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# Optimization and comparison of three methods for extraction of volatile compounds from *Cyperus rotundus* evaluated by gas chromatography–mass spectrometry

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**Abstract**

The essential oil of *Cyperus rotundus* has multiple pharmacological activities. Therefore, the extraction with high yield and quality is very important for preparation of essential oil of *C. rotundus*. In this paper, three methods, namely hydrodistillation (HD), pressurized liquid extraction (PLE) and supercritical fluid extraction (SFE), for extraction of volatile compounds from *C. rotundus* were optimized and compared by gas chromatography–mass spectrometry. Among eight identified compounds in *C. rotundus*, five components including α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone were quantitatively determined or estimated using α-cyperone as standard, which showed that PLE had the highest extraction efficiency, while SFE had the best selectivity for extraction of β-cyperone and α-cyperone. The contents of ingredients from *C. rotundus* extracted with HD, PLE and SFE are significantly different, which suggest that comparison of chemical components and pharmacological activities of different extracts is helpful to elucidate the active components in *C. rotundus* and control its quality.


*Keywords:* Gas chromatography–mass spectrometry; Hydrodistillation; Pressurized liquid extraction; Supercritical fluid extraction; *Cyperus rotundus*

## 1. Introduction

*Cyperus rotundus*, a traditional medicinal plant in China, India and Japan, is encountered in tropical, subtropical and temperate regions. It is used against spasms and stomach disorders [1]. The essential oil of *C. rotundus* is reported possess analgesic, anti-inflammatory, antipyretic [2–4] and antifungical activity [5]. Several sesquiterpenes including cyperone, cyperene and patchoulenone are thought to be the biological active ingredients in the essential oil [6–8]. Therefore, the extraction with high yield and quality is very important for preparation of essential oil of *C. rotundus*. Conventionally, the essential oil of plants is isolated by either hydrodistillation (HD) or solvent extraction [9–13]. These techniques present some shortcomings, namely losses of volatile compounds, low extraction efficiency, long extraction time, degradation of unsaturated compounds and toxic solvent residue. Thus, developing alternative extraction techniques with better selectivity and efficiency are highly desirable. Consequently, supercritical fluid extraction (SFE) as an environmentally responsible and efficient extraction technique for solid materials was introduced and extensively studied for separation of active compounds from herbs and other plants [14–20], where oils and essential oils (26%) is one of the two main fields of application for supercritical fluid extraction [21].

In addition, pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) is another promising and recent sample preparation technique, which offers the advantages of reducing solvent consumption and allowing for automated sample handling [22]. It is being exploited in diverse areas, including the extraction of chemical constituents from plants or herbal materials [23–33]. However, because the extraction is performed at elevated temperatures using PLE, thermal degradation should be a concern though it is available for extraction of some heat labile compounds in plants [34,35].

The aim of the present work was to investigate the extraction efficiency of the main volatile constituents including α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone in *C. rotundus* obtained by HD, SFE and PLE, respectively. Gas

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chromatography–mass spectrometry assay was used to quantify the compounds under the investigation. The difference of three extraction methods was also compared.

## 2. Materials and methods

### 2.1. Materials

Four batches (CR1–CR4) of *C. rotundus* rhizomes were obtained from Anhui, Shandong, Hubei and Zhejiang Province, respectively. The voucher specimens of *C. rotundus* rhizomes were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

α-Cyperone was separated and purified in our lab. In brief, commercial essential oil (500 ml) of *C. rotundus* was added into the silica gel column, and eluted with petroleum ether, ethyl acetate and methanol, respectively. The ethyl acetate eluent was then separated by medium pressure liquid chromatography (BÜCHI Labortechnik AG, Flawil, Switzerland) with gradient elution of water and methanol. The final fraction with a major compound was further purified using preparative HPLC (Agilent Technologies, Palo Alto, CA), where Alltima C18 column (250 mm × 22 mm I.D., 10 μm) and mobile phase of methanol and water (9:1) was used. The fraction was collected based on the peak detected at 246 nm. A yellow oily compound with the purity of more than 95.7% tested by HPLC was obtained from the second concentrated fraction. The structure is confirmed as α-cyperone by comparing its EI–MS (Table 1) and NMR data with the reference [36]. Methanol, ethyl acetate and petroleum ether for GC were purchased from Merck (Darmstadt, Germany). The reagents not mentioned here were from standard sources.

### 2.2. Hydrodistillation

HD was performed according to the method described in China Pharmacopoeia (2005). Twenty gram of *C. rotundus* powder (0.2–0.3 mm) was placed in the flask of a Clevenger extractor and extracted with 200 ml of water for 8 h. The water suspension was extracted with 25 ml ethyl acetate thrice. The ethyl acetate extract and the essential oil obtained were pooled and transferred into a 500 ml volumetric flask and made up to its volume with methanol. The sample solution was dehydrated with sodium sulphate anhydrous and filtered through a 0.45 μm Econofilter (Agilent Technologies) before GC–MS analysis.

### 2.3. Pressurized liquid extraction

Pressurized liquid extractions were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. In brief, raw materials of *C. rotundus* were dried at 40 °C for 12 h and were ground into powder of 0.2–0.3 mm. Powder of *C. rotundus* (1.0 g) was mixed with diatomaceous earth (1.0 g) and placed into 11 ml stainless steel extraction cell, respectively. The parameters include the type of solvent (methanol, ethyl acetate and petroleum ether), temperature (100–180 °C) and static extraction time (5–15 min) of PLE were optimized using univariate approach. The sample was extracted under the optimized conditions. Then, the extract was transferred to a 25 ml volumetric flask, which was made up to its volume with methanol and filtered through a 0.45 μm Econofilter (Agilent Technologies) prior to injection into the GC–MS system.

### 2.4. Supercritical fluid extraction

SFT-250 SFE/SFR system (Supercritical Fluid Technologies, Newark, DE, USA) was used for all the extractions. The parameters, including pressure, temperature, static time and volume of ethanol (modifier, it is selected based on the preliminary investigation), which influence the extraction efficiency of SFE, were optimized. The best conditions for extraction were studied by the orthogonal test. The investigated variables and their test levels are listed in Table 2. Reference to the experimental design theory, the orthogonal array L9($3^4$) was selected to arrange the test program (Table 3). The extraction was performed by filling the 100 ml extraction vessel with 20.0 g *C. rotundus* powder under certain conditions, and the extracted analytes were col-

Table 1
Mass data of eight compounds identified from *Cyperus rotundus*

| Peak number | Compound | $R_t$ (min) | Mass data[a] |
|---|---|---|---|
| 1 | α-Copaene | 13.2 | 204 (M+, 21), 161 (100), 120 (24), 119 (99), 105 (84), 93 (41), 91 (35), 81 (20), 55 (9), 41 (15) |
| 2 | Cyperene | 14.4 | 204 (M+, 100), 189 (67), 175 (16), 161 (32), 147 (16), 133 (20), 119 (32), 105 (29), 91 (22), 79 (9), 55 (7), 41 (10) |
| 3 | β-Selinene | 20.0 | 204 (M+, 65), 189 (66), 175 (26), 161 (64), 147 (49), 133 (51), 121 (62), 105 (100), 93 (83), 79 (69), 67 (49), 53 (26), 41 (46) |
| 4 | Selina-4,11-diene | 20.6 | 204 (M+, 60), 189 (100), 147 (34), 133 (57), 119 (26), 107 (54), 105 (48), 93 (57), 91 (49), 81 (42), 79 (36), 55 (20), 41 (28) |
| 5 | Aristol-9-en-8-one | 34.4 | 218 (M+, 100), 203 (25), 175 (91), 161 (27), 147 (40), 133 (19), 119 (17), 105 (29), 91 (28), 77 (19), 41 (19) |
| 6 | Aristol-9-en-3-one | 43.3 | 218 (M+, 13), 203 (34), 175 (87), 161 (41), 147 (100), 133 (60), 122 (68), 105 (64), 91 (59), 77 (35), 41 (32) |
| 7 | β-Cyperone | 45.0 | 218 (M+, 100), 203 (13), 189 (10), 175 (38), 161 (25), 147 (36), 133 (28), 119 (22), 105 (23), 91 (24), 77 (12), 55 (9), 41 (14) |
| 8 | α-Cyperone | 54.3 | 218 (M+, 100), 203 (45), 185 (15), 175 (53), 161 (60), 147 (65), 133 (46), 121 (59), 105 (45), 91 (58), 79 (42), 55 (24), 41 (30) |

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

Table 2
The investigated variables and their levels

| Factors | Variables investigated levels of each variable | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| A: pressure (bar) | 150 | 200 | 250 |
| B: temperature (°C) | 35 | 40 | 45 |
| C: volume of ethanol (ml) | 20 | 40 | 80 |
| D: static time (h) | 1 | 2 | 3 |

lected in a 500 ml volumetric flask and made up to its volume with methanol and then filtered through a 0.45 μm Econofilter (Agilent Technologies) prior to injection into the GC–MS system.

### 2.5. GC–MS analysis

GC–MS was performed with an Agilent 6890 gas chromatography instrument coupled to 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 set at 100 °C and held for 3 min for injection, then programmed at 5 °C $min^{-1}$ to 110 °C, then at 1 °C $min^{-1}$ to 120 °C and held for 20 min, then at 1 °C $min^{-1}$ to 130 °C and held for 15 min, and finally, at 20 °C $min^{-1}$ to 290 °C and held for 2 min. Split injection (5 μl) was conducted with a split ratio of 5:1 and high purity helium was used as carrier gas of 1.0 ml $min^{-1}$ flow-rate. The spectrometers were operated in electron-impact (EI) mode, the scan range was 40–550 amu, the ionization energy was 70 eV and the scan rate was 0.35 s per scan. The inlet, ionization source temperature were 150 °C and 280 °C, respectively.

## 3. Result and discussion

### 3.1. Identification of components in C. rotundus

Total ion chromatograms of PLE extracts from *C. rotundus* rhizomes are shown in Fig. 1. All the main components were

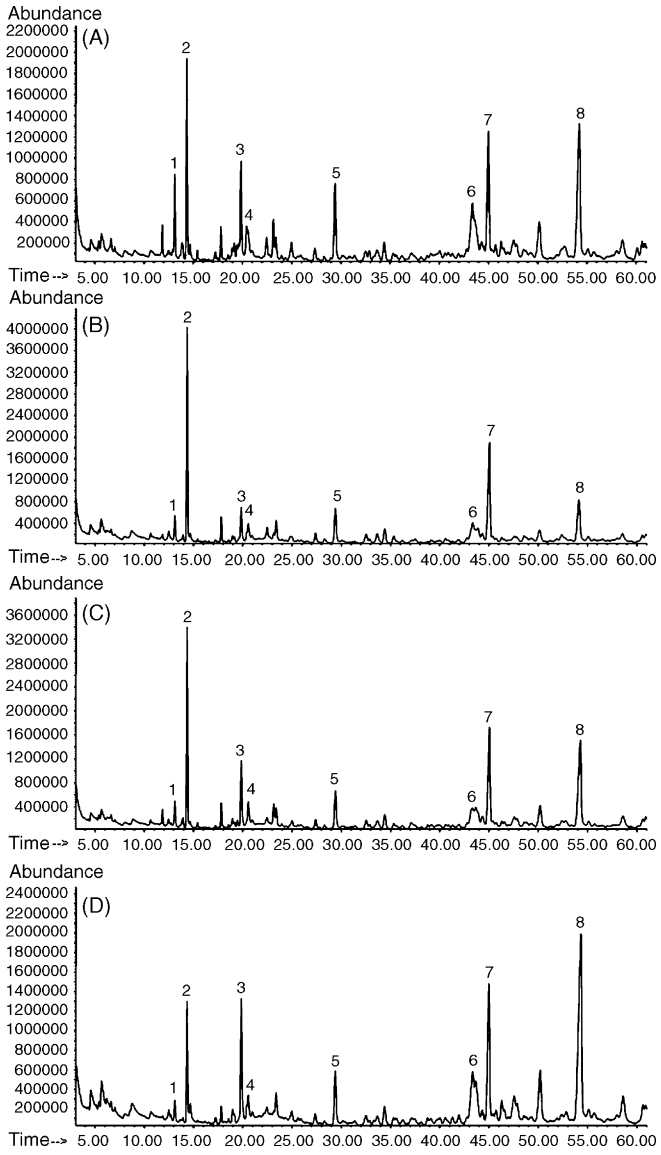


Fig. 1. GC–MS total ion chromatograms for PLE extracts of *C. rotundus* derived from (A) Anhui, (B) Shandong, (C) Hubei, and (D) Zhejiang Province. 1, α-copaene; 2, cyperene; 3, β-selinene; 4, selina-4,11-diene; 5, aristol-9-en-8-one; 6, aristol-9-en-3-one; 7, β-cyperone; 8, α-cyperone.

Table 3
Experimental arrangement and test result

| Test number | A | B | C | D | TA[a] |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 991,781,108 |
| 2 | 1 | 2 | 2 | 2 | 968,839,052 |
| 3 | 1 | 3 | 3 | 3 | 1,071,884,604 |
| 4 | 2 | 1 | 2 | 3 | 1,106,285,753 |
| 5 | 2 | 2 | 3 | 1 | 1,014,123,052 |
| 6 | 2 | 3 | 1 | 2 | 1,315,361,146 |
| 7 | 3 | 1 | 3 | 2 | 1,064,013,920 |
| 8 | 3 | 2 | 1 | 3 | 1,132,983,127 |
| 9 | 3 | 3 | 2 | 1 | 1,109,855,595 |
| $K_1$ | 3,032,504,764 | 3,162,080,781 | 3,440,125,381 | 3,115,759,755 | |
| $K_2$ | 3,435,769,951 | 3,115,945,231 | 3,184,980,400 | 3,348,214,118 | |
| $K_3$ | 3,306,852,642 | 3,497,101,345 | 3,150,021,576 | 3,311,153,484 | |
| $K_{max} - K_{min}$ | 134,421,729 | 127,052,038 | 96,701,268 | 77,484,788 | |

[a] Total peak area of α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone.

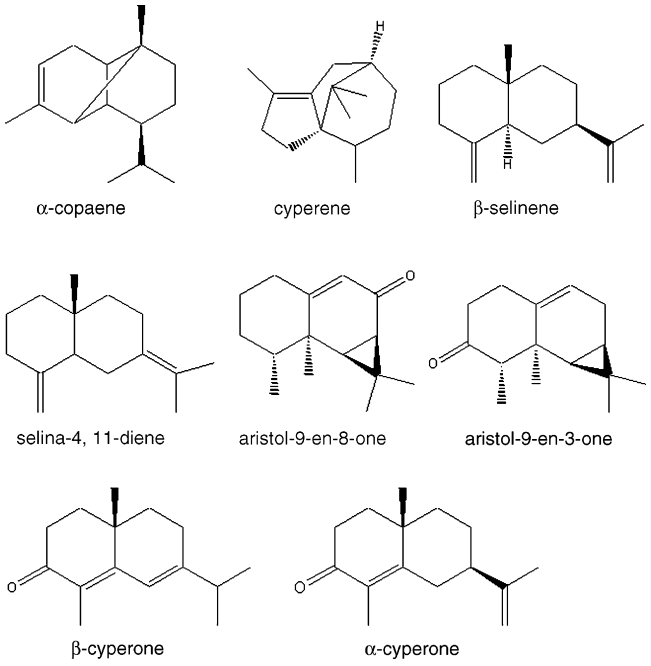


Fig. 2. The structures of eight identified compounds in *Cyperus rotundus*.

separated completely, and eight of them were identified according to the mass spectra. By comparing with literatures [36–43] and standard, peaks 1–8 were identified as α-copaene, cyperene, β-selinene, selina-4,11-diene, aristol-9-en-8-one, aristol-9-en-3-one, β-cyperone and α-cyperone, respectively (Table 1 and Figs. 1 and 2).

### 3.2. Quantitation of five compounds in C. rotundus

The selected ion monitoring (SIM) method was used for the quantification of five compounds in *C. rotundus*. The fragment ions of *m/z* 161, 204, 105, 218 and 218 were used for α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone, respectively. The contents of α-copaene, cyperene, β-selinene and β-cyperone in *C. rotundus* rhizomes were estimated by using calibration curve of α-cyperone, which is one of the major components in *C. rotundus*.

The calibration curve, which obtained from the selected ion peak area, of α-cyperone was linear over the range of 44.9–179.5 μg/ml with slope of $1.91 \times 10^7$. The coefficient of correlation ($r$) was 0.9992. The limits of detection (LOD) and quantification (LOQ) for α-cyperone were 2.6 μg/ml and 3.8 μg/ml, respectively. The injection precision was determined by injecting successively standard for six times. The relative standard deviation (R.S.D.) was 1.8%, 2.6% and 3.8% at the concentration of 128.7 μg/ml, 83.3 μg/ml and 45.1 μg/ml, respectively.

The short-term (six runs in 12 h) repeatability as well as the long-term (six runs in 24 h) repeatability of α-cyperone was calculated. The peak area of selected ion was relatively stable. The R.S.D.s of short- and long-term repeatability were 1.8–3.8% and 1.9–4.0% at the concentration of 128.7–45.1 μg/ml, respectively.

It is difficult for GC or HPLC to identify the peaks without standards. However, it is much easer using GC–MS. The content of five compounds including α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone in *C. rotundus* rhizomes was determined or estimated using α-cyperone as standard. Table 4 presents the summary results, which shows the extractable contents of five compounds in *C. rotundus* are greatly variant among three extraction methods and/or different locations of *C. rotundus* rhizomes.

### 3.3. Optimization of PLE procedure

PLE procedure was optimized. The pressure applied usually does not have a significant effect on the extraction efficiency as it is used to keep the solvent in the liquid state. Herein, 1000 p.s.i. was set as the default level. Total amount of five investigated compounds, i.e. α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone, was used as the marker for evaluation of the extraction efficiency (Fig. 3). The recovery efficiency for 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

Table 4
Extractable contents (mg/g) of five compounds in *Cyperus rotundus* using HD, SFE and PLE

| Analytes | CR1[a] | | | CR2 | | | CR3 | | | CR4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HD | PLE | SFE | HD | PLE | SFE | HD | PLE | SFE | HD | PLE | SFE |
| α-Copaene[b] | 2.61[c] | 1.86 | +[d] | 0.58[e] | 1.03 | + | + | 0.96 | + | + | 0.48 | + |
| Cyperene | 3.34 | 3.28 | 1.55 | 2.66 | 6.80 | 1.92 | 5.30 | 5.45 | 0.97 | 1.99 | 2.29 | 1.27 |
| β-Selinene | 1.08 | 0.96 | 0.64 | 0.99 | 0.86 | 0.57 | 1.03 | 1.09 | 1.51 | 1.25 | 1.27 | 0.84 |
| β-Cyperone | 2.91 | 3.73 | 2.56 | 2.70 | 5.83 | 3.46 | 3.05 | 5.05 | 1.70 | 3.86 | 4.44 | 3.15 |
| α-Cyperone | 1.78 | 2.41 | 1.79 | 1.98 | 1.55 | 1.05 | 2.09 | 2.61 | 2.31 | 2.98 | 3.55 | 2.42 |
| Total | 11.72 | 12.24 | 6.54 | 8.91 | 16.07 | 7.00 | 11.47 | 15.16 | 6.49 | 10.08 | 12.03 | 7.68 |

[a] CR1, CR2, CR3 and CR4 are *Cyperus rotundus* derived from Anhui, Shandong, Hubei and Zhejiang Province, respectively.
[b] α-Copaene, cyperene, β-selinene and β-cyperone were determined using α-cyperone as reference.
[c] The data was presented as average of three replicates (R.S.D. < 3%). Injection volume 5 μl with split ratio of 5:1.
[d] Under the limit of quantitation.
[e] For test the content less than 1.1 mg/g, injection volume 5 μl with split ratio of 1:1. While the content more than 5.0 mg/g, injection volume 5 μl with split ratio 10:1.

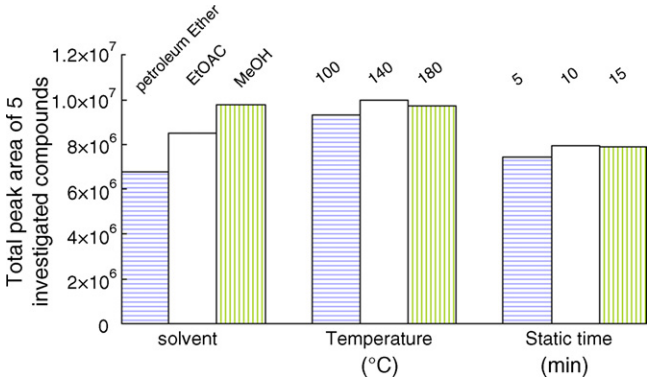


Fig. 3. Effects of solvent, temperature and static extraction time on PLE of *Cyperus rotundus*.

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; static extraction time, 10 min; pressure, 1000 p.s.i.; static cycle, 1% and 60% of the flush volume.

### 3.4. Optimization of SFE procedure

The total area of five peaks including α-copaene, cyperene, β-selinene, β-cyperone and α-cyperone was considered for estimating the results of orthogonal test. As Table 3 shows, the optimum level of each variable is: pressure, 200 bar; temperature, 45 °C; volume of ethanol as modifier, 20 ml; static time, 2 h. However, the less volume of ethanol, the higher extraction efficiency during the investigated ranges (20–80 ml). Therefore, the effect of the volume of ethanol (5–20 ml) on the extraction efficiency was further investigated. Finally, 10 ml ethanol was used as modifier for increasing the extraction efficiency. Thus, the optimum conditions of SFE are as follows: pressure, 200 bar; temperature, 45 °C; volume of modifier, 10 ml; static extraction time, 2 h.

### 3.5. Comparison on three methods for extraction of five compounds in C. rotundus

It is thought that interaction of multiple chemical compounds contributes to the therapeutic effects of Chinese medicines. The contents of the five investigated volatile components in *C. rotundus* extracted by HD, PLE and SFE are obviously different (Fig. 4). PLE had the highest extraction efficiency, while SFE had the best selectivity for extraction of β-cyperone and α-cyperone though its extraction yield was lower. HD may have more variation or be easy influenced by sample matrix because of its non-automated controlled parameters. Therefore, HD extraction efficiency was variant for individual compound in different raw materials (Fig. 4). The details need to be further investigated. In addition, the overall clinical efficacy of these extracts has not been determined. Therefore, comparison of chemical components and pharmacological activities of these extracts is helpful to elucidate the mechanism of therapeutic effects and active components in *C. rotundus* and control its quality.

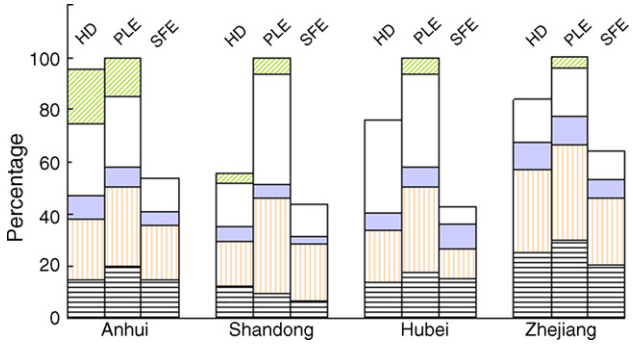


Fig. 4. Comparison of five volatile compounds in different *C. rotundus* extracted by HD, SFE and PLE. (▨) α-copaene, (□) cyperene, (▩) β-selinene, (▥) β-cyperone and (▤) α-cyperone.

## 4. Conclusion

The contents of volatile components extracted from *C. rotundus* using HD, PLE and SFE are obviously different, which suggests that comparison of chemical components and pharmacological activities of different extracts is helpful to elucidate the active components in *C. rotundus* and control its quality.

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