Static Headspace GC-MS Analysis for Volatile Components in *Eleocharis Tuberosa* Peel

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**Abstract.** Volatile components of *Eleocharis tuberosa* peels were extracted with absolute ether by ultrasonic wave assisted evaporating. The experiment mainly researches on three positive factors solid-liquid ratio, extraction time and ultrasonic power influencing the extraction efficiency. According to the results of one factor optimization, the orthogonal experiment L⁹(3⁴) was designed to confirm the optimum extraction conditions. The volatile chemical constituents of the extract were separated and identified by GC-MS. The optimal extraction conditions were as follows: solid-liquid ratio was 1:25 g/mL, extraction time was 60 min and ultrasonic power was 250 w. Confirmed by the practice, the average extraction rate was 3.25%, the conditions shown that the method was credible and reliable. There were 10 volatile compositions in the extract from *E. tuberosa* peels by the ultrasonic assisted extraction method. The relative content of volatile compositions were 2,6-Di-tert-butyl-4-methoxyphenol (45.24%), Ageratrochromene II (23.17%), 3,5-Bis(1,1-dimethylethyl) phenol (9.18%), 9,12-Octadecadienoic acid ethyl ester (5.84%), Ethyl palmitate (4.94%), 1-Linolenoylglycerol (4.11%), Dimethyl phthalate (3.30%), 1,3-Dimethyl-5-tert-butyl-2-acetophenone (2.10%), Phenylacetaldehyde (1.42%), Cedrol (0.70%).

1. **Introduction**

*Eleocharis tuberosa*, also known as water chestnut, is a minor but popular aquatic plant distributed in various parts of the world. This plant is a widespread species native to fresh-water or brackish marshes of the old world tropics from Madagascar to India, south-eastern Asia, the Philippines, Malaya, Indonesia, Melanesia and Fiji[1-2]. In China, it is estimated that the annual production of *E. tuberosa* was about 900,000 tons in 2015[3]. The fruits of water chestnuts contain high starch levels and are most often eaten after boiling or are used as sources of alcohol[4]. Moreover, the husk of *E. tuberosa* contains many dietary fibres and polyphenols[5]. *E. tuberosa* has also been used as a folk medicine to treat hypertension, chronic nephritis, constipation, pharyngitis, laryngitis and enteritis[6-7].

In recent years, fresh-cut *E. tuberosa* corms are in high demand because of their unique taste and medicinal properties[3]. A large number of peels were wasted. It was reported that the extracts of *E. tuberosa* peels showed strong bactericidal and antioxidant activities[8-9]. To comprehensive utilize it, the chemical constitutes of *E. tuberosa* peels were investigated. The peels were found to contain puchiin, cytokinin, flavonoids and phenolics[9,10]. However, little research was reported about its volatile component. In this paper, the volatile components of *E. tuberosa* peels collected by static
headspace injection were separated and identified by GC-MS, which played the certain roles of guarding and reference for the future further research and development.

2. Materials and methods

2.1. Preparation of E. tuberosa peels
Fresh E. tuberosa peels were purchased from the Hezhou Central Market. The peels were derived and in addition to decayed, washed and dehydrated 24 h within air dry oven at 40 °C. The E. tuberosa peels were sifted by 60 mesh sieve after fine crushing, and then stored them at 40 °C in storage locker (Shanghai Qixin Scientific Instruments Co., Ltd. China) until use.

2.2. Extraction of volatile components from E. tuberosa peels
10 g powders of E. tuberosa peels were accurately quantified and placed in 500 mL round-bottom flask. A certain proportional absolute ether was added in the flask and installed the condense pipe to build reflux condenser system in a water bath at 70 °C. Volatile components were extracted with ultrasonic wave assisted evaporating under specified time, solid-liquid ratio and ultrasonic power. After the end of extraction, all filtrate were collected, concentrated under vacuum, dried and weighed. The extraction rate was calculated by the following formula.

The extraction rate (%) = \((\frac{m_2-m_1}{m}) \times 100\)

Notes: \(m\) indicated the quality of E. tuberosa peels, in g; \(m_1\) indicated the quality of the flask before extraction, in g; \(m_2\) indicated the quality of the flask after extraction, in g.

2.3. Single factor test
Extraction of volatile components from E. tuberosa peels by ultrasonic assisted extraction. Under extraction time 60 min and ultrasonic power 60 w, the effect of solid-liquid ratio (1:5, 1:10, 1:20, 1:25, 1:30 g/mL) on the extraction of volatile components was detected to determine the optimum solid-liquid ratio. Under extraction this optimum solid-liquid ratio and ultrasonic power 60 w, the effect of extraction time (20, 40, 60, 80, 100 min) on the extraction of volatile components was detected to determine the optimum time. Under this time and the solid-liquid ratio, the effect of ultrasonic power (50, 100, 200, 250, 300 w) on the extraction of volatile components was detected to determine the optimum ultrasonic power.

2.4. Orthogonal experiment
On the basis of the results of one factor optimization, an orthogonal experiment of three factors and three levels L9(3^4) was designed to study the optimum requirements of extraction time, solid-liquid ratio and ultrasonic power in extraction of volatile components of E. tuberosa peels, in which the three conditions were varied, and the extraction rate as response values. The factors and levels of L9(3^4) orthogonal experiment as shown in Table 1.

| Levels | A (Solid-liquid Ratio, g/mL) | B (Extraction Time, min) | C (Ultrasonic Power, w) |
|--------|-----------------------------|--------------------------|------------------------|
| 1      | 1:5                         | 20                       | 50                     |
| 2      | 1:10                        | 40                       | 100                    |
| 3      | 1:25                        | 60                       | 250                    |

2.5. Static Headspace GC-MS Analysis
The extract of E. tuberosa peels was individually balanced 30 min at 40 °C. Volatile components were investigated by GC-MS with headspace sampling. The cryogenic trap was coupled to an analytical fused silica capillary column HP-5 MS, 30 m × 0.25 mm × 0.25 μm (Agilent Technologies, USA). Helium was the carrier gas (purity ≥ 99.999%). The temperature program was as follows: 40 °C held for 3 min, then raised at 2 °C/min to 150 °C held for 2 min, at 10 °C/min to 250 °C and finally held for
1 min. MSD conditions were set as follows: capillary direct interface temperature was 250 °C, scanning mass range was 35~450 amu, the ionization energy was 70 eV, and the detection voltage was 1.32 kV.

The GC-MS system was controlled by the Chem-Station software (Agilent Technologies, Version D.03.00, USA). The volatile components were initially identified by comparison of their mass spectra with those contained in the NIST Chemistry WebBook 6th edition (NIST Standard Reference Database number 69 containing 129,000 mass spectra). The retrieval results were compared with available literature. Chromatographic peak area normalization method was used for calculating their relative content of compositions.

2.6. Statistical analysis
All experimental data were presented as the mean ± standard deviation of the mean, data were analysed using IBM SPSS Statistics 22.0 (IBM Corporation, USA). Analysis of variance (ANOVA) was used to evaluate differences between groups: *P<0.05 was considered significant.

3. Results

3.1. Effect of solid-liquid ratio on the extraction of volatile components
As shown in Figure 1, while the ratio of solid-lipoid increases, the extraction rate of volatile components of *E. tuberosa* peels also increases. When solid-liquid ratio reached 1:25 g/mL, the extraction rate was no longer increased and tended to the same level. Therefore, under extraction time 60 min and ultrasonic power 60 w, the optimum solid-liquid ratio was 1:25 g/mL.

Figure 1. Effect of solid-liquid ratio on the extraction of volatile components.

Figure 2. Effect of extraction time on the extraction of volatile components.

Figure 3. Effect of ultrasonic power on the extraction of volatile components.

Figure 4. The total ion chromatogram of volatile compositions of the extract from *E. tuberosa* peels.
3.2. Effect of extraction time on the extraction of volatile components

As shown in Figure 2, along with extraction time increasing, the extraction rate of volatile components of *E. tuberosa* peels also increased. The extraction rate was increased slowly and tended to the same level when the extraction time reached 60 min. Therefore, under solid-liquid ratio 1:25 g/mL and ultrasonic power 60 w, the optimum extraction time was 60 min.

3.3. Effect of ultrasonic power on the extraction of volatile components

As shown in Figure 3, the extraction rate of volatile components of *E. tuberosa* peels was decreased along with the increase of ultrasonic power. When the ultrasonic power reached 250 w, the extraction rate was no longer increased and tended to the same level. Therefore, under solid-liquid ratio 1:25 g/mL and extraction time 60 min, the optimum ultrasonic power was 250 w.

3.4. Orthogonal experiment analysis

The results of orthogonal experiments were shown in Table 2. Variance analysis of L₉(3⁴) orthogonal experiment were shown in Table 3.

Table 2. Results of orthogonal experiment.

| Number | Factors | The extraction rate (x ± s) |
|--------|---------|-----------------------------|
|        | A  | B  | C  | D  |                          |
| 1      | 3  | 3  | 1  | 3  | 2.80±0.02                |
| 2      | 1  | 2  | 3  | 3  | 2.70±0.04                |
| 3      | 3  | 1  | 3  | 2  | 2.87±0.05                |
| 4      | 2  | 1  | 2  | 3  | 2.32±0.02                |
| 5      | 1  | 1  | 1  | 1  | 1.42±0.09                |
| 6      | 2  | 2  | 1  | 2  | 2.10±0.02                |
| 7      | 1  | 3  | 2  | 2  | 2.58±0.04                |
| 8      | 2  | 3  | 3  | 1  | 3.10±0.06                |
| 9      | 3  | 2  | 2  | 1  | 2.91±0.05                |
|        | 2.233 | 2.203 | 2.110 |
|        | 2.507 | 2.570 | 2.603 |
|        | 2.863 | 2.830 | 2.890 |
| R      | 0.630 | 0.627 | 0.780 |

According to the results of the range value (R), it is shown that the degree of the extraction rate impact of all factors could be ranked in a decreasing order as C (Ethanol volume fraction) > A (Ultrasonic power) > B (Extraction time).

The average extraction rate (K) showed that the extraction rate of volatile components of *E. tuberosa* peels was up to the highest under solid-liquid ratio A₃, extraction time B₃, ultrasonic power C₃. Therefore, the optimal extraction conditions were as follows: solid-liquid ratio was 1:25 g/mL, extraction time was 60 min and ultrasonic power was 250 w.
Table 3. Variance analysis of L9(3^4) orthogonal experiment.

| Source                     | Type III Sum of Squares | df | Mean Square | F     | Sig. |
|----------------------------|-------------------------|----|-------------|-------|------|
| Corrected Model            | 2.124                   | 6  | 0.354       | 26.612| 0.037* |
| Intercept                  | 57.760                  | 1  | 57.760      | 4342.857 | <0.001* |
| A(Solid-liquid Ratio, g/mL)| 0.592                   | 2  | 0.296       | 22.266| 0.043* |
| B(Extraction Time, min)    | 0.589                   | 2  | 0.294       | 22.138| 0.043* |
| C(Ultrasonic Power, w)     | 0.942                   | 2  | 0.471       | 35.431| 0.027* |
| Error                      | 0.027                   | 2  | 0.013       |       |      |
| Total                      | 59.910                  | 9  |             |       |      |
| Corrected Total            | 2.150                   | 8  |             |       |      |

a. R Squared 0.998

Notes: *P<0.05 was considered significant.

Repeated measurement ANOVA indicated that C (ultrasonic power) was the main factor that affected the extraction rate of volatile components of *E. tuberosa* peels, and there was a significant relationship between them (*P<0.05*); then A (solid-liquid ratio) and B (extraction time) also were a significant impact on the extraction rate (*P<0.05*).

In order to confirm the model was accurate and reliable, the optimal experimental conditions were the 3 times repetition test. And the average extraction rate of volatile components of *E. tuberosa* peels was 3.25% under the optimal conditions.

3.5. Analysis of volatile components

The total ion chromatogram of volatile compositions of the extract from *E. tuberosa* peels was shown in Figure 4. Volatile compositions of the extract from *E. tuberosa* peels were shown in Table 4. The ten volatile components were identified, and the retrieval results were compared with available literature. The relative content of volatile composition was calculated by chromatographic peak area normalization method. The results were as follows: 2,6-Di-tert-butyl-4-methoxyphenol (45.24%), Ageratochromene II (23.17%), 3,5-Bis(1,1-dimethylethyl) phenol (9.18%), 9,12-Octadecadienoic acid ethyl ester (5.84%), Ethyl palmitate (4.94%), 1-Linolenoylglycerol (4.11%), Dimethyl phthalate (3.30%), 1,3-Dimethyl-5-tert-butyl-2-acetophenone (2.10%), Phenylacetaldehyde (1.42%), Cedrol (0.70%).

Table 4. Volatile compositions of the extract from *E. tuberosa* peels.

| Number | R.T.(min) | Name                  | CAS         | Molecular formula | Pet Total (%) |
|--------|-----------|-----------------------|-------------|-------------------|---------------|
| 1      | 9.721     | Phenylacetaldehyde    | 122-78-1    | C_6H_5O         | 1.42          |
| 2      | 10.332    | Dimethyl phthalate    | 131-11-3    | C_{10}H_{10}O_4  | 3.30          |
| 3      | 11.333    | 1,3-Dimethyl-5-tert-butyl-2-acetophenone | 2040-10-0 | C_{14}H_{20}O | 2.10          |
| 4      | 11.664    | Cedrol                | 77-53-2     | C_{15}H_{20}O   | 0.70          |
| 5      | 12.329    | Ageratochromene II   | 644-06-4    | C_{13}H_{16}O_3 | 23.17         |
| 6      | 12.81     | 3,5-Bis(1,1-dimethylethyl)phenol  | 1138-52-9 | C_{14}H_{20}O   | 9.18          |
| 7      | 13.315    | 2,6-Di-tert-butyl-4-methoxyphenol | 489-01-0 | C_{15}H_{20}O_2 | 45.24         |
| 8      | 14.226    | Ethyl palmitate       | 628-97-7    | C_{16}H_{36}O_2 | 4.94          |
| 9      | 15.301    | 9,12-Octadecadienoic acid ethyl ester | 544-35-4 | C_{20}H_{36}O_2 | 5.84          |
| 10     | 15.33     | 1-Linolenoylglycerol  | 18465-99-1  | C_{21}H_{36}O_4 | 4.11          |
4. Discussion
Volatile components of *E. tuberosa* peels were extracted with ultrasonic wave assisted evaporating. According to the results of one factor optimization, the orthogonal experiment L9(3^4) was designed to confirm the optimum extraction conditions, which were as follows: solid-liquid ratio was 1:25 g/mL, extraction time was 60 min and ultrasonic power was 250 W. Confirmed by the practice, the average extraction rate of volatile components of *E. tuberosa* peels was 3.25%, the conditions shown that the method was credible and reliable.

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