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Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 486–492
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Quantitative determination of eight components in rhizome (*Jianghuang*) and tuberous root (*Yujin*) of *Curcuma longa* using pressurized liquid extraction and gas chromatography–mass spectrometry

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Received 14 June 2006; received in revised form 16 July 2006; accepted 17 July 2006
Available online 23 August 2006

Abstract

Curcuma longa (Zingiberaceae) is a native plant of southern Asia and is cultivated extensively throughout the warmer parts of the world. *Jianghuang* and *Yujin* are rhizome and tuberous root of *C. longa*, respectively, which were traditionally used as two Chinese medicines. In this paper, pressurized liquid extraction (PLE) and gas chromatography–mass spectrometry (GC–MS) were developed for quantitative determination/estimation of eight characteristic compounds including β-caryophyllene, ar-curcumene, zingiberene, β-bisabolene, β-sesquiphellandrenendrene, ar-turmerone, α-turmerone and β-turmerone in *Jianghuang* and *Yujin*. A HP-5MS capillary column (30 m × 0.25 mm i.d.) coated with 0.25 μm film 5% phenyl methyl siloxane was used for separation and selected ion monitoring (SIM) method was used for quantitation. Hierarchical cluster analysis based on characteristics of eight identified peaks in GC–MS profiles showed that 10 samples were divided into two main clusters, *Jianghuang* and *Yujin*, respectively. Four components such as ar-curcumene, ar-turmerone, α-turmerone and β-turmerone were optimized as markers for quality control of rhizome (*Jianghuang*) and tuberous root (*Yujin*), which are two traditional Chinese medicines, from *Curcuma longa*.

Keywords: Pressurized liquid extraction (PLE); Gas chromatography–mass spectrometry (GC–MS); *Curcuma longa* L.; *Jianghuang*; *Yujin*

1. Introduction

Curcuma longa L. (Zingiberaceae) is a native plant of southern Asia and is cultivated extensively throughout the warmer parts of the world. It is grown on a large scale in India and China. Its rhizome, in powder form (turmeric) is widely used as a food additive (to impart flavor and a yellow color) and has been also very popular in Asian medicine for the treatment of coryza, hepatic disorders and rheumatism [1]. *C. longa* is reported possess multiple pharmacological activities, including antioxidation [2–4], antimicrobial [5–7], antiatherosclerotic [8,9], anti-inflammatory [10,11], antidepressant [12], antiplatelet [13] and immune activation [14] activities. The essential oil of *C. longa* is considered as one of the major active fractions [2,5,6,13]. Actually, rhizome and tuberous root of *C. longa* are used as two Chinese medicines, e.g. *Jianghuang* and *Yujin*, respectively [15]. Traditionally, they have different pharmacological activities [16] and clinical indications [15]. Therefore, the difference of chemical components between them may contribute to their efficacies. Up to date, no components in essential oil of *C. longa* was quantitatively determined because of absence of chemical standards, though identification and relative amount of components in turmeric oil have been determined by LC–MS [17], GC and GC–MS [6,18,19]. Unfortunately, the results of relative amount could not be used for evaluating the quality of different samples or batches [20]. In addition, it is difficult to determine clinic dose of herbs without accurate amount of the active components.

In present study, pressurized liquid extraction (PLE) and GC–MS was developed for simultaneous determination/estimation of eight compounds, including β-caryophyllene, ar-curcumene, zingiberene, β-bisabolene, β-sesquiphellandrenendrene, ar-turmerone, α-turmerone and β-turmerone, in rhizome (*Jianghuang*) and tuberous root (*Yujin*) of *C. longa*. The chemical difference between *Jianghuang* and *Yujin* were also compared using hierarchical cluster analysis.

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doi:10.1016/j.jpba.2006.07.034

2. Materials and methods

2.1. Materials

Each five samples for *Jianghuang* (J1–J5) and *Yujin* (H1–H5) were collected from Wuning, Jiangxi Province; Quanzhou, Fujian Province; Shuangliu, Qianwei and Chongzhou, Sichuan Province, respectively. ar-curcumene, β-turmerone and α-turmerone were separated and purified ourselves (Fig. 1). The structures were confirmed by comparing their EI-MS and NMR data with references [21–24]. ar-turmerone and methanol for GC were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany), respectively.

2.2. Pressurized liquid extraction

Pressurized liquid extractions were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. Powder of *Jianghuang* (0.2 g) or *Yujin* (0.4 g) was mixed with diatomaceous earth at the ratio of 1:1, and placed into 11 ml stainless steel extraction cell, respectively. The sample was extracted under the optimized conditions: solvent, methanol; temperature, 140 °C; particle size, 0.15–0.20 mm; static extraction time, 5 min; pressure, 1000 p.s.i.; static cycle, 1; and 60% of the flush volume. Then, extract was transferred to a 25 ml volumetric flask which was made up to its volume with extraction solvent and filtered through a 0.45 μm Econo filter (Agilent Technologies) prior to injection into the GC–MS system.

2.3. GC–MS analysis

GC–MS was performed on an Agilent 6890 gas chromatography instrument coupled with an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies, Palo Alto, CA). A HP-5MS capillary column (30 m × 0.25 mm i.d.) coated with 0.25 μm film 5% phenyl methyl siloxane was used for separation. The column temperature was at 80 °C for injection, then programmed at 20 °C min^{-1} to 120 °C, then at 1 °C min^{-1} to 130 °C and held for 5 min, then at 4 °C min^{-1} to 160 °C, finally, at 20 °C min^{-1} to 280 °C. Split injection (2 μl) was conducted with a split ratio of 10:1 and high purity helium was used as carrier gas of 1.0 ml min^{-1} flow-rate. The mass spectrometer was operated in electron-impact (EI) mode, the scan range was 40–550 amu, the ionization energy was 70 eV and the scan rate was 0.34 s per scan. The inlet, ionization source temperature were 250 and 280 °C, respectively.

3. Result and discussion

3.1. Optimization of PLE procedure

PLE procedure was optimized. The parameters including the type of solvent (methanol, ethanol, ethyl acetate

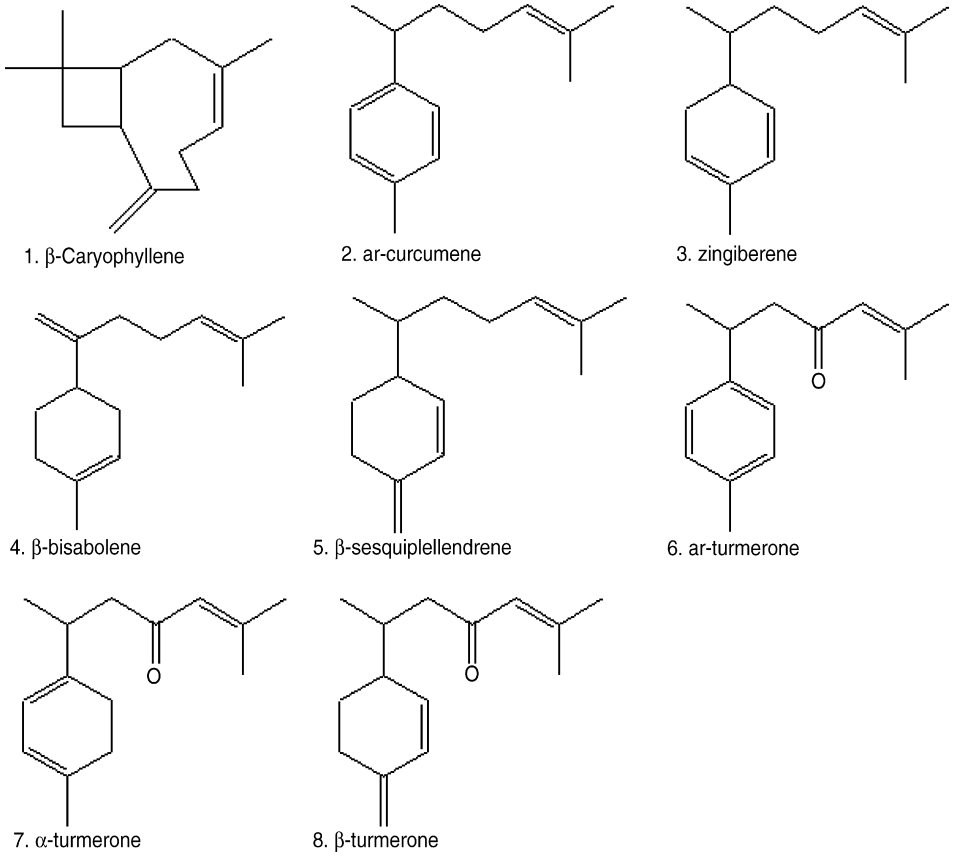

Fig. 1. Structures of eight investigated volatile compounds in *Curcuma longa*.

and chloraform), particle size (0.15–0.45 mm), temperature (80–160 °C), static extraction time (5–15 min), extraction pressures (500–1500 p.s.i.), flush volume (20–60%), extraction cycles (one to three cycles) and number of extraction (one to three times) were studied by using univariate approach. Total amount of investigated compounds were used as marker for evaluation of extraction efficiency. The recovery efficiency for the PLE procedure was determined by performing consecutive pressurized liquid extractions on the same sample under the optimized PLE conditions, until no investigated compounds were detected by the analysis. The recovery was calculated based on the total amount of individual investigated components. Taking into account the results of optimization and recovery experiment (data not shown), the conditions of the PLE method proposed were: solvent, methanol; temperature, 140 °C; particle size, 0.15–0.20 mm; static extraction time, 5 min; pressure, 1000 p.s.i.; static cycle, 1; number of extraction, 1; and 60% of the flush volume.

3.2. Identification of compounds in Jianghuang and Yujin

Total ion chromatograms of *Jianghuang* and *Yujin* were shown in Fig. 2. Eight characteristic peaks were identified in *Jianghuang* and *Yujin* by comparing the mass spectra with literature [21–27] or standards, which were β-caryophyllene, ar-curcumene, zingiberene, β-bisabolene, β-sesquiphellandrene, ar-turmerone, α-turmerone and β-turmerone, respectively. The MS data are summarized in Table 1.

3.3. Quantitative determination of investigated components in Jianghuang and Yujin

The selected ion monitoring (SIM) method was used for quantitation of eight investigated compounds (Table 1). The peaks of ar-turmerone and α-turmerone could not be baseline separated, so the characteristic fragment ions of *m/z* 132 and 111 were selected for their quantitation, respectively, which could completely separated the two peaks with higher selectivity (Fig. 3). The contents of β-caryophyllene, zingiberene, β-bisabolene, and β-sesquiphellandrenendrene were estimated by using calibration curve of ar-curcumene which is one of the major components in *C. longa*.

A series of concentrations for standards were obtained by dissolving the compounds in methanol, and then performed

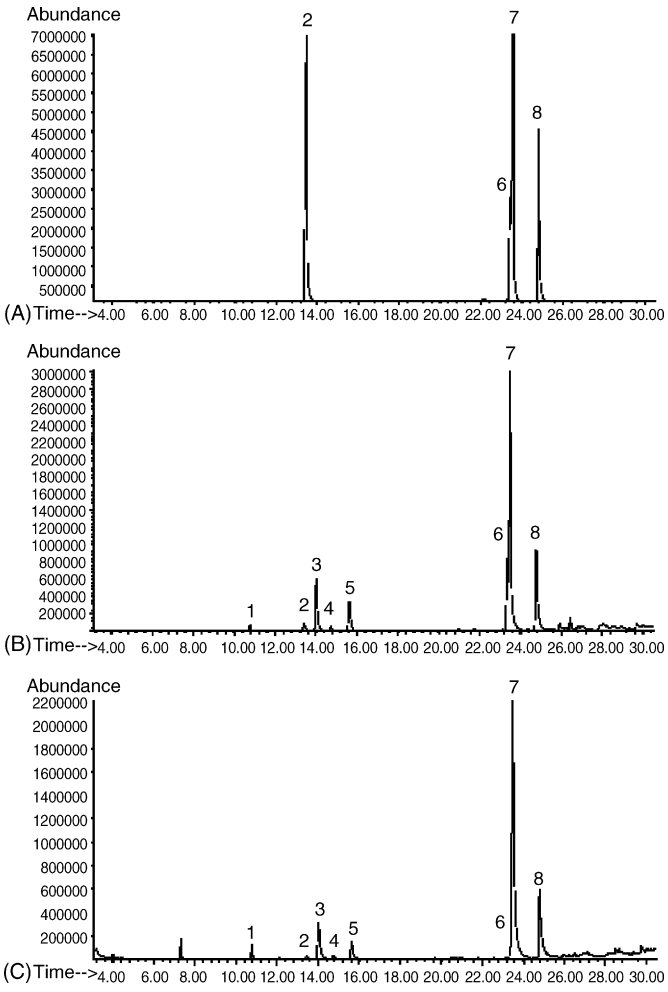

Fig. 2. GC–MS total ion chromatograms of (A) mixed standards, (B) *Jianghuang* and (C) *Yujin*. (1) β-caryophyllene; (2) ar-curcumene; (3) zingiberene; (4) β-bisabolene; (5) β-sesquiphellandrene; (6) ar-turmerone; (7) α-turmerone and (8) β-turmerone.

the analysis as mentioned above. The calibration curves, which obtained from the selected ions peak area, for ar-curcumene, ar-turmerone, α-turmerone and β-turmerone were linear over the ranges 1.71–54.70, 2.35–75.00, 1.91–61.20 and 7.42–118.80 ng absolute on the column, with the coefficient of correlation (*r*) higher than 0.9997. The limits of detection (LOD) and quantification (LOQ) for each analyte were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively. The data were shown in Table 2.

Table 1
Mass data of eight compounds identified in *Curcuma longa*

Peak no.	RT (min)	Compound	Mass data[a]
1	10.80	β-Caryophyllene	204(M+, 12), 161(38), 133(95), 119(35), 105(54), 93(100), 91(88), 79(81), 69(79), 55(36), 41(77)
2	13.75	ar-Curcumene	202(M+, 31), 145(25), 132(98), 131(26), 120(27), 119(100), 117(23), 105(47), 91(24), 41(22)
3	14.02	Zingiberene	204(M+, 11), 119(100), 93(82), 91(35), 77(23), 69(26), 56(10), 55(9), 41(19)
4	14.75	β-Bisabolene	204(M+, 29), 161(23), 135(13), 121(7), 119(69), 109(28), 93(81), 79(37), 69(100), 67(39), 41(71)
5	15.63	β-Sesquiphellandrene	204(M+, 27), 161(49), 133(36), 120(36), 93(64), 91(55), 77(37), 69(100)
6	23.34	ar-Turmerone	216(M+, 30), 201(20), 132(20), 120(7), 119(72), 117(14), 115(8), 105(11), 91(14), 83(100), 55(15)
7	23.75	α-Turmerone	218(M+, 4), 120(55), 119(50), 111(27), 105(97), 93(19), 91(32), 85(15), 83(100), 77(23), 55(23),
8	24.78	β-Turmerone	218(M+, 2), 121(10), 120(100), 105(15), 93(3), 92(6), 91(13), 83(25), 79(4), 77(7), 55(9)

[a] *m/z*, relative intensity shown in parenthesis, and the ion of relative intensity 100 was used for the quantification except for ar-turmerone and α-turmerone.

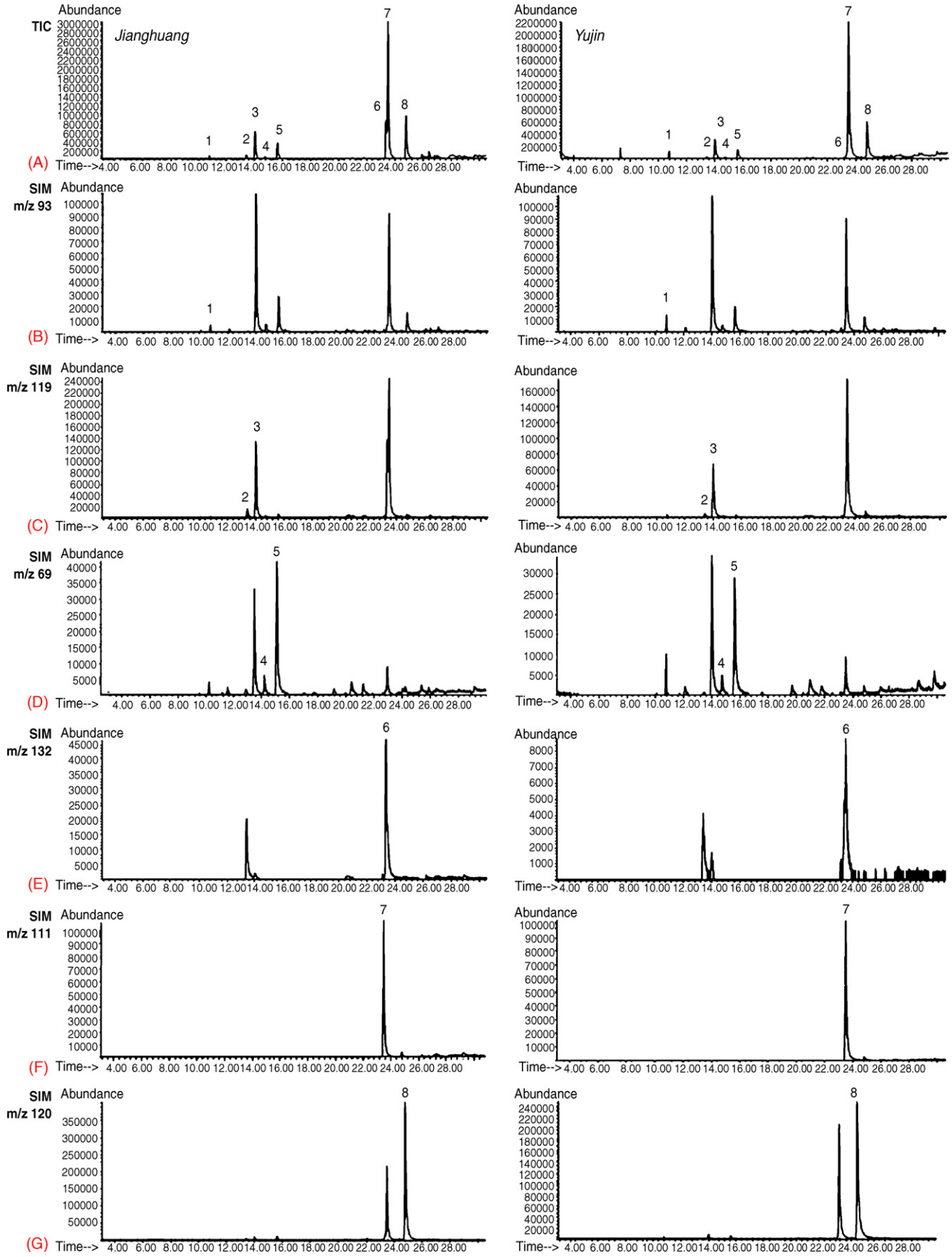

Fig. 3. GC–MS total ion chromatograms of (A) PLE extract and the selected ion chromatograms for (B) β-caryophyllene, (C) ar-curcumene + zingiberene, (D) β-bisabolene + β-sesquiphellandrene, (E) ar-turmerone, (F) α-turmerone and (G) β-turmerone.

Table 2
Linear regression data, LOD and LOQ of four investigated compounds in rhizome (*Jianghuang*) and tuberous root (*Yujin*) from *Curcuma longa*

Analytes	SIM	Linear regression data		*r*	LOD (ng)	LOQ (ng)
		Regression equation	Linear range (ng)			
ar-Curcumene	119	$y = 48220x - 79773$	1.71–54.70	0.9998	0.257	0.428
ar-Turmerone	132	$y = 22923x - 47571$	2.35–75.00	0.9999	0.411	0.587
α-Turmerone	111	$y = 25290x - 35022$	1.91–61.20	0.9997	0.574	0.957
β-Turmerone	120	$y = 75256x - 440624$	7.42–118.80	0.9998	0.519	0.743

Table 3
Intra-and inter-day variability for the assay of four investigated compounds

Analytes	Conc. (μg/ml)	Intra-day ($n = 6$)			Inter-day ($n = 12$)		
		Found (μg/ml)	R.S.D. (%)	Accuracy[a] (%)	Found (μg/ml)	R.S.D. (%)	Accuracy (%)
ar-Curcumene	8.55	9.21	0.73	107.72	9.41	1.65	110.06
	34.19	34.43	0.25	100.56	37.53	1.00	109.76
	136.75	139.42	0.53	101.95	138.01	2.82	100.92
ar-Turmerone	11.72	12.52	0.77	106.83	12.92	1.23	110.26
	46.88	44.73	1.10	95.39	45.31	1.87	96.52
	187.50	185.91	0.73	99.19	188.29	1.49	100.43
α-Turmerone	9.56	10.00	1.77	104.91	10.43	2.76	108.99
	38.25	36.21	1.05	94.44	36.61	1.40	95.48
	153.00	148.79	0.74	96.99	151.24	1.53	98.29
β-Turmerone	17.43	18.40	0.72	105.75	18.92	1.60	108.62
	69.81	69.30	0.41	99.28	70.19	1.96	100.57
	279.00	293.20	0.59	105.09	296.60	1.36	106.31

[a] Accuracy (%) = 100 × mean of measured concentration/nominal concentration.

Intra- and inter-day variations were chosen to determine the precision of the developed assay. For intra-day variability test, three concentrations of the mixed ar-curcumene, ar-turmerone, α-turmerone and β-turmerone solution were analyzed for six replicates within 1 day; while for inter-day variability test, the solution was examined in triplicates for consecutive 2 days. For every calibration curve, the calibration concentrations were back-calculated from the related peak area of the analytes. The deviation from the nominal concentration defined as accuracy (Table 3).

The stability of ar-curcumene, ar-turmerone, α-turmerone and β-turmerone were also determined by injecting freshly prepared standard solution for three times at 0, 1, 2, 4, 8 and 12 h, respectively. The R.S.D.% of ar-curcumene, ar-turmerone and α-turmerone and β-turmerone was 0.84, 1.22, 0.96 and 1.38% at the concentration of 34.19, 46.88, 38.25 and 69.81 μg/ml, respectively.

The recovery was preformed by adding known amount of individual standards into an accurately weighed sample (*Jianghuang* produced from Chongzhou, Sichuan Province).

Table 4
Recoveries for the assay of four investigated compounds

Analytes	Added (mg)	Found (mg)	Average recovery[a] (%)	R.S.D. (%)
ar-Curcumene	0.173	0.178	102.78	1.91
	0.345	0.363	105.25	2.67
	0.650	0.666	102.49	3.46
ar-Turmerone	0.400	0.390	97.09	2.44
	0.800	0.777	97.39	2.78
	1.600	1.522	95.07	1.77
α-Turmerone	1.063	1.022	96.29	1.21
	2.125	2.084	98.03	0.71
	4.250	4.193	98.65	1.71
β-Turmerone	1.305	1.317	100.92	2.20
	2.608	2.509	96.20	4.00
	5.215	5.300	101.63	3.58

Recovery (%) = 100 × (amount found − original amount)/amount spiked.
[a] The data was present as average of three determinations.

Table 5
The contents (mg/g) of eight investigated compounds in rhizome (*Jianghuang*) and tuberous root (*Yujin*) from *Curcuma longa*

Sample[a]	β-Caryophyllene	ar-Curcumene	Zingiberene	β-Bisabolene	β-Sesquiphellandrene	ar-Turmerone	α-Turmerone	β-Turmerone	Total
J1	+[b]	3.19[c]	3.63	3.43	2.81	9.02	14.58	17.27	53.93
J2	+	3.32	8.67	1.49	4.78	12.55	18.14	28.33	77.27
J3	+	3.48	11.86	1.57	5.92	12.63	21.85	31.43	88.74
J4	+	2.67	13.19	1.59	5.17	8.52	21.87	27.70	80.71
J5	+	2.55	12.84	1.52	5.47	7.55	21.31	26.49	77.73
H1	+	+	1.94	+	1.02	+	4.27	5.86	13.09
H2	0.75	0.85	4.02	0.78	1.59	+	9.91	11.14	29.02
H3	0.86	+	6.30	0.89	2.21	1.03	10.86	9.53	31.66
H4	0.88	+	5.17	0.95	2.41	0.92	10.01	8.26	28.61
H5	0.85	+	6.61	0.79	2.15	+	9.32	11.23	30.94

[a] *Jianghuang* (J1–J5) and *Yujin* (H1–H5) are rhizome and tuberous root of *C. longa*, which were collected from Wuning, Jiangxi Province; Quanzhou, Fujian Province; Shuangliu, Sichuan Province; Qianwei, Sichuan Province; Chongzhou, Sichuan Province, respectively.
[b] Below the limit of quantitation.
[c] The data was presented as average of three replicates (R.S.D.% < 4%).

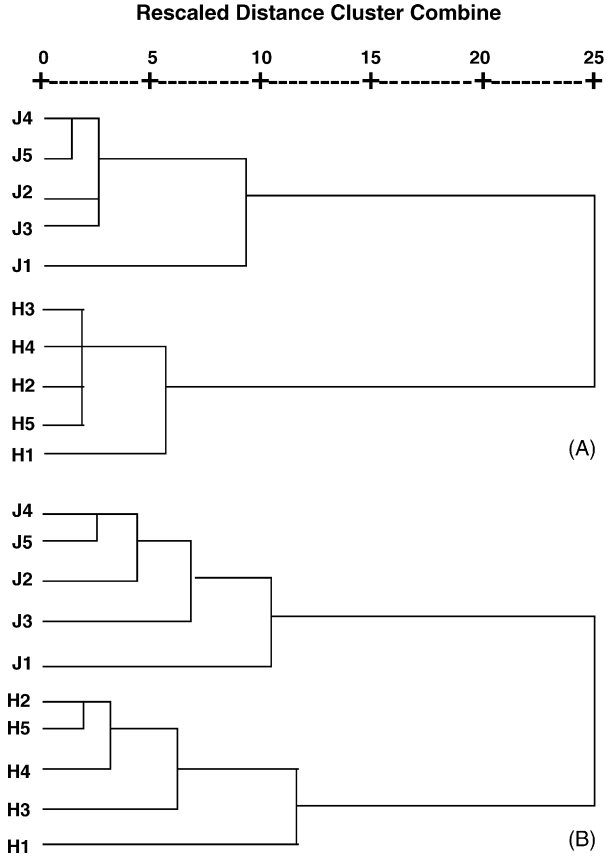

Fig. 4. Dendrograms resulting from average linkage between groups hierarchical cluster analysis. The hierarchical clustering was done by SPSS software. A method named as average linkage between groups was applied, and Squared Euclidean Distance was selected as measurement. (A) Dendrogram resulting from eight peaks, their retention times and peak area, derived from GC fingerprints of the tested 10 samples. (B) Dendrogram resulting from the characteristics of ar-curcumene, ar-turmerone, α-turmerone and β-turmerone. Five samples of *Jianghuang* (J1–J5) and *Yujin* (H1–H2) were collected from Wuning, Jiangxi Province; Quanzhou, Fujian Province; as well as Shuangliu, Qianwei and Chongzhou, Sichuan Province, respectively.

The mixture was extracted and analyzed using the method mentioned above. Table 4 shows the recoveries of the four investigated compounds.

It is difficult to use GC or HPLC to identify the peaks without standards. However, it is easier using GC–MS. The content of eight identified compounds in *C. longa* were determined or estimated. Table 5 shows the summary results. The data could be used for evaluating the quality of samples, though some errors for quantification of β-caryophyllene, zingiberene, β-bisabolene, and β-sesquiphellandrenendrene were existent because of using ar-curcumene as standard. The results showed the contents of eight investigated compounds were greatly variant in *Jianghuang* and *Yujin*.

3.4. Comparison of Jianghuang and Yujin

In order to differentiate the *Jianghuang* and *Yujin*, hierarchical cluster analysis was performed based on the eight identified characteristic peaks from GC–MS profiles. A method named as average linkage between groups was applied, and Squared Euclidean Distance, allow for distances between clusters, was selected as Interval measurement. Fig. 4A shows the result on the tested 10 samples, which are divided into two main clusters, *Jianghuang* and *Yujin*. Among eight identified peaks, two peaks including ar-curcumene and ar-turmerone were obviously different between *Jianghuang* and *Yujin*. Using the peak's characteristics of ar-curcumene, ar-turmerone, α-turmerone and β-turmerone, hierarchical cluster analysis was also performed as mentioned above. The result was the same as the one derived from eight peaks characteristics (Fig. 4B). Therefore, ar-curcumene, ar-turmerone, α-turmerone and β-turmerone could be used as markers for discrimination of *Jianghuang* and *Yujin*, as well as quality control of rhizome and tuberous root, which are used as two Chinese medicines, of *C. longa*.

4. Conclusion

The developed PLE and GC–MS method is simple, rapid and accurate for quantitative determination of major volatile compounds in rhizome (*Jianghuang*) and tuberous root (*Yujin*) of

Curcuma longa, which is helpful to control the quality of the two Chinese medicines.

Acknowledgements

We are grateful to P. Li and J. B. Wang from our institute, Prof. C.M. Fu from Chengdu University of TCM for their expert technical assistance.

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