Gas Chromatography–Mass Spectrometry for Quantitative and Qualitative Analysis of Essential Oil from Curcuma wenyujin Rhizomes

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Abstract

Objectives: A rapid and sensitive gas chromatography–mass spectrometry (GC–MS) method for quantitative and qualitative analysis of essential oil from Curcuma wenyujin rhizomes was established. Methods: The essential oil of C. wenyujin rhizomes was extracted by supercritical CO2 extraction (SFE). Six main bioactive compounds (eucalyptol, β-elemene, curzerene, germacrone, curdione, and curcumol) were analyzed in selected ion monitoring mode (SIM). Results: Curzerene is not originally present in C. wenyujin rhizomes, but is a product of the transformation of furanodiene at high temperature. The six target components demonstrated good linearity (R2 > 0.9979) over a relatively wide concentration range. The interday and intraday variations had relative standard deviation values less than 5% and the average recovery ranged from 96.95% to 100.04%. The limit of quantitation ranged from 0.032 to 0.235 μg/mL. The developed method was successfully used to analyze the six compounds in 17 samples collected from different origins. Significant variation was observed for the concentrations of the six compounds. In addition, 51 constituents were identified in C. wenyujin rhizome essential oil, consisting of 87.66% of the total essential oil, including curdione, curzerene, dehydrocurdione, germacrone, 1,4-bis(2-benzimidazoyl)benzene, neocurdione, curcumone, and β-elemene. Conclusions: The proposed method will be useful in the quality control of C. wenyujin rhizome essential oil production.

Keywords: Curcuma wenyujin, essential oil, gas chromatography–mass spectrometry, quantitative analysis, supercritical CO2 extraction

Introduction

Curcuma wenyujin Y. H. Chen et C. Ling is a perennial plant belonging to the family Zingiberaceae and is mainly cultivated in the Chinese provinces of Zhejiang, Guangdong, Guangxi, Jiangxi, Fujian, and Hainan. The rhizomes of C. wenyujin are used as a traditional Chinese medicine for the treatment of jaundice, thoracic-abdominal pain, arthralgia, hematuria, dysmenorrhea, epilepsy, and psychataxia.1-3 Modern pharmacological studies have revealed that the essential oil of C. wenyujin rhizomes possesses various biological activities, including antimicrobial, anti-inflammatory, anticancer, antiviral, and antidepressant activity.4-6 The essential oil from C. wenyujin rhizomes is considered to be an effective product and is listed in the Chinese Pharmacopoeia (ChP) as an antiviral remedy to treat pediatric diseases, such as acute upper respiratory infections, viral myocarditis, and acute pneumonia.1,9,10 Hainan Bikai Pharmaceutical produces the drug “bao fu kung shuan,” which contains C. wenyujin rhizome essential oil as the main ingredient. This drug is used primarily...

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as a treatment for vaginitis and cervical erosion. Therefore, the quantitation of the functional components of *C. wenyujin* rhizome essential oil is desirable to ensure the efficacy of drugs produced from the oil.

Phytochemical investigations have demonstrated that eucalyptol, β-elemene, curzerene, germacrone, curdione, furanodiene, and curcumol are the main bioactive ingredients of *C. wenyujin* rhizome essential oil.[11-13] Germacrone, furanodiene, and β-elemene are sesquiterpenoids that show antitumor activity by inhibiting cell proliferation, arresting the cell cycle, inducing cell apoptosis, exerting antiangiogenesis and antimetastasis effects, and enhancing the immune system.[14-16] Germacrone and furanodiene can be used as marker components to assess the quality of *C. wenyujin* rhizome essential oil as indicated in the ChP.[1] Eucalyptol, curdione, and curcumol are the key active components used for the treatment of blood stasis.[17] Eucalyptol has been studied for its anti-inflammatory, expectorant, and hypotensive effects.[18,19] In blood system diseases, eucalyptol has a therapeutic effect. Curcumol has been shown to have strong antiplatelet aggregation (clinically used to treat blood stasis), antifibrosis, analgesic, and anti-inflammatory activities, and inhibits cell proliferation. In addition, curdione also has an antitumor effect through inhibiting proliferation and inducing the apoptosis of a variety of malignant tumor cells (e.g., A549 and CEN-2 cells).[20,21] Curdione has been reported to have a strong antiplatelet aggregation effect and is mainly used to treat blood stasis.[22] At present, most studies of *C. wenyujin* rhizome essential oil are focused on the identification of compounds and the determination of their relative amounts.[23] The results from qualitative research cannot accurately assess the quality of *C. wenyujin* rhizome essential oil.

Supercritical fluid extraction (SFE) has great potential as an alternative process to conventional solvent extraction and steam distillation (SD) to produce essential oils, especially in the herb and spice industries. The advantage of SFE regarding essential oil extraction is that it is free of solvent residues, and the process can be conducted at low temperature, which is especially useful for thermolabile compounds.[24]

The objective of this study was to develop a rapid and efficient gas chromatography–mass spectrometry (GC–MS) method for the simultaneous determination of six bioactive components [eucalyptol, β-elemene, curzerene, germacrone, curdione, and curcumol; Figure 1] in the essential oil of *C. wenyujin* rhizomes extracted by SFE. This article describes the comprehensive investigation of the essential oil from *C. wenyujin* rhizomes, and provides a theoretical basis for the development and utilization of *C. wenyujin*.

**Materials and Methods**

**Chemical and reagents**

Ethyl acetate and methanol (high-performance liquid chromatography grade) were purchased from Merck (Darmstadt, Germany). Reference standards of eucalyptol, β-elemene, curzerene, furanodiene, germacrone, curdione, curcumol, and eugenol (internal standard [IS]) were obtained from Chengdu Chroma-Biotechnology (Chengdu, China). The purities of the reference compounds were >98%.

The rhizomes of *C. wenyujin* were collected from the Chinese provinces of Zhejiang, Jiangxi, Fujian, and Hainan. All samples were authenticated by research associate Rongtao Li, Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China. Voucher specimens were deposited in the Resource Center for Chinese Materia Medica, Hainan Branch Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College, China. The samples were chopped into small pieces, dried in the air, and ground in a high-speed rotary cutting mill. The powdered samples were stored at −20°C in a refrigerator until analysis.

**Gas chromatography–mass spectrometry conditions**

Chromatographic separation was conducted on an Agilent 7890A gas chromatograph coupled to a 5975C quadrupole mass spectrometer and automated 7683B sample injector system (Agilent, Santa Clara, CA, USA). Chromatography was performed on an HP-5MS column (30 m × 0.25 mm i.d. × 0.5 μm; Agilent). The carrier gas was helium at a constant flow rate of 1.0 mL/min. The GC oven temperature program was: Initial temperature of 60°C, increased to 100°C at 10°C/min; increased to 200°C at 4°C/min; increased to 280°C at 20°C/min, and held for 7 min. The injection volume was 1.0 μL using split mode (20:1) at 220°C. The mass selective detector was operated with electron energy of 70 eV in electron ionization mode. The ion source and quadrupole temperatures were 230 and 150°C, respectively. The selected ion monitoring (SIM) conditions for the selected components were determined after identification of the most abundant and characteristic ions in full scan mode. Three ions were selected for each compound for SIM mode, one of which was used for quantitation [Table 1] with a solvent delay of 3.0 min. The main compounds in the essential oil

![Figure 1: The structures of six target compounds](image-url)
were identified using the scan mode in the mass range m/z 50–550 amu.

### Preparation of standard solutions

Stock solutions of each target compound and eugenol (IS) were prepared in ethyl acetate. The six stock solutions were mixed and diluted with ethyl acetate to prepare a final mixed standard containing 63.8 μg/mL of eucalyptol, 86 μg/mL of β-elemene, 316 μg/mL of curdione, 94 μg/mL of curcumol, 156 μg/mL of germacrone, and 134 μg/mL of curzerene. A series of working solutions of these compounds was obtained by diluting the mixed standard solutions with ethyl acetate at the appropriate concentrations. The concentration of the IS solution was 10 μg/mL. All solutions were stored at 4°C.

### Preparation of sample solutions

Extraction was accomplished using a supercritical fluid extractor (SFT-110, Supercritical Fluid Technologies, Newark, DE, USA). Approximately 100 g of sample was loaded into the 500-mL supercritical CO$_2$ vessel. The highest yield of essential oil was obtained under the optimized conditions of pressure 25 MPa, temperature 50°C, time 120 min, and flow of CO$_2$ 15 L/h. The essential oil was collected, dried over anhydrous Na$_2$SO$_4$, and stored in a refrigerator (4°C). Ten microliters of the *C. wenyujin* rhizome essential oil was added to a 10-mL volumetric flask and diluted to volume with ethyl acetate. The concentration of IS solution was 10 μg/mL. The solution was then filtered through a 0.22-μm membrane filter before injection into the GC system. All samples were analyzed in triplicate.

### Method validation

The GC–MS method was validated in terms of linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, repeatability, stability, and accuracy for all target compounds. Data for calibration curves were generated from six concentrations for each analyte (n = 3). Calibration curves were plotted as peak-area ratios of the analyte to IS (Y) against the ratios of analyte concentration to IS (X). The LOD was defined as the concentration with a signal-to-noise (S/N) ratio of 3; for LOQ, S/N = 10. Interday and intraday precision were determined by analyzing six replicates of a mixed standard solution during a single day and by duplication of the experiments on 3 consecutive days. To further confirm the repeatability of the developed method, six replicates of the *C. wenyujin* rhizome essential oil were analyzed. The relative standard deviation (RSD) was calculated as a measurement of precision and repeatability. Stability was evaluated under two different storage conditions (n = 3). The solutions were kept at room temperature (25°C) for 48 h and in a refrigerator (–4°C) for 5 days. The solution was considered to be stable when it presented a recovery >90%. Accuracy was evaluated using a recovery test in which the mean recovery of the target compounds was divided into three levels (low, 80% of known amount; mid, same as known amount; high, 120% of the known amount).

### Results and Discussion

#### Optimization of gas chromatography conditions

To achieve the resolution and separation of analytes in the essential oil, the chromatographic conditions were optimized, including the column type and injector temperature. First, different columns (HP-5 [30 m × 0.25 mm × 0.25 μm], DB1701 [30 m × 0.32 mm × 0.25 μm], and HP-INNOWax [30 m × 0.25 mm × 0.25 μm]) were compared for separation of compounds. The results showed that the HP-5 column provided the best resolution of the analytes. Given that the essential oil of *C. wenyujin* rhizomes contains heat-sensitive components such as furanodiene, consideration was given to the choice of injector temperature. Furanodiene is known to degrade to curzerene on exposure to heat through a [3,3]-sigmatropic reaction [Cope rearrangement, Figure S1]. Thus, effort was made to lower the injector temperature to avoid the degradation of heat-labile components in *C. wenyujin* rhizome essential oil [Figure S2]. However, when the injector temperature is >190°C, furanodiene was completely degraded to curzerene in a result similar to that from a previous report.[25] Thus, we considered that GC analysis was not viable for the determination furanodiene. To ensure the volatility of the other compounds, the injector temperature was set at 220°C. As a result, although furanodiene could not be directly quantitated, we determined the content of curzerene, and used this analysis as an indicator of furanodiene content.

#### Optimization of extraction method

Supercritical CO$_2$ extraction is a common method for the industrial extraction of volatile oils. The most important advantage of SFE regarding essential oil extraction is that this method can be performed at low temperature and thus thermal degradation of more sensitive compounds is avoided. Given that curzerene can be considered a representative component in *C. wenyujin* rhizome essential oil, the effects of different extraction methods (SD, ultrasonic-assisted ethyl acetate extraction) on the curzerene content were evaluated by ultraperformance liquid chromatography with photodiode array detection. The results showed that curzerene was not an original component of *C. wenyujin* rhizome essential oil [Figure S3]. High extraction temperature also led to

### Table 1: Gas chromatography-mass spectrometry selected ion monitoring mode conditions for the target compounds and internal standard

| Compounds   | Rt (min) | Ions (m/z) |
|-------------|----------|------------|
| Eucalyptol  | 5.003    | 81.10*;108.10;71.10 |
| β-Elemene   | 12.144   | 93.10*;81.10;67.10 |
| Curzerene   | 16.854   | 108.10*;148.05;79.10 |
| Curcumol    | 20.776   | 121.10*;107.10;93.10 |
| Germacrone  | 24.028   | 107.10*;135.05;67.10 |
| Curdione    | 24.890   | 180.20*;69.10;109.10 |
| Eugenol (IS)| 14.711   | 164.10*;149.05;103.10 |

*Extracted ion was used for quantification. IS: Internal standard
the degradation of furanodiene to curzerene. This result confirmed previous conjecture.\(^{[27]}\) Compared with other extraction methods, SFE is more suitable for the extraction of \textit{C. wenyujin} rhizome essential oil. To identify the optimal extraction conditions, the crucial factors of SFE were varied: Extraction time (1, 2, 3 h), temperature (40, 50, 60\(^\circ\)C), and pressure (15, 25, 35 MPa) were examined. When one factor was tested, the others were set at the default (extraction time, 2 h; temperature 50\(^\circ\)C; pressure, 25 MPa). The results showed that the yield of essential oil did not increase significantly for pressures above 25 MPa [Figure S4]. After testing, the favored SFE conditions were selected as: Extraction temperature, 50\(^\circ\)C; pressure, 25 MPa, extraction time, 2 h. Under optimal extraction conditions, the average yield of essential oil from \textit{C. wenyujin} rhizomes was 4.03\% (v/w).

### Method validation

The optimized GC–MS method was validated to determine the linearity, LOD, LOQ, precision, repeatability, accuracy, and stability. The results are summarized in Table 2. The calibration curves of the target compounds exhibited good linearity (\(R^2 > 0.9970\)) within the tested range. For the six components, the LOD ranged from 0.011 to 0.085 \(\mu\)g/mL and the LOQ ranged from 0.032 to 0.235 \(\mu\)g/mL. The intraday precision (measured as RSD) ranged from 0.87\% to 2.95\%, while the interday precision ranged from 1.56\% to 4.04\%. For the repeatability of the six analytes, the RSD values were all <5\%. Stability tests showed that the six components were stable under the two different conditions (25\(^\circ\)C or -4\(^\circ\)C) with recoveries above 90\%. The mean recoveries at three spiked levels varied from 96.95\% to 100.04\%, with RSD values of <5.0\%. These data indicated that the developed method is precise, accurate, and sensitive for the simultaneous determination of six components in the essential oil of \textit{C. wenyujin} rhizomes.

### Sample analysis

The developed GC–MS method was used to analyze six bioactive compounds in 17 batches of \textit{C. wenyujin} rhizome essential oil. A typical SIM chromatogram is shown in Figure 2. Each sample was tested in triplicate to determine the mean concentration. The concentrations of the six selected components were calculated using IS methods based on the respective calibration curves [Table 2], and the results are presented in Table 3. These results indicated that there were significant differences in the contents of eucalyptol, \(\beta\)-elemene, curzerene, curcumol, germacrone, and curdione among the 17 samples. Curzerene, germacrone, and curdione were found to be the most abundant in all samples, with respective concentration ranges of 105.96–174.89 mg/mL, 66.42–90.28 mg/mL, and 82.31–146.53 mg/mL. The relative amounts of germacrone and furanodiene in \textit{C. wenyujin} rhizome essential oil (extracted by SD) were reported as >7.5\% and >10\%, respectively, according to the ChP.\(^{[11]}\) Curzerene is a product of the transformation of furanodiene at high temperature. Therefore, the content of curzerene can reflect the content of furanodiene in \textit{C. wenyujin} rhizome essential oil. In this study, the amount of curzerene in all the samples was above this level (>10\%). Although the content of germacrone in three batches (S4, S12, S15) did not meet the level reported in the ChP, the values were close to this standard (7.5\%). The content

| Compounds     | Calibration curves         | \(R^2\) | Linear range (\(\mu\)g/mL) | LOD (\(\mu\)g/mL) | LOQ (\(\mu\)g/mL) | Precision (RSD, %) | Repeatability (RSD, %) | Recovery (\%, RSD) |
|---------------|---------------------------|---------|----------------------------|-------------------|------------------|---------------------|-----------------------|---------------------|
| Eucalyptol    | \(Y = 0.5067X - 0.0009\)  | 0.9994  | 0.032–31.9                 | 0.011             | 0.032            | 1.87                | 100.04                | 97.72 (3.78)        |
| \(\beta\)-Elemene | \(Y = 0.4117X + 0.0018\) | 0.9999  | 0.086–43.0                 | 0.034             | 0.086            | 1.74                | 99.24                 | 99.24 (4.26)        |
| Curzerene     | \(Y = 0.7969X + 0.0033\)  | 0.9987  | 0.158–316                  | 0.050             | 0.158            | 2.65                | 100.04                | 99.24 (4.26)        |
| Curcumol      | \(Y = 0.2062X - 0.0007\)  | 0.9979  | 0.235–47.0                 | 0.085             | 0.235            | 0.87                | 100.04                | 99.24 (4.26)        |
| Germacrone    | \(Y = 0.5297X - 0.0236\)  | 0.9990  | 0.156–156                  | 0.052             | 0.156            | 1.99                | 100.04                | 99.24 (4.26)        |
| Curdione      | \(Y = 0.6687X - 0.027\)   | 0.9987  | 0.067–134                  | 0.022             | 0.067            | 2.95                | 100.04                | 99.24 (4.26)        |

LOD: Limit of detection, LOQ: Limit of quantitation, RSD: Relative standard deviation.
of curdione in most batches was >10%. The trends for the three compounds (curzerene, germacrone, and curdione) in our results were similar to those found in previous research.\cite{23,28}

For example, Deng et al. analyzed curcumin, curdione, and germacrone in the rhizomes of three Curcuma species by microwave-assisted extraction followed by headspace solid-phase microextraction and GC–MS, and found that germacrone and curdione were the dominant sesquiterpenes in \textit{C. wenyujin} rhizomes.\cite{28} In another study, 11 sesquiterpenes were extracted from the rhizomes of three \textit{Curcuma} species by pressurized liquid extraction and analyzed by GC–MS. The study identified furanodiene, germacrone, and curdione as the main compounds in \textit{C. wenyujin} rhizomes.\cite{23} These results showed that curdione has the highest concentration of all components in \textit{C. wenyujin} rhizomes. Therefore, curdione can be recommended as a quality control marker in \textit{C. wenyujin} rhizome essential oil. Eucalyptol, β-elemene, and curcumol were relatively abundant in \textit{C. wenyujin} rhizome essential oil, with respective concentration ranges of 2.11–15.42 mg/mL, 8.09–25.03 mg/mL, and 2.71–25.34 mg/mL. In a previous study, Yang et al.\cite{25} did not detect curcumol in \textit{C. wenyujin} rhizomes. However, Deng et al. reported a concentration of 0.162–0.261 mg/g for curcumol in \textit{C. wenyujin} rhizomes.\cite{28}

To better comprehend the relationship between various samples from different origins, hierarchical cluster analysis was performed on standardized data to investigate the similarities between different samples. Average linkage clustering between groups was applied, and squared Euclidean distance, allowing for the distance between clusters, was selected for interval measurement. Figure 3 shows that the 17 samples analyzed can be divided into two main clusters (clusters I and II) based on the contents of the six components selected as variables. Furthermore, cluster I can then be divided into two subgroups (groups I\textsubscript{A} and I\textsubscript{B}). It is worth noting that the distance when all samples are divided into two clusters is 10, which suggests that the quality of all the tested samples was similar. As can be seen in Table 3, the concentrations of curzerene in cluster II samples (collected from Jiangxi and Fujian provinces) were significantly higher than in cluster I. In addition, the concentrations of curdione in group I\textsubscript{A} were significantly higher than in group I\textsubscript{B}. The samples in group I\textsubscript{A} were mainly collected from Zhejiang province, while group I\textsubscript{B} samples were mainly from Hainan province. These results indicated that the source of \textit{C. wenyujin} may have an influence on the contents of some of the main components in the essential oil.

**Qualitative analysis of components in \textit{Curcuma wenyujin} rhizome essential oil**

GC–MS was used for qualitative analysis of \textit{C. wenyujin} rhizome essential oil extracted by SFE. Most of the volatile

### Table 3: The amounts of 6 target compounds in \textit{Curcuma wenyujin} rhizomes essential oil (n=3, mg/mL)

| Samples | Origins | Eucalyptol | β-Elemene | Curzerene | Curcumol | Germacrone | Curdione |
|---------|---------|------------|-----------|-----------|----------|-------------|----------|
| S1      | Zhejiang| 2.45±0.03  | 14.13±0.56| 100.96±2.04| 12.92±0.24| 77.53±1.73 | 136.88±2.47|
| S2      | Zhejiang| 8.53±0.45  | 10.21±0.23| 126.63±1.76| 11.70±0.65| 76.12±1.78 | 146.53±2.58|
| S3      | Zhejiang| 4.88±0.05  | 16.63±0.74| 130.78±2.76| 6.28±0.26 | 75.02±2.22 | 139.72±3.13|
| S4      | Zhejiang| 2.11±0.02  | 16.43±0.78| 125.17±2.09| 7.64±0.18 | 66.80±1.48 | 133.06±2.94|
| S5      | Zhejiang| 3.29±0.02  | 10.15±0.14| 114.05±3.06| 7.84±0.08 | 77.15±1.76 | 135.07±3.96|
| S6      | Zhejiang| 8.28±0.32  | 23.30±0.99| 129.96±2.38| 9.21±0.61 | 78.14±2.06 | 132.63±6.02|
| S7      | Zhejiang| 14.08±0.52 | 16.87±0.76| 123.85±2.43| 12.67±0.38| 89.84±1.89 | 100.84±2.65|
| S8      | Zhejiang| 7.04±0.06  | 11.57±0.61| 121.57±2.18| 7.84±0.16 | 90.28±1.95 | 93.90±2.77|
| S9      | Jiangxi | 13.48±0.33 | 25.03±1.02| 174.86±3.89| 9.23±0.35 | 82.69±2.75 | 90.98±1.91|
| S10     | Fujian  | 15.42±0.37 | 23.92±0.92| 155.57±2.87| 10.84±0.29| 79.76±2.07 | 82.31±0.87|
| S11     | Hainan  | 4.69±0.04  | 22.40±0.89| 145.18±2.79| 9.37±0.34 | 88.99±2.06 | 127.16±2.87|
| S12     | Hainan  | 2.45±0.03  | 12.08±0.18| 105.39±0.88| 10.34±0.73| 66.42±1.68 | 108.33±2.87|
| S13     | Hainan  | 3.03±0.04  | 9.03±0.08 | 109.82±1.97| 4.46±0.05 | 80.52±2.05 | 107.60±2.02|
| S14     | Hainan  | 3.04±0.05  | 17.24±0.36| 123.97±2.89| 2.71±0.02 | 82.98±2.03 | 97.44±2.23|
| S15     | Hainan  | 4.22±0.06  | 9.79±0.08 | 102.39±2.28| 4.42±0.03 | 65.48±1.89 | 94.19±1.93|
| S16     | Hainan  | 2.54±0.03  | 16.62±0.24| 124.79±1.92| 4.82±0.05 | 75.44±1.94 | 120.02±2.59|
| S17     | Hainan  | 3.43±0.06  | 8.09±0.05 | 105.96±2.05| 3.64±0.03 | 76.82±2.07 | 121.76±2.98|
components were identified by qualitative analysis methods using standards and the similarity percentage of the spectra of each compound with mass spectra searches (NIST 14).

Typical total ion chromatograms of the essential oil (S6) are shown in Figure 4. In total, 51 volatile compounds were identified, representing 87.66% of the total oil composition.
The chemical composition of the oil, retention time, molecular formula, and the percentage relative to each constituent are presented in Table 4. The amounts of curdione (13.11%) and curzerene (10.41%) were the highest. The total amount of these two compounds accounted for nearly a quarter of the total amount, which was in agreement with previous studies.\(^{[29,30]}\) Other compounds included dehydrocurdione (8.69%), germacrone (7.72%), 1,4-bis(2-benzimidazoyl) benzene (6.95%), neocurdione (5.67%), β-acetoxy-16,17-m ethylenepregn-5-en-20-one (4.94%), curcumene (4.22%), β-elemene (3.80%), curcumol (2.54%), γ-elemene (1.74%), β-stigmasterol (1.68%), γ-sitosterol (1.46%), curcumol (1.42%), eucalyptol (1.25%), and germacrene D (1.04%). The main volatile components of C. wenyujin rhizome essential oil determined in this study were similar to those found in previous reports.\(^{[29,30]}\) However, the relative quantities of some of the compounds were different. Zhang et al. reported that the major compounds in C. wenyujin rhizome essential oil extracted by SD were curdione (19.53%), curzerene (15.80%), germacrone (9.98%), curcumol (9.49%), neocurdione (4.54%), β-elemene (3.98%), eucalyptol (3.14%), and camphor (2.27%).\(^{[30]}\) In another study, the components in C. wenyujin rhizome essential oil extracted by SD and SFE were similar; the main constituents were curzerene (SD: 11.39%, SFE: 14.58%), γ-elemene (3.99%, 3.57%), caryophyllene (4.16%, 1.04%), germacrone D (4.44%, 3.96%), β-selinene (6.04%, 1.95%), curdione (1.56%, 3.82%), and germacrone (3.96%, 2.33%).\(^{[31]}\) An analogous result was reported by Nie et al.\(^{[32]}\) The differences in the relative amounts of the major components in C. wenyujin rhizome essential oils can be attributed to many factors, including extraction and detection methods, type of cultivar, adaptive metabolism, and harvest time.

**Conclusion**

A simple GC–MS quantitative method for the simultaneous determination of six active components in C. wenyujin rhizome essential oil was established. The proposed method was successfully applied to the determination of eucalyptol, β-elemene, curzerene, curcumol, germacrone, and curdione in 17 samples collected from different regions of China. The results showed that there were remarkable differences in the contents of the six target compounds. Curzerene is not originally present in C. wenyujin rhizomes, but is a product of the transformation of furanodione at high temperature. SFE is suitable for the extraction of C. wenyujin rhizome essential oil. The source of C. wenyujin was a factor affecting the contents of some components in the essential oil. GC–MS was used to identify the main components in the volatile oils. The results obtained in this work show that this quantitation and identification method will be useful for quality control in the production of C. wenyujin rhizome essential oil.

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**Conflicts of interest**

There are no conflicts of interest.

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