Quantitative Analysis and Discrimination of Steamed and Non-Steamed Rhizomes of *Curcuma wenyujin* by GC–MS and HPLC

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Simple gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) methods were developed for quantifying eight volatile compounds and 10 sesquiterpenoids, respectively. GC–MS analysis was performed on an HP-5MS capillary column (30 m × 0.25 mm i.d.) coated with 0.25 μm film 5% phenyl–95% methylpolysiloxane and selected ion monitoring was used for quantification. Four volatile and previously unquantified monoterpenoids were determined. HPLC analysis was performed on a C18 column with water and acetonitrile as mobile phase. The proposed method, determined 10 non-polar and polar sesquiterpenoids simultaneously, which covered a wider polar range of analytes and had a more perfect resolution. Among them, five sesquiterpenoids were not determined before and some specific components, (4S,5S)-germacrone-4,5-epoxide, curcumenone and dehydrocurdione were completely separated for the first time. Both methods were validated for linearity, limit of detection and quantification, precision, accuracy, recovery and system suitability. The methods were simple, effective, reliable and successfully applied to global detection and analysis of volatile and non-volatile components of steamed and non-steamed rhizomes of *Curcuma wenyujin* (Wen-E-Zhu (WEZ) and Pian-Jiang-Huang (PJH)). Multivariate statistical analysis was employed to distinguish PJH and WEZ and seven chemical components, including (4S,5S)-germacrone-4,5-epoxide, curcumenone, β-elemene, curzerene, germacrone, curdione, neocurdione and curcumene were the dominant and bioactive ingredients (6–8). The present study provided a promising method for accurate discrimination of the herbal medicines with the same origin.

Introduction

The crude traditional Chinese medicine (TCM) or its processed products were used clinically for thousands of years. A proper pharmaceutical processing method may significantly alter the pharmacological properties of the crude TCM, such as reducing toxicity or altering pharmaceutical efficacy. In China, the processing methods for crude TCM have been practiced since the Tang Dynasty and well documented in the Chinese Pharmacopoeia. Nowadays, in order to further use crude TCM and the processed products rationally and safely, a deeper understanding of chemical composition of TCM is important, which relies on the more sensitive and accurate analytical methods.

*Curcuma wenyujin* Y. H. Chen et C. Ling is cultivated in southeast China, which has wide applications in pharmaceutical industry in China because of its wide biological activities. Steamed and non-steamed rhizomes of *C. wenyujin* are traditionally used as two different herb medicines in clinic, named as Wen-E-Zhu (WEZ) and Pian-Jiang-Huang (PJH), respectively (1). The PJH is used for alleviating pain of arthrosis and skin infection with the prominent antimicrobial and anti-inflammatory activities. The WEZ is used for treating cancer, virus and gynecological diseases. Due to the same origin, the use of the two medicines is rather confused. Therefore, a detailed chemical comparative study is necessary to elucidate the difference of two medicines and construct their recognition model.

Previous studies showed that the rhizome of *C. wenyujin* possessed wide activities, such as anti-tumour (2, 3), anti-inflammatory (4) and antiviral activities (5). The rhizome of *C. wenyujin* was rich in volatile components consisted of monoterpenes and sesquiterpenes, and its polar extract mainly contained sesquiterpenes. It was reported that eucalyptol, camphor, isoborneol, borneol, β-elemene, curzerene, germacrone, curdione, neocurdione and curcumene were the dominant and bioactive ingredients (6–8). Previous phytochemical investigations were confined to report the chemical composition of WEZ and little information was available about that of PJH. Especially, no report was to investigate the effect of steaming process on the chemical constituents of *C. wenyujin* rhizomes.

In recent years, analysis of *Curcuma* extracts was generally accomplished by GC–FID (9), GC–MS (7) and high-performance liquid chromatography (HPLC) (10). The GC–MS and HPLC, coupled with pattern recognition, had been proved to be good choices for metabolic profiling analysis (11).

In this study, based on the isolation of some compounds by our lab, GC–MS and HPLC methods were developed for quantitative determination of 16 volatile and non-volatile compounds in PJH and WEZ, respectively. Among them, four monoterpenoids including eucalyptol, camphor, isoborneol and borneol and five more polar sesquiterpenoids, e.g., zedoarondiol, isozaedoarondiol, aerugidiol, (4S,5S)-germacrone-4,5-epoxide and dehydrocurdione were not separated and determined before. It was found that the contents of most of these analytes were greatly changed after steam processing and could be used as the potential characteristic chemical markers to discriminate the steamed and non-steamed samples.

Multivariate statistical analysis, including principle component analysis (PCA) and partial least squares discriminate analysis (PLS-DA), had been employed to show the differences between PJH and WEZ. The aims of this study include: (i) to depict the chemical characteristics of steamed and non-steamed rhizomes...
of C. wenyujin, (ii) to screen the potential chemical markers for discrimination and quality control of PJH and WEZ and (iii) to elucidate the effects of steaming process on the chemical constituents.

Experimental

Plant material

In this study, 11 batches of non-steamed and steamed samples were collected from Rui-an, Zhejiang, China in February 2011, respectively. Among them, eight batches of samples were collected as the individual sampling (No. 1–8), and three batches of samples were obtained as bulk sampling (No. 9–11). The voucher specimens (C. wenyujin Y. H. Chen et C. Ling, No: WYJ-201005) were deposited in the Herbarium of the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, PR China. Each batch of the rhizome by single sampling.

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Chemicals and reagents

Eucalyptol, camphor, isoborneol, borneol, β-elemene, germacrone and furanodiene were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Curzerene, curdione, zedoarondiol, isozedoarondiol, aerugidiol, (4S,5S)-germacrone-4,5-epoxide, curcumenone, dehydrocurdione and neocurdione were accurately weighed and dissolved in cyclohexane. The extract was filtered through a 0.22-µm membrane. Then 1 µL of the filtrate was injected into the GC–MS system for each analysis.

Sample preparation

The samples were powdered to a homogeneous size in a mill and passed through 40-mesh sieve. For GC–MS analysis, ~1.0 g of the pulverized sample was weighed accurately and then extracted by ultrasonic techniques with 10 mL of cyclohexane at the temperature of 20°C for 30 min. The sample was cooled, and the loss of weight due to evaporation of solvent was replenished with cyclohexane. The extract was filtered through a 0.22-µm membrane. Then 1 µL of the filtrate was injected into the HPLC system.

Standard solution preparation

For GC–MS analysis, a mixed stock solution-containing reference standards of seven compounds (eucalyptol, camphor, isoborneol, borneol, β-elemene, germacrone and curdione) was prepared by dissolving weighed accurately standard of each compound in cyclohexane at a concentration of 0.08–2.94 mg mL⁻¹. In addition, a single standard stock solution of curcumenone was prepared by dissolving accurately weighed compound in cyclohexane at a concentration of 1.94 mg mL⁻¹.

For HPLC analysis, 0.50 g of the pulverized sample was weighed accurately and macerated in 10 mL of methanol. The sample was extracted for 30 min in an ultrasonic bath at 20°C and the loss of weight due to evaporation of solvent was replenished with methanol. The supernatant was filtered through a 0.22-µm filter membrane. Then 10 µL of the filtrate was injected into the HPLC system.

Apparatus and chromatographic conditions

GC–MS was performed with an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer and an Agilent Chemstation software (Agilent Technologies, Palo Alto, CA, USA). An HP-5MS capillary column (30 m × 0.25 mm i.d.) coated with a 0.25-µm film of 5% phenyl and 95% methyl siloxane was used for separation. The column temperature program was as follows: 2 min at 45°C, then to 250°C at 4°C min⁻¹, held at temperature of 250°C for 1 min. The temperature of the split injector was 240°C and the split ratio was 1:20. The injection volume was 1 µL. High-purity helium was used as carrier gas at a flow rate of 0.9 mL min⁻¹. The spectrometer was operated in electron-impact mode, and the ionization energy was 70 eV. The GC–MS interface, ion source and quadrupole temperatures were 280, 200 and 150°C, respectively. The selected ion monitoring (SIM) method was used for quantification of eight analytes. Fragment ions were m/z 154 for eucalyptol, m/z 95 for camphor, isoborneol and borneol, m/z 93 for β-elemene, m/z 108 for curzerene, m/z 107 for germacrone and m/z 180 for curdione, respectively.

HPLC analysis was performed on a Waters e2695–2998 series, including a quaternary pump, a photodiode array detector (PDA), a vacuum degasser, a thermostat autosampler, a column compartment and Empower work station. A Diamonsil C₁₈ column (250 × 4.6 mm i.d., 5 µm) was used for separation. The mobile phase consisted of water (A) and acetonitrile (B). The gradient program was as follows: 20% B in 0–10 min, 20–46% B in 10–15 min, 46–50% B in 15–35 min, 50–55 min, 54–90% B in 55–70 min, 90–100% B in 70–75 min and 100% B in 75–85 min. The flow rate was 1 mL min⁻¹, and the column temperature was kept constant at 35°C. The injection volume was 10 µL. The detection wavelength of the PDA detector was 256 nm for zedoarondiol, isozedoarondiol, aerugidiol, (4S,5S)-germacrone-4,5-epoxide as well as curcumenone, and 214 nm for dehydrocurdione, neocurdione and furanodiene, respectively.

Sample preparation

The samples were powdered to a homogeneous size in a mill and passed through 40-mesh sieve. For GC–MS analysis, ~1.0 g of the pulverized sample was weighed accurately and then extracted by ultrasonic techniques with 10 mL of cyclohexane at the temperature of 20°C for 30 min. The sample was cooled, and the loss of weight due to evaporation of solvent was replenished with cyclohexane. The extract was filtered through a 0.22-µm membrane. Then 1 µL of the filtrate was injected into the GC–MS system for each analysis.

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For HPLC analysis, 10 reference compounds including zedoarondiol, isozedoarondiol, aerugidiol, (4S,5S)-germacrone-4,5-epoxide, curcumenone, dehydrocurdione, curdione, neocurdione, germacrone and furanodiene were accurately weighed and dissolved in methanol. The concentration of the compounds in mixed stock solution ranged from 0.04 to 0.69 mg mL⁻¹.
Curdione and germacrone could be quantified both by GC–MS and by HPLC. In this paper, the data of curdione and germacrone obtained by GC–MS was used for multivariate analysis.

**Validation of GC–MS and HPLC methods**

The validation parameters for the assay included linearity, sensitivity, precision, stability, repeatability and accuracy. According to the concentration of samples, the mixed stock solutions of reference standards were diluted to the appropriate concentration for establishing calibration curves. Six concentrations of the mixed standard solution were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak areas ($y$) versus the amount ($x$, μg) of each analyte and by the linear regression analysis. The limit of detection (LOD) and limit of quantification (LOQ) under the present chromatographic conditions were determined at signal-to-noise ratios of $\sim 3$ and 10, respectively. Intra- and inter-day variations were used to determine the precision. For intra-day tests, a certain concentration solution was analyzed for six times within 1 day, while for inter-day tests, the solution was examined twice per day for three consecutive days. The stability was evaluated by analyzing the same sample solution at the time points of 0, 2, 4, 8, 12 and 18 h, which was placed at room temperature after preparation. To confirm the repeatability, six replicates of the sample were extracted and analyzed. The relative standard deviation (RSD) value was calculated to evaluate method repeatability. Recovery test was used to evaluate the accuracy of GC–MS and HPLC methods. Three different concentrations (high, middle, low) of the standards were added to the known sample. Three replicates were performed for each level test. The mixture was extracted and analyzed as described for normal samples. The recovery was calculated by the formula as follows:

$$\text{Recovery (\%)} = 100 \times \frac{\text{amount found} - \text{original amount}}{\text{amount spiked}}.$$ 

**Data processing and multivariate analysis**

External standard method was used to calculate the contents of 16 analytes in non-steamed and steamed rhizomes of *C. wenyujin*. The data were expressed as average of duplicate samples. Based on the content data of 16 compounds in the samples, multivariate analysis techniques, including PCA and PLS-DA, were used to discriminate between the samples and to find out the most important components for their difference. Potential chemical markers were selected according to variable importance in the project (VIP) value, and the S-plot. PCA and PLS-DA analyses were applied using SIMCA-P11.0 software (Umetrics, Umea, Sweden).

**Results**

**Optimization of extraction conditions**

For GC–MS analysis, the samples collected by single plant generally ranged from 1.5 to 20.0 g, which was not enough for extracting the essential oil by steam distillation. Therefore, organic solvents were selected and an ultrasonic bath was utilized to extract the volatile components for analysis (12). Several experiments were carried out in order to optimize extraction conditions. The peak areas of eight tested volatile compounds were evaluated in each test condition. Extract solvents such as diethyl ether, petroleum ether, hexane and cyclohexane were used in extraction. Finally, cyclohexane was adopted due to its high extraction efficiency for most tested volatile compounds (Figure 1). The effects of the extraction time were studied from

![Figure 1. The extraction efficiency of some compounds using different extraction solvents determined by GC–MS.](image-url)
15 to 60 min with other conditions fixed. In this study, the optimal extract method was obtained by extracting samples in cyclohexane for 30 min with ultrasound-assisted method.

For HPLC analysis, some researchers used methanol as extraction solvent to extract sesquiterpenes in plants (10, 11). In order to study the most efficient medium for extraction, methanol of 30, 50, 70 and 100, and 100% ethanol were used. As a result, 100% methanol showed higher extraction efficiency for the investigated sesquiterpenes than other solvents (Figure 2). Extract methods such as ultrasonic extraction, reflux and Soxhlet’s extraction were compared. The proper time was also optimized. The optimal extract method was obtained by extracting in methanol for 30 min with ultrasonic assistance, which was the most efficient and convenient.

**Optimization of analysis conditions**

For GC–MS analysis, column temperature program, the temperature of the split injector and the split ratio were optimized. The total ion monitoring and SIM method were compared for quantification of eight analytes. And the optimal fragment ion for every analyte was selected.

For HPLC analysis, based on UV spectra of the investigated compounds, 214 and 256 nm were chosen for detection of the analytes. Several preliminary experiments were performed for testing different chromatographic conditions. A solvent system consisting of acetonitrile and water was ultimately selected, which provided lower pressure, greater baseline stability and complete separation between analytes. Especially, complete separation between (4S,5S)-germacrone-4,5-epoxide, curcumenone and dehydrocurdione was achieved for the first time. And an optimum gradient was finally picked out upon a large number of empirical attempts. The peaks of these analytes were identified by comparing the retention times with those of the reference compounds.

**Method validation**

The regression equations and linear ranges of all investigated components by GC–MS or HPLC were given in Table I. Calibration curves of the 16 analytes showed good linearity in relatively wide dynamic ranges, with the squares of correlation coefficients for the linear regression analysis falling into the range of 0.9944–1.0000. The LODs and LOQs of all analytes for the GC–MS or HPLC method were also summarized in Table I. The results showed that the GC–MS and HPLC methods were sensitive. The overall intra- and inter-day variations (Table II), which were expressed by RSD, of the 16 analytes were <5.65 and 5.91%, respectively. All analytes were found to be stable within 18 h with RSD <5.46%, and the methods exhibited good repeatability with RSD <5.02%. Detailed data were available in Table II. The present quantitative methods (GC–MS and HPLC) had satisfactory accuracy, with the overall recoveries of the investigated components ranging from 95.72 to 107.54% and their RSD values <7.40%, which were shown in Table II.

**Analysis of samples**

Sixteen characteristic components in 22 batches of PJH and WEZ samples were determined in duplicate, with eucalyptol, camphor, isoborneol, borneol, β-elemene, curzerene, germacrone and curdione quantified by GC–MS and zedoarandiol, isozedoarandiol, aerugidiol, (4S,5S)-germacrone-4,5-epoxide, curcumenone, dehydrocurdione, neocurdione as well as furanodiene quantified by HPLC, respectively. GC–MS SIM chromatograms were displayed in Figures 3 and 4 showed the HPLC chromatograms.

![Figure 2](https://academic.oup.com/chromsci/article-abstract/52/9/961/274166)

**Figure 2.** The extraction efficiency of some sesquiterpenoids using different extraction solvents determined by HPLC.
Table I. Linear Regression Data, LOD and LOQ of the Investigated Compounds

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>Linear regression data</th>
<th>LOD (ng)</th>
<th>LOQ (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Regression equation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$y = 1.84 \times 10^x - 1.15 \times 10^2$</td>
<td>1.26–409.00</td>
<td>0.9993</td>
</tr>
<tr>
<td>1</td>
<td>Eucalytol</td>
<td>$y = 4.19 \times 10^x - 6.87 \times 10^2$</td>
<td>0.79–161.44</td>
<td>0.9997</td>
</tr>
<tr>
<td>2</td>
<td>Camphor</td>
<td>$y = 4.40 \times 10^x - 2.97 \times 10^2$</td>
<td>0.33–66.72</td>
<td>0.9998</td>
</tr>
<tr>
<td>3</td>
<td>Isoborneol</td>
<td>$y = 4.51 \times 10^x - 3.72 \times 10^2$</td>
<td>0.40–25.40</td>
<td>0.9986</td>
</tr>
<tr>
<td>4</td>
<td>Borneol</td>
<td>$y = 4.77 \times 10^x - 1.22 \times 10^2$</td>
<td>3.17–203.00</td>
<td>0.9891</td>
</tr>
<tr>
<td>5</td>
<td>β-Elemene</td>
<td>$y = 3.12 \times 10^x - 2.93 \times 10^2$</td>
<td>9.69–1937.22</td>
<td>0.9944</td>
</tr>
<tr>
<td>6</td>
<td>Curzerene</td>
<td>$y = 3.05 \times 10^x - 1.07 \times 10^2$</td>
<td>31.29–998.50</td>
<td>0.9994</td>
</tr>
<tr>
<td>7</td>
<td>Curdione</td>
<td>$y = 2.43 \times 10^x - 3.48 \times 10^1$</td>
<td>91.88–2940.00</td>
<td>0.9996</td>
</tr>
<tr>
<td>8</td>
<td>(4S,5S)-Germacrone-4,5-epoxide</td>
<td>$y = 1.44 \times 10^x + 2.33 \times 10^3$</td>
<td>23.60–1180.00</td>
<td>0.9999</td>
</tr>
<tr>
<td>9</td>
<td>Zedoarondiol</td>
<td>$y = 1.34 \times 10^x + 5.78 \times 10^4$</td>
<td>8.09–808.98</td>
<td>1.0000</td>
</tr>
<tr>
<td>10</td>
<td>Isoezodarondiol</td>
<td>$y = 2.16 \times 10^x - 1.83 \times 10^5$</td>
<td>4.00–400.00</td>
<td>1.0000</td>
</tr>
<tr>
<td>11</td>
<td>(4S,5S)-Germacrone-4,5-epoxide</td>
<td>$y = 6.58 \times 10^x + 4.69 \times 10^7$</td>
<td>6.58–6580.00</td>
<td>1.0000</td>
</tr>
<tr>
<td>12</td>
<td>Curcumeneone</td>
<td>$y = 2.09 \times 10^x + 1.24 \times 10^1$</td>
<td>4.52–4452.30</td>
<td>1.0000</td>
</tr>
<tr>
<td>13</td>
<td>Dehydrocurdione</td>
<td>$y = 1.07 \times 10^x - 2.68 \times 10^0$</td>
<td>6.24–624.00</td>
<td>1.0000</td>
</tr>
<tr>
<td>14</td>
<td>Neocurdione</td>
<td>$y = 7.99 \times 10^x + 1.40 \times 10^1$</td>
<td>58.60–2930.20</td>
<td>0.9999</td>
</tr>
<tr>
<td>15</td>
<td>Zedoarondiol</td>
<td>$y = 2.86 \times 10^x + 6.16 \times 10^3$</td>
<td>137.00–6850.00</td>
<td>0.9996</td>
</tr>
</tbody>
</table>

All the main compounds were well resolved and the summary results were shown in Table III.

Quantitative comparison of the components between PJH and WEZ

Quantitative comparison of chemical components between PJH and WEZ was conducted. As shown in Figures 3 and 4, the characteristic chemical compositions of PJH and WEZ were similar, but their contents were greatly various due to the steam processing after harvest (Table III). It showed that the average contents of eucalyptol, camphor, isoborneol and borneol in WEZ were about three times lower than those in PJH, which elucidated that the monoterpenoids with lower boiling point in WEZ were distilled off during the steaming process with high temperature, leaving behind the higher boiling point constituents (6). For the investigated sesquiterpenoids, the levels of β-elemene and curzerene in WEZ increased almost 3-fold. Meanwhile, in WEZ, the content of (4S,5S)-germacrone-4,5-epoxide amazingly decreased nearly 13 times, while curcumeneone revealed to be four times higher, compared with PJH.

Multivariate analysis

PCA was used as an unsupervised method to study the differences between PJH and WEZ samples for it had been universally used for achieving the natural interrelationship within the data without prior knowledge of the data set. The PCA score plot (Figure 5a) obtained by all observations from the two medicines showed clear classification of the samples from PJH and WEZ. Sixty-nine percent of the total variables could be explained by the first two components, indicating a significant chemical difference between PJH and WEZ. Almost all the samples from the first group (PJH, No. P1–P11) were distributed at the right of the plot, except for the sample of P8, and samples from the second group (WEZ, No. W1–W11) were distributed at the left of the

n.d.: not determined.
The quality of the models was described by the cross-validation parameter $Q^2$ (the predictability of the model) and $R^2_Y$ (the total explained variation for the $X$ matrix). The $R^2_Y$ and $Q^2$ were 0.888 and 0.805, respectively, indicating the proposed PLS-DA model (Figure 5c) was reliable and sufficiently discriminated between PJH and WEZ. Due to the same origin, identification between them only by macroscopic and microscopic authentication was difficult and the use of the two medicines was rather confused. In this work, based on the chemical data, PCA and PLS-DA analyses were successfully applied to discriminate the samples from PJH and WEZ.

The loading plots of both PCA (Figure 5b) and PLS-DA (figure not shown), in which each data point represented a component (variable), revealed that the phytochemical components that contributed significantly to the intergroup differences were those farthest from the main cluster of phytochemical components. Potential chemical markers were selected according to VIP value (Figure 5d). According to VIP $> 1$, the most important chemical markers were ($4S,5S$)-germacrone-4,5-epoxide, curcumene, $\beta$-elemene, curzerene, borneol, isoborneol and camphor, which indicated that the selected seven components might had a marked effect on the discrimination among different group samples. $\beta$-Elemene, curzerene and curcumene were found to be specific markers for WEZ, while ($4S,5S$)-germacrone-4,5-epoxide was a potential marker for PJH.

**Figure 3.** GC–MS SIM chromatograms of (a) mixed standards, (b) the sample of PJH and (c) the sample of WEZ. Peak identities: 1, eucalyptol; 2, camphor; 3, isoborneol; 4, borneol; 5, $\beta$-elemene; 6, curzerene; 7, germacrone; 8, curcumene.
epoxide, borneol, isoborneol and camphor were characteristic markers for PJH. Compared with WEZ, PJH contained more \((4S,5S)\)-germacrone-4,5-epoxide, borneol, isoborneol and camphor but less \(\beta\)-elemene, curzerene and curcumenone. This was broadly consistent with the statistical analysis results mentioned above. Curdione and germacrone could be quantified both by GC–MS and HPLC. In this paper, the data of curdione and germacrone obtained by GC–MS were used for multivariate analysis. The similar results of multivariate analysis could be obtained if the content data of curdione and germacrone was obtained by HPLC (see the Supplementary data, Table SI and Figure S1).

**PCA and PLS-DA analysis of the chemical markers**

As mentioned above, the PCA and PLS-DA loading plots indicated that \((4S,5S)\)-germacrone-4,5-epoxide, curcumenone, \(\beta\)-elemene, curzerene, borneol, isoborneol and camphor might be the chemical markers for the discrimination of different groups. In present study, PCA and PLS-DA analyses based on the seven chemical markers were further performed to assess their discriminative ability. The PCA score plot was displayed in Figure 5e. And it indicated that the samples were successfully separated when the seven chemical markers were used as bases. 90.9% of the total variables could be explained by the first two components, indicating a significant chemical difference between PJH and WEZ. In the PLS-DA analysis, the PLS-DA score plots of all the samples were shown in Figure 5f. The \(R^2\) and \(Q^2\) were 0.872 and 0.850, respectively, indicating excellent prediction ability of the model. And the correct rate of the classification of PJH and WEZ by PLS-DA method was 100%, which suggested that the PLS-DA model was as good as that from all common components. The model further proved the discriminative ability of the seven chemical markers.

**Discussion**

**Improvement in chromatographic methods**

In this paper, a global detection and quantitative analysis of volatile and non-volatile components of steamed and non-steamed rhizomes of *C. wenyujin* were conducted by GC–MS and HPLC. It is a specific, effective and reliable method for discrimination of the steamed and non-steamed rhizomes of *C. wenyujin*.

First, the investigation is more comprehensive than previous report. The contents of nine components were reported for the first time. In GC–MS analysis, four monoterpenoids including...
The components: 1, eucalyptol; 2, camphor; 3, isoborneol; 4, borneol; 5, \(\beta\)-elemene; 6, curzerene; 7, germacrone; 8, curdione; 9, zedoarondiol; 10, isozedoarondiol; 11, aerugidiol; 12, (4,5S)-germacrone-4,5-epoxide; 13, curcumene; 14, dehydrocurdione; 15, neocurdione; 16, furanodiene.

No. P1–P11 were non-steamed rhizomes of \(C.\) wenyujin (PJH) and No. W1–W11 were steamed rhizomes of \(C.\) wenyujin (WEZ).

### Potential Pharmacological Difference

Previous report showed that monoterprenoids including eucalyptol, camphor, isoborneol and borneol in WEZ were lower than those in PJH, which elucidated that the monoterprenoids with lower boiling point in WEZ were distilled off during the steaming process with high temperature, leaving behind the higher boiling point constituents.

As for the investigated sesquiterpenoids, the levels of \(\beta\)-elemene, curzerene and curcumene in WEZ increased obviously which suggested some thermo-sensitive components in \(C.\) wenyujin might transform when steamed in boiling water, and furanodiene was found to degrade to curzerene under heat through a [3,3]-sigmatropic reaction (Cope rearrangement) (10, 13, 14). It had also been found that transannular cyclization of (4,5S)-germacrone-4,5-epoxide to curcumene could occur under thermal condition (15), which might be the primary reason of the much lower amount of (4,5S)-germacrone-4,5-epoxide and higher level of curcumene in WEZ. Nevertheless, composition changes during processing remained to be explored in-depth.

**Potential chemical transform during steam processing**

Quantitative comparison of chemical components between PJH and WEZ showed that their contents were greatly various due to the steam processing after harvest. The contents of eucalyptol, camphor, isoborneol and borneol in WEZ were lower than those in PJH, which elucidated that the monoterprenoids with lower boiling point in WEZ were distilled off during the steaming process with high temperature, leaving behind the higher boiling point constituents.

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**Potential pharmacological difference**

Previous report showed that monoterprenoids including eucalyptol, camphor, isoborneol and borneol had antimicrobial activities (16–18), and anti-inflammatory and analgesic effects (19–23). The higher monoterprenoids in PJH suggested that the oil of PJH might be with prominent antimicrobial and anti-inflammatory activities and was worthy of a further research and development. The conclusion also supported the clinical application of PJH which was commonly applied to alleviate pain of arthrosis and skin infection. While among these sesquiterpenoids, the levels of \(\beta\)-elemene and curzerene in WEZ increased obviously during the steaming process. Besides, \(\gamma\)-elemene and \(\delta\)-elemene were also higher in steamed samples, but no quantization in this paper
for the lack of the reference substances. It had been well known that \(\beta\)-elemene and its derivatives possessed evident activity against a broad spectrum of cancers including brain, breast, liver, lungs, prostate and drug-resistance tumors with few side effects (5, 24, 25). So our results agreed with the clinical practice of WEZ with anticancer activity (2, 3). Meanwhile, the contents of \((4S,5S)\)-germacrone-4,5-epoxide and curcumenone in PJH and WEZ varied significantly and curcumenone was found to possess hepatoprotective effect (26), but little information was available about the bioactivity of \((4S,5S)\)-germacrone-4,5-epoxide. Especially, no report was to investigate the pharmacological difference between \((4S,5S)\)-germacrone-4,5-epoxide and curcumenone. In order to elucidate the specific therapeutic application of PJH and WEZ, further research on the relationship between the differences in chemical compositions and pharmacological activities was necessary.

**Conclusion**

Simple, accurate, precise and reproducible GC–MS and HPLC–DAD methods for determination of 8 volatile and 10 non-volatile components were developed and validated in PJH and WEZ,
respectively. The application of our proposed methods can quantify the analytes with a wider polar range and had a more perfect resolution. Based on the data of the chemical difference, PCA was used to obtain an overview of the distribution of all the samples from PJH and WEZ. PLS-DA was then employed to construct the recognition model of PJH and WEZ. Furthermore, the chemical markers, (4S,5S)-germacrone-4,5-epoxide, curcumenone, β-elemene, curzerene, borneol, isoborneol and camphor were screened. These can be applied in the accurate discrimination and quality control of PJH and WEZ. The combination of multivariate statistical technique and chemical composition data proved to be a powerful tool for the discrimination and quality control of TCM.

Supplementary data
Supplementary data are available at Journal of Chromatographic Science online.

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