.418	1.397
6	16.65	1.588	1.973	1.930	3.045	3.236	3.350	1.777	2.291	1.358	1.108	0.271
7	16.75	0.884	0.687	0.937	2.527	3.475	3.234	1.537	2.100	1.203	0.861	–
8	17.43	2.870	3.361	2.802	3.302	4.127	3.662	3.571	5.667	5.422	5.710	1.924
9	17.53	12.42	12.05	12.76	11.29	10.73	12.87	13.20	10.68	9.331	11.00	8.118
10	18.55	0.732	0.933	0.854	0.434	0.263	0.305	0.309	0.384	0.274	0.259	–
11	18.84	0.146	0.112	0.126	0.415	0.209	0.212	0.232	0.486	0.428	0.418	0.337
12	19.76	3.149	3.626	2.889	5.119	3.042	2.999	4.130	4.217	5.119	4.607	6.316
13	20.40	1.710	1.775	2.082	1.204	1.906	2.234	2.788	2.384	1.878	2.539	3.109
14	21.39	2.183	1.645	2.168	1.216	1.146	1.286	1.051	1.225	0.856	0.888	0.349
15	22.71	13.92	17.13	13.99	20.01	15.75	13.47	21.17	19.73	23.94	24.93	32.19
16	22.83	3.522	4.258	3.406	5.865	6.642	3.495	6.245	4.408	10.81	10.90	10.96
17	23.33	2.018	1.862	1.416	1.922	1.965	1.956	1.801	2.212	2.582	2.864	3.792
18	25.75	0.785	0.740	0.693	0.434	0.550	0.564	0.679	0.580	0.741	0.859	–
19	26.19	1.517	1.608	1.503	1.052	1.488	1.248	1.423	1.092	1.219	1.427	–
20	27.97	1.300	0.826	0.913	1.553	0.815	1.080	0.956	1.441	1.735	1.548	1.823
21	29.23	0.559	0.866	0.856	0.509	0.873	0.748	1.202	0.728	1.053	1.301	2.130
22	30.21	31.19	27.17	31.01	20.43	25.92	28.46	16.54	17.94	10.44	7.425	2.277
23	30.37	2.271	1.925	1.820	4.521	3.461	2.661	1.219	2.831	2.002	1.308	–
24	30.55	3.929	3.275	3.747	3.563	3.952	4.108	2.275	3.007	1.642	–	–
U1	14.27	–	–	–	–	0.485	0.586	2.527	2.127	3.725	4.086	8.694
U2	27.36	–	–	–	–	0.431	0.501	1.708	2.608	3.552	3.709	4.345

"–", not determined.
"U", new components only appeared in the toasting process.

acids in *F. xanthii* were hard to evaporate due to their high boiling points and would not decompose in the toasting process. In the toasting process, some common peaks could be detected in decreasing abundance, which might tentatively explain why the similarity degree of the processed drugs constantly reduced.

At the beginning of the toasting process, the contents of components did not undergo obvious change. After 3 min, the components changed not only in content but also in species. The intensities of 2-Keto-D-gluconic acids at retention times 14.93 and 15.10 min could not be detected. Meanwhile, two new components appeared (retention times 14.27 and 27.36 min), and their abundance gradually strengthened with the prolonging of the toasting time. The observation suggested that the gluconic acid with ketonic groups was a thermally unstable substance and the metamorphosis was associated with the disintegration of ketonic groups at high temperatures in toasting process [40]. The reason why the abundance of glycosides such as β-D-Fructofuranosyl-α-D-glucopyranoside decreased and rapidly weakened may be related with the ketonic and the multi-carboxy groups in these compounds' structure, which caused charring of the glycosides in a long thermal environment. Considering the effects of toasting upon the toxicity of *F. xanthii*, it is not difficult to make a primary conclusion that the great changes in these compounds are related with the toxicity decrease in *F. xanthii*.

4. Conclusion

F. xanthii is a common Traditional Chinese Medicine. Although there are morphological differences and qualitative and quantitative variations in chemical compositions as a result of different origins and toasting methods, the chromatograms of different products were found generally consistent with some common characteristics both in retention times and constitution of components.

Microwave assisted extraction could be considered as an effective technique for rapid and selective extraction for chemical components in *F. xanthii*. In the investigation of optimizing conditions of MAE, liquid/solid ratio was found to be the most

important factor to influence the MAE efficiency. Coupled with GC–MS, the fingerprint of *F. xanthii* could be established. The method was simple, efficient and stable; RSDs of retention time and peak area of each component were less than 0.1% and 6%, respectively.

By comparing the components of different habitat samples and different toasted samples, the chromatographic fingerprint could properly reveal the quality characteristics of *F. xanthii*. Although the relationship between bioactive/toxicity effect and the components was not studied in detail, β-D-Fructofuranosyl-α-D-Glucopyranoside and other glycosides could be considered as tentative pivotal substances whose concentration was related with *F. xanthii*'s bioactivity and toxicity.

It is strongly believed that microwave assisted extraction coupled with GC–MS is a suitable method for fingerprint characteristic analysis and quality assessment of *F. xanthii*.

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References

[1] The State of Pharmacopoeia Commission of People's Republic of China, Pharmacopoeia of People's Republic of China, vol. 1, Chemical Industry Press, 2000, p. 128.
[2] Jiang Su New Medical College, Dictionary of Chinese Meteria Mediaca, Shanghai Scientific Technological Publishers, Shanghai, 1985, p. 1071.
[3] T. Mehmet, C.A. Cafer, G. Metin, K. Abdullah, D. Yasar, T. Muhittin, A. Nusret, A. Denizmen, Ann. Trop. Paediatr. 25 (2005) 125.
[4] Y.T. Ma, M.C. Huang, F.L. Hsu, H.F. Chang, Phytochemistry 48 (1998) 1083.
[5] C. Roussakis, I. Chinou, C. Vayas, Planta Med. 60 (1994) 473.
[6] M. Calvin, J.M. Tom, Pedro I. Chavez, Am. J. Bot. 63 (1976) 317.
[7] Y. Gaillard, G. Pepin, J. Chromatogr. B 733 (1999) 181.
[8] D.K. Obatomi, P.H. Bach, Food Chem. Toxicol. 36 (1998) 335.
[9] Y. Liang, P. Xie, K. Chan, J. Chromatogr. B 812 (2004) 53.
[10] L. Yang, D. Wu, X. Tang, W. Peng, X. Wang, Y. Ma, W. Su, J. Chromatogr. A 1070 (2005) 35.
[11] S. Yan, W. Xin, G. Luo, Y. Wang, Y. Cheng, J. Chromatogr. A 1090 (2005) 90.
[12] G. Lu, K. Chan, Y. Liang, K. Leung, C. Chan, Z. Jiang, Z. Zhao, J. Chromatogr. A 1073 (2005) 383.
[13] X. Fan, Y. Cheng, Z. Ye, R. Lin, Z. Qian, Anal. Chim. Acta 555 (2006) 217.
[14] P. Xie, S. Chen, Y. Liang, X. Wang, R. Tian, R. Upton, J. Chromatogr. A 1112 (2006) 171.
[15] Q. Liu, C.M. Wai, Talanta 53 (2001) 771.
[16] J.D. Denery, K. Dragull, C.S. Tang, Q.X. Li, Anal. Chim. Acta 501 (2004) 175.
[17] E.S. Ong, J. Chromatogr. B 812 (2004) 23.
[18] J.C. Young, J. Agric. Food Chem. 43 (1995) 2904.
[19] X. Pan, G. Niu, H. Liu, J. Chromatogr. A 922 (2001) 371.
[20] X. Pan, H. Liu, G. Jia, Y.Y. Shu, Biochem. Eng. J. 5 (2000) 173.
[21] J. Huang, Z. Zhang, Anal. Sci. 20 (2004) 395.
[22] Y.Y. Shu, M.Y. Ko, Y.S. Chang, Microchem. J. 74 (2003) 131.
[23] F. Gong, Y.Z. Liang, C. Hui, F.T. Chau, B.T.P. Chan, J. Chromatogr. A 905 (2001) 193.
[24] F. Gong, Y.Z. Liang, Q.S. Xu, F.T. Chau, J. Chromatogr. A 909 (2001) 237.
[25] A. Velasco-Negueruela, M.J. Perez-Alonso, P.L. Perez de Paz, J. Pala-Paul, J. Sanz, J. Chromatogr. A 984 (2003) 159.
[26] S. Shen, Y. Sha, C. Deng, X. Zhang, D. Fu, J. Chen, J. Chromatogr. A 1047 (2004) 281.
[27] C. Deng, Y. Mao, N. Yao, X. Zhang, Anal. Chim. Acta 575 (2006) 120.
[28] C. Deng, N. Yao, B. Wang, X. Zhang, J. Chromatogra. A 1103 (2006) 15.
[29] Z. Zhang, J. Cai, R. Ruan, G. Li, J. Chromatogr. B 822 (2005) 244.
[30] H. Li, G. Li, Z. Zhang, Chinese J. Anal. Chem. 31 (2003) 1341.
[31] W. Vongsangnak, J. Gua, S. Chauvatcharin, J. Zhong, Biochem. Eng. J. 18 (2004) 115.
[32] W.J. Sun, J.F. Sheng, A Handbook of Bioactive Compounds from Plants, first ed., China Medical-Pharmacological Science and Technology Publishing House, Beijing, 1998.
[33] X.D. He, J.H. Zou, Chinese Materia Medica, first ed., Chemistry Press, Beijing, 1998.
[34] N.P. Wang, W. Wei, Chin. Pharmacol. Bull. 19 (2003) 366.
[35] http://www.scorecard.org/chemical-profiles/.
[36] Y. Cao, L. Wang, X. Yu, J. Ye, J. Pharm. Boimed. Anal. 41 (2006) 845.
[37] R.G. Brereton, Chemometrics: Data Analysis for the Laboratory and Chemical Plant, John Wiley & Sons, Chichester, 2003.
[38] S. Yan, W. Xin, G. Luo, Y. Wang, Y. Cheng, J. Chromatogr. A 1090 (2005) 90.
[39] D.R. Zhang, J. Chin. Med. Mater. 19 (1996) 347.
[40] C. Xu, S. Sun, C. Guo, Q. Zhou, J. Tao, I. Noda, Vib. Spectro. 41 (2006) 118.