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气相色谱-质谱结合化学计量学分析莪术挥发油成分

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摘　要:中药市场上的莪术来自不同的官方品种和炮制品种,因而存在着一定的混用情况。为了初步比较研究市售莪术的化学成分,我们对其主要的活性成分莪术挥发油进行了研究。采用气相色谱-质谱结合化学计量学方法对来自于16个批次的不同官方品种和炮制品种的挥发油进行了化学分析和MCF-7细胞毒性检测。鉴定出53个化学成分,其中包括11个首次鉴定出的化合物。结果表明16批样品显示相似的抗肿瘤活性,而其化学成分在种间存在显著差异,而炮制品间未见差异。因此,来自于 *Curcuma kwangsiensis* 和 *C. phaeocaulis* 的挥发油应该与 *C. wenyujin* 区分开来。三个官方品种应当被进行深入的比较研究和区别应用。

关键词:挥发油;莪术;气相色谱-质谱联用技术;化学计量学

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Analysis of Essential Oil Composition of Commercial Curcumae Rhizoma from Different Processed Products and Sources by GC-MS coupled with Chemometrics

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Abstract: The three official species and processed products of Chinese medicine Curcumae Rhizoma were commonly mixed used. In order to preliminary study and compare the chemical components of Curcumae Rhizoma commercially available, the essential oil, which was reported to be the main active components, was taken as the research object in this study. The essential oil composition from different processed products and sources of Curcumae Rhizoma was analyzed by GC-MS coupled with chemometrics. In addition, their cytotoxic activity on MCF-7 cell lines was also assessed. As a result, 53 components, including 11 components for the first time, were identified from Curcumae Rhizoma volatile oil. In the comparative study of species, although their cytotoxic activity on MCF-7 cell lines were equally effective, significant differences were found intergroup by SPSS analysis; and the comparative study of processed products showed no significant difference. The essential oils from *Curcuma kwangsiensis* and *C. phaeocaulis* should be distinguished from *C. wenyujin*, the official source of essential oil of Curcumae Rhizoma.

Key words: essential oil; Curcumae Rhizome; GC-MS; chemometrics

Introduction

Curcumae Rhizoma called E'zhu in Chinese, belonged to the *Curcuma* genus (Zingiberaceae family), is regulated to be the dry rhizomes of *Curcuma wenyujin* (CW), *C. kwangsiensis* (CK) and *C. phaeocaulis* (CP) in the Chinese Pharmacopoeia [1]. It is a commonly-used Chinese medicine, initially recorded in Leigong Paozhi Lun during the southern and northern dynasties, which is deemed as the earliest monograph of science of processing Chinese medicine in the world. In Rihuazi Bencao, Cucumae Rhizoma was described to stimulate one's appetite, help digestion, promote menstruation, eliminate blood stasis and alleviate pain

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caused by injuries from falls [2].

Owing to its good performance in clinics and favorable pharmacokinetic characteristics, Curcumae Rhizoma is always the hot point, and has shown a variety of activities, such as antitumor [3], antivirus [4], anti-inflammatory [5-6], *etc*. It has been developed into a variety of formulations in clinical treatment. Most of all, Curcumae Rhizoma oil injection has recorded in Chinese Pharmacopoeia and can effectively treat acute upper respiratory tract infection and shorten the main symptoms duration of patient condition [7]. Sesquiterpenes, the main constituents of essential oil, are reported to be responsible for their extraordinary anti-cancer, anti-inflammatory, antiviral, neuroprotective and anti-thrombotic effects [8].

Through medical market investigation in Chinese mainland, there commonly exist mixed usage of Curcumae Rhizoma species and processed products. However, essential oil of Curcumae Rhizoma as an important effective role, quality investigation on it is few. What's more, the official source of Curcumae Rhizoma oil is regulated to be only CW according to China Pharmacopeia Version 2005 and 2010. Whether the factors of species and processing influence the composition of saled Curcumae Rhizoma oil or not? And if CK and CP can also be the source of Curcumae Rhizoma oil? We collected samples nationwide commercially available and determined the essential oil composition from different processed products and sources of Curcumae Rhizoma by GC-MS coupled with chemometrics.

Materials and Methods

Materials and reagents

All reagents used in the experiments were of analytical grade. *n*-Alkane, the internal standard (IS), was purchased from Beijing Chemical Works. Ethyl acetate was from Fisher (Emerson, IA, USA).

Plant materials

16 batches of Curcumae Rhizome samples were commercially available from different provinces in China. Their species and sources were shown in Table 1.

Table 1 Information of Curcumae Rhizoma samples used in this study

No.	Species	Raw or processed[a]	Sold districts
S1	CP	R	Sichuan Chongzhou
S2	CP	R	Sichuan Chongzhou
S3	CP	R	Sichuan Chongzhou
S4	CP	R	Sichuan Chengdu
S5	CK	R	Fujian Fuzhou
S6	CK	P	Beijing
S7	CK	R	Beijing
S8	CK	P	Zhejiang Hangzhou
S9	CK	R	Zhejiang Hangzhou
S10	CK	R	Guangxi Nanjing
S11	CK	P	Guangxi Nanjing
S12	CK	R	Guangdong Guangzhou
S13	CK	P	Guangdong Guangzhou
S14	CP	P	Sichuan Chongzhou
S15	CP	P	Sichuan Chongzhou
S16	CK	P	Fujian Fuzhou

[a] R for raw materials and P for vinegar processed materials.

Essential oil samples extraction

100 g of Curcumae Rhizoma samples were soaked in tenfold deionized water at room temperature for 24 h, and then subjected to hydrodistillation in a modified

clevenger-type apparatus for 10 h. The essential oils were exsiccated by anhydrous sodium sulphate, stored in a stopper vial at 4 ℃ until analysis and dissolved in ethyl acetate for GC-MS analysis.

Chemical characterization of the essential oil from *Curcumae Rhizoma*

Essential oils were analyzed by an Agilent 5975C (GC) equipped with a HP-5MS capillary column (5% phenyl methyl Siloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness) and an HP 5973 mass selective detector (FINNIGAN TRACE-MS 2000, USA) in the electron impact ionization mode (70 eV) under the following operating conditions: injection mode: split ratio, 1∶20; injection volume, 1 μL (TBME solution); inlet temperature, 260 ℃; detector temperature, 260 ℃; The oven temperature was programmed to start at 50 ℃ and holding for 2 min, increased at 5 ℃/min to 250 ℃. The Helium (99.999%) carrier gas was kept with a constant flow of 1.0 mL/min.

Statistical analysis

ANOVA analysis was conducted to find out the factors that influence the object significantly, the interaction of each factor and the best level of various factors. Using ANOVA analysis of the chemical content of essential oil in this experiment was hoping to study the otherness among the samples. Getting rid of the constituents that had low component content or only in the minority samples, 30 constituents were tested among the 16 batches. Besides, in order to evaluate and distinguish the source of *Curcumae Rhizoma* samples commercially available, PCA (principal component analysis) of relative content was conducted among the 30 constituents.

Statistical analysis on the essential oil contents and their activities were measured by SPSS (Version 20.0 IBM), and were used to seek out the features among the batches of samples.

Cytotoxic activity on MCF-7 cell lines of the essential oil

Human breast carcinoma cell lines (MCF-7) were purchased from Cell Resource Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing, China. RPMI 1640 medium supplemented with 10% FBS, 100 units/mL penicillin and 100 μg/mL streptomycin was used for the cultures of MCF-7. Inhibition of cellular proliferation was assessed by MTT [3-(4, 5-dimethylthiazol-2-yl)-5-3 (carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] reduction method (AMRESCO, America), based on the measurement of mitochondrial respiration in living cells. The extent of the reduction of MTS to formazan is proportional to the viability of the cells. Optical density was read at 560 nm with a microplate reader (Thermo Multiskan FC, Shanghai, China) [9]. Cytotoxicity was expressed as the concentration of drug inhibiting cell growth by 50% (IC_{50} value).

Results and Discussion

Content of essential oil

53 components in total were identified including 11 firstly identified components from Curcumae Rhizoma (tetramethylpyrazine, 32-hexenylmethyl carbonate, *exo*-fenchol, pinocarveol, camphene hydrate, isobornyl formate, dauca-5, 8-diene, hedycaryol, carotol, selin-11-en-4-α-ol and eudesm-7(11)-en-4-ol) (Table 2). It might supply more chemical information and provide possible guide for the study of Curcumae Rhizoma.

It was also found that the content of Curcumae Rhizoma oil was the highest in curzerenone, content of 30%-60%, followed by germacrone (2%-10%) and curcumol (0.5%-7%). While almost all the samples cannot be up to the quality standard of Curcumae Rhizoma oil according to China Pharmacopeia, version 2010: furanodiene (not less than 10%) and germacrone (not less than 7.5%). It is the fact that CK, CP and CW are all regarded as the official species of Curcumae Rhizoma, and only CW is the regulated source of Curcumae Rhizoma oil. Therefore, confusion occurs when Curcumae Rhizoma decoction is used in medication. Further study should focus on this problem to clarify the differences in these three species.

ANOVA analysis

In the comparative study of species, S1-S4 (CP) and S5, S7, S9, S10 and S12 (CK) showed that, 15 constituents in total exist significant difference: 1, 8-cineole,

Table 2 Main chemical composition of essential oil from different batches of Curcumae Rhizoma

No.	KI	Compound	% of Total															
			S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
1	943	Camphene	Tr	Tr	Tr	–	–	–	–	–	–	–	–	–	–	–	–	–
2	1024	*p*-Cymene	–	Tr	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	1029	Limonene	–	Tr	–	Tr	–	–	–	–	–	–	–	–	–	–	–	–
4	1033	1,8-Cineole	0.1	0.4	0.1	0.1	Tr	–	–	–	Tr	Tr	Tr	–	–	Tr	Tr	0.1
5	1082	Tetramethylpyrazine	Tr	Tr	–	–	–	–	–	–	–	–	–	–	–	Tr	–	–
6	1089	2-Nonanone	–	Tr	Tr	–	Tr	Tr	Tr	Tr	Tr	Tr		Tr		Tr	Tr	–
7	1096	32-Hexenyl methyl carbonate	Tr	Tr	Tr	–	–	–	–	–	–	–	–	–	–	Tr	–	–
8	1098	Linalool	–	–	–	0.1	–	–	–	–	–	–	–	–	–	–	–	–
9	1121	*exo*-Fenchol	–	–	–	–	Tr	–	–	–	–	–	–	–	–	–	–	–
10	1142	Pinocarveol	–	Tr	Tr	–	–	–	–	–	–	–	–	–	–	Tr	–	–
11	1148	Camphor	1.7	1.2	1.2	0.9	0.1	0.1	0.1	Tr	0.1	0.1	0.1	0.1	tr	0.7	0.1	1.1
12	1147	Camphene hydrate	Tr	Tr	Tr	Tr	–	–	–		Tr	Tr	–	–	–	Tr	–	–
13	1164	Isoborneol	0.9	0.6	0.7	1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.1	0.4	0.2	1.2
14	1159	δ-Terpineol	Tr	Tr	Tr	–	Tr	–	Tr	Tr	Tr	Tr	Tr	Tr	–	Tr	Tr	–
15	1173	Borneol	0.4	0.2	0.3	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.3
16	1181	Terpinen-4-ol	0.1	0.1	0.1	Tr	Tr	Tr	Tr	Tr	0.1	Tr	Tr	Tr	–	Tr	0.1	0.1
17	1190	2-Decanone	–	Tr	Tr	–	–	–	–	–	–	–	–	–	–	Tr	–	–
18	1195	α-Terpineol	0.2	0.2	0.2	–	0.2	0.2	0.3	0.1	0.3	0.3	0.1	0.1	0.1	0.2	0.3	0.3
19	1215	*trans*-Carveol	–	–	–	–	Tr		Tr	Tr	Tr	Tr	–	–	–	–	Tr	–
20	1239	Isobornyl formate	–	–	Tr		–	–	–	–	Tr	–	–	–	–	Tr	–	–
21	1244	Carvone	–	Tr	Tr	–	–	–	–	–	Tr	–	–	–	–	Tr	–	–
22	1291	2-Undecanone	0.1	0.1	0.1	–	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	–	0.1	Tr	–
23	1287	Isobornyl acetate	–	–	Tr	0.3	Tr	Tr	– -	Tr	Tr	Tr	–	–	–	–	Tr	0.4
24	1296	Carvacrol	–	–	Tr	Tr	Tr	Tr	–	Tr	Tr	Tr	Tr	–	–	–	–	–
25	1336	δ-Elemene	Tr	0.1	Tr	0.2	0.1	0.1	0.1	0.2	0.1	0.1	Tr	0.1		Tr	0.2	0.4
26	1391	β-Elemene	1.7	2.5	0.6	1.9	0.4	0.4	0.4	0.9	0.5	0.4	0.4	0.4	0.2	1	0.6	5.1
27	1408	(*Z*)-Caryophyllene	–	–	–	– -	0.1	0.3	0.1	0.1	Tr	0.1	Tr	–	–	–	0.1	–
28	1422	(*E*)-Caryophyllene	–	0.1	Tr	–	0.2	0.2	0.4	0.1	0.1	0.1	0.2	0.1	–	0.1	0.4	–
29	1430	γ-Elemene	0.1	0.2	Tr	–	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	–	0.1	0.3	0.1
30	1451	Aromadendrene	Tr	0.1	Tr	–	0.1	0.1	0.1	0.1	Tr	Tr	0.1	Tr	–	Tr	0.1	0.1
31	1458	Alloaromadendrene	–	–	–	–	0.1	0.1	0.1	0.1	0.1	0.1	Tr	Tr	–	–	0.1	–
32	1457	α-Humulene	Tr	Tr	–	–	–	–	–	–	–	–	–	0.7	0.1	Tr	–	0.1
33	1475	β-Chamigrene	0.1	0.1	Tr	0.1	0.4	0.6	0.3	0.3	0.3	0.3	0.4	0.2		0.1	0.4	0.4
34	1477	Dauca-5,8-diene	–	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–
35	1481	α-Curcumene	0.1	0.1	0.3	0.3	0.2	0.3	0.2	0.2	0.1	0.1	0.2	0.1	0.2	0.3	0.2	0.4
36	1491	β-Selinene	0.3	0.3	0.2	0.5	0.8	1.3	0.8	0.9	0.8	0.8	1.2	0.7	0.3	0.2	1.3	1.1
37	1494	Curzerene	0.1	0.8	0.1	–	0.3	0.3	1.8	0.4	0.3	0.2	0.2	0.5	0.6	1.2	1.5	0.9
38	1498	*cis*-β-Guaiene	0.6	0.7	0.4	0.5	1.2	1.8	1.3	1	0.9	0.8	1.3	0.7	0.4	0.2	1.6	1
39	1543	Selina-3,7(11)-diene	–	0.2	–	–	0.5	0.5	1.2	0.3	0.5	0.4	0.6	0.5	0.2	–	0.7	–

40	1548	Hedycaryol	–	–	–	0.2	–	–	–	–	–	–	–	–	0.3	0.1	–	0.2
41	1549	Elemol	0.1	0.2	0.1	–	0.2	–	0.4	0.2	0.3	0.2	0.3	0.3		0.1	0.3	0.2
42	1584	Caryophyllene oxide	0.2	0.1	0.3	0.2	0.4	0.4	0.2	0.3	0.5	0.4	0.4	0.4	0.3	0.3	0.3	0.3
43	1590	Carotol	0.1	0.4	0.2	0.2	0.5	0.7	0.5	0.5	0.4	0.5		0.4	0.7	0.3	0.6	0.5
44	1602	*trans*-β-Elemenone	–	–	–	0.8	–	–	–	–	–	–	–	–	5.8	–	–	2.5
45	1609	Curzerenone	43.1	59	52.5	0.7	35.6	32.1	39.5	46.6	40	42	38.9	44.4	–	43.5	40.1	–
46	1641	Aromadendrene epoxide	1	0.9	0.8	1.6	1.3	1.4	0.9	0.7	0.9	1	1.2	0.8	3.7	5.7	1.1	2.1
47	1632	γ-Eudesmol	–	–	–	–	–	–	–	–	–	–	–	–	1.5	–	–	1.1
48	1657	β-Eudesmol	0.8	0.7	0.8	1.9	3.7	3.9	3.1	2.8	2.4	2.8	3.3	2.3	3.1	0.8	3.1	2.4
49	1660	Selin-11-en-4-α-ol	–	–	–	2.8	–	–	–	–	–	–	–	–	1.7	–	–	3
50	1663	α-Turmerone	Tr	0.4	–	0.2	–	–	–	–	–	–	–	–	–	–	–	–
51	1696	Germacrone	2.4	4.1	1.3	1.6	8.2	7.3	9.2	4.3	3.2	3.3	5.3	3.9	5.1	3.9	7.8	2.8
52	1700	Eudesm-7(11)-en-4-ol	–	–	0.2	0.2	–	–	–	–	–	–	–	–	0.5	–	–	–
53	1723	Curcumenol	6.8	2.5	3	5	0.5	0.4	0.4	0.4	0.7	0.7	0.6	0.6	5.5	10.8	0.4	6.6
Total		61	76.3	63.7	21.6	55.5	52.8	61.9	61	53.6	55.2	55.2	57.8	30.6	70.5	62.4	34.7	

[a] "-" means the compound was not identified and "Tr" means the content was less than 0.04%.

camphor, camphene hydrate, isoborneol, borneol, β-elemene, (*E*)-caryophyllene, β-chamigrene, β-selinene, cis-β-guaiene, selina-3, 7(11)-diene, elemol, carotol, β-eudesmol and curcumenol. Thereinto, curcumenol, β-eudesmol and β-elemene have already proved to be the main components possessing anti-tumor bioactivities [10]. These two species were both regarded as the official sources of Curcumae Rhizoma recorded in the Chinese Pharmacopoeia, while their main effective constituents of essential oil may exist difference. Thus, the two species of essential oil and herbal sample of Curcumae Rhizoma were speculated to be treated separately in scientific study and even in the medical market.

In the comparative study of processed products, samples S1-S4 (raw CP) and S14-S15 (vinegar processed samples from CP) showed no significant difference, except isoborneol only; samples S5, S7, S9, S10 and S12 (raw CK) and samples S6, S8, S11, S13 and S16 (vinegar processed samples from CK) neither showed significant difference, except α-terpineol only.

PCA analysis

98% of the total variance was accumulated in two principal components (PC). As shown in Fig. 1, samples could be clearly grouped into two groups A (CK) and B (CP). However, the raw and processed products existed no difference. The results stayed the same with ANOVA analysis result. Excluding geographical distribution and environmental factors, we could speculate that Curcumae Rhizoma commercially available may need to be distinguished with species. Moreover, in consideration of the imbalance of the three species recorded according to Chinese Pharmacopoeia in the medical market, the mixed usage in clinical was common. More studies should focus on the degree of variance from the three species, so as to regulate clinical medication.

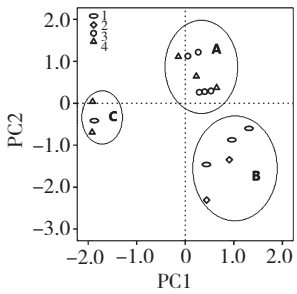

Fig. 1 PCA plot of 16 batches of Curcumae Rhizoma samples

vinegar processed samples from CP; 2: samples from CP; 3: vinegar processed samples from CK; 4: samples from CK.

Exceptionally, three other samples (S2, S13 and S16) got to group *C. curzerenone* [11] as the most constituent found in the essential oil of Curcumae Rhizoma, 32.1%-59.0% of content in most of the samples, with germacrone (1.3%-9.2%) [12] and curcumenol (0.4%-10.8%) [13], was reported to possess excellent antitumor

activity. However, the content of curzerenone varied greatly among the samples commercially available. Thereinto, the content of curzerenone in sample 2 sold in Sichuan Chongzhou was the highest, reaching 59.0%, while sample 13 (Guangdong Guangzhou) and 16 (Fujian Fuzhou) nearly did not contain it and only 0.7% in sample 4. Thus it can be seen, quality control in samples commercially available existed some problems needing solved urgently.

There was no significant difference between raw and processed products of Curcumae Rhizoma, being agreement with above result in ANOVA analysis. According to TCM theory, the usage of the raw sample and vinegar-processed sample was different in clinics. The raw one emphasised on promoting the circulation of qi and removing food retention. While the vinegar processed one can help it work through liver meridian and blood system, thus enhanced the activity of activating blood and resolve lumps. It was also reported that vinegar processing only affected the content of essential oil from Curcumae Rhizoma, instead of its chemical ingredients[14]. Hence, it was possible that the non-volatile ingredient was the main factor of the difference between raw and processed product. According to Chinese Pharmacopoeia, it does not mention the preparation method of Curcumae Rhizoma essential oil. Therefore, comparatively chemical and effective study on non-volatile ingredient of the raw and processed product should be carried out, so as to provide a guide to clarify the relationship of the TCM theory and modern science, and standard medication.

Cytotoxic activity on MCF-7 cell lines of the essential oil

As a preliminary data for analyzing the essential oils, their anticancer effect was conducted. Different batches of essential oil were tested *in vitro* for the inhibitory effect on human tumor cell lines MCF-7, using MTT assay. The concentration that inhibited cell vitality by 50% (IC_{50}) was represented in Table 3. Apparently, all batches were almost equally effective and presented IC_{50} values ranging from 40.2-62.9 μg/mL (no significant intergroup difference).

Table 3 ***In vitro*** **cytotoxic activity of the essential oils from different batches of Curcumae Rhizoma**

Samples	MCF-7 IC_{50} (μg/mL)	Samples	MCF-7 IC_{50} (μg/mL)
S1	41.5	S9	49.5
S2	53.6	S10	49.3
S3	44.4	S11	49.5
S4	40.2	S12	55.8
S5	42.3	S13	49.5
S6	45.7	S14	62.9
S7	54.9	S15	50.5
S8	48.0	S16	51.3

Conclusion

This is the first report regarding the volatile profiles of essential oil from Curcumae Rhizoma commercially available. The results showed that volatile compounds were in? uenced by species. There were notable quantitative differences in prominent compounds such as 1, 8-cineole, camphor, camphene hydrate, isoborneol, borneol, β-elemene, (*E*)-caryophyllene, β-chamigrene, β-selinene, *cis*-β-guaiene, selina-3, 7(11)-diene, elemol, carotol, β-eudesmol and curcumenol. The essential oils from CP and CK were distinguished from CW oils, the official source of essential oils of Curcumae Rhizoma. With the use of multivariate analysis, Curcumae Rhizoma from different species could be differentiated through the formation of groups based on the volatile compounds identified from chromatography. Otherwise, the non-volatile ingredients may be the main factor of the difference between the raw and processed products. Research on their efficacy evaluation may explore more sensitive pharmacodynamic indexes, which the mechanism of action of the principle component can be one direction. More study on it could provide a guide to clarify the relationship of the TCM theory and modern science, and help regulate clinical medication of Curcumae Rhizoma.

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