The Difference of Volatile Compounds in Female and Male Buds of *Herpetospermum pedunculosum* Based on HS-SPME-GC-MS and Multivariate Statistical Analysis

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**Abstract:** *Herpetospermum pedunculosum* (Ser.) C. B. Clarke (Family Cucurbitaceae) is a dioecious plant and has been used as a traditional Tibetan medicine for the treatment of hepato-biliary diseases. The component, content, and difference in volatile compounds in the female and male buds of *H. pedunculosum* were explored by using headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) technology and multivariate statistical analysis. The results showed that isoamyl alcohol was the main compound in both female and male buds and its content in males was higher than that in females; 18 compounds were identified in female buds including 6 unique compounds such as (E)-4-hexenol and isoamyl acetate, and 32 compounds were identified in male buds, including 20 unique compounds such as (Z)-3-methylbutyraldehyde oxime and benzyl alcohol. (Z)-3-methylbutyraldehyde oxime and (E)-3-methylbutyraldehyde oxime were found in male buds, which only occurred in night-flowering plants. In total, 9 differential volatile compounds between female and male buds were screened out, including isoamyl alcohol, (Z)-3-methylbutanal oxime, and 1-nitropentane based on multivariate statistical analysis such as principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA). This is the first time to report the volatile components of *H. pedunculosum*, which not only find characteristic difference between female and male buds, but also point out the correlation between volatile compounds, floral odor, and plant physiology. This study enriches the basic theory of dioecious plants and has guiding significance for the production and development of *H. pedunculosum* germplasm resources.

**Keywords:** *Herpetospermum pedunculosum*; HS-SPME-GC-MS; dioecious plant; bud; volatile compound; multivariate statistical analysis

1. **Introduction**

*Herpetospermum pedunculosum* (Ser.) C. B. Clarke (Family Cucurbitaceae) is an annual climbing herb, distributed in Tibet, Yunnan, Sichuan, and other high-altitude areas in China, Nepal, and northeastern India [1–3]. As a traditional Tibetan medicine, it has the functions of clearing away heat and detoxification, and removing the gallbladder and internal heat for its bitter taste and cool potency [3,4], and is widely used for the treatment of hepato-biliary diseases [5].

*H. pedunculosum* is a dioecious plant, whose bisexual flower is shown in Figure 1. Its flowering period is June to September, and it blooms at night. Its flowers are golden-yellow with five petals, trumpet-shaped, eventually tubular, and covered with fine hairs. The
male flower is usually solitary with sparsely pubescent hairs or concomitant with the same raceme; pedicels with 2.0–6.0 cm length and sparse villous hairs; calyx tube with 2.0–2.5 cm length, enlarged similarly to a funnel, tubular lower part, and lanceolate lobes; yellow corolla with 2.0–2.2 cm in length and 1.2–1.3 cm in width, and with elliptic lobes and sharp tips. The female flower is solitary with the same perianth as male flowers and with three staminodes or none, and oblong ovary with three rooms. The mature fruit is 7–8 cm in length and 3–4 cm in width with long pubescent hairs.

![Figure 1. The flowers of H. pedunculosum (Left: female; right: male).](image)

There were many studies of *H. pedunculosum*. Its seeds had some pharmacological activities of hepatoprotection, anti-hepatitis B virus, and anti-liver fibrosis [6,7], contained chemical constitutes of fatty acid, polysaccharides, coumarin, and spinasterol glycoside [6–9], and had 156,531 bp length of the whole genome [10]. Because the seeds have medicinal, economic, and sowing values, the female plant is more popular with farmers [11,12]. Fifty metabolites including carbohydrates, amino acids, organic acids, lipids, and polyamine were identified in its leaves [13]. Twelve compounds including n-benzyltyramine, 1H-indol-3-carboxylic acid, rhodiocyanoside B, and matteflavoside A were isolated from its stems [14]. The moisture, total ash, acid-insoluble ash, and alcohol-soluble extract were determined as quality indexes of its flower [15–17]. However, there were a few studies on the gender difference in the dioecious plant of *H. pedunculosum*.

In this study, the female and male buds of *H. pedunculosum* were collected. Headspace solid-phase microextraction (HS-SPME) was used to extract their volatile components. Gas chromatography-mass spectrometry (GC-MS) was used to separate and identify these volatile compounds. The multivariate statistical analysis was chosen to screen the differential compounds, aiming to explore the difference in volatile compounds between female and male buds of this dioecious plant.

2. Results

2.1. Volatile Compounds of GC-MS Analysis

The total ion chromatogram obtained by GC-MS analysis was shown in Figure 2. The results showed that there were no significant differences among the three female buds (f1, f2, and f3) nor among the three male buds (m1, m2, and m3), but there were significant differences between female and male buds. It was indicated that there were no significant differences of buds in the same sex, but there were essential differences between different sexes regarding their volatile constitutes.
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Figure 2. GC-MS total ion chromatogram of volatile components from female and male buds of H. pedunculosum.

The results of volatile compounds by GC-MS analysis were shown in Table 1. The volatile compounds were identified by comparing retention time and mass spectra. For example, compound 1 (4.044 min) yielded a parent ion at m/z 88 and fragment ions at m/z 77, 55, and 42, and was identified as isoamyl alcohol according to NIST database. Compound 3 (8.675 min) displayed a parent ion at m/z 101 and fragment ions at m/z 86, 59, and 41, and was identified as (Z)-3-methylbutanal oxime. Compound 9 (10.578 min) produced a parent ion at m/z 117 and fragment ions at m/z 71, 43, and 29, and was identified as 1-nitropentane.

Table 1. Volatile compounds of GC-MS analysis from female and male buds of H. pedunculosum.

| No. | Rt/min | Compound                          | F   | CAS       | MW | Fragment (m/z) | Relative Content% |
|-----|--------|----------------------------------|-----|-----------|----|----------------|-------------------|
|     |        |                                  |     |           |    |                | Female | Male | 1  | 2  | 3  | 1  | 2  | 3  | 1  | 2  | 3  |
| 1   | 4.044  | Isoamyl alcohol                  | C8H15O | 122-51-3 | 88 | 70/55/42       | 90.19 | 83.29 | 90.17 | 43.23 | 57.90 | 64.01 |
| 2   | 8.355  | (E)-4-Hexenol                    | C8H14O | 928-92-7 | 100| 82/67/55/41    | 1.24  | 0.64 | 1.92  | 1.98  | 1.95  | 1.98  |
| 3   | 8.675  | (Z)-3-Methylbutanal oxime        | C8H14NO | 5780-40-3 | 101| 86/59/41       | -     | -     | -     | 7.15  | 6.71  | 6.66  |
| 4   | 8.960  | p-Xylene                         | C8H10 | 106-42-3 | 106| 91/76          | -     | -     | 2.57  | 2.11  | 1.83  |
| 5   | 9.513  | 3-Methyl-1-butanol acetate       | C8H14O | 123-92-2 | 130| 70/55/43       | 1.66  | 1.31  | 1.69  | 1.83  |
| 6   | 9.438  | (E)-3-Methylbutanal oxime        | C8H14NO | 5775-74-6 | 101| 86/59/41       | -     | -     | 2.11  | 2.13  | 1.94  |
| 7   | 10.000 | o-Xylene                         | C8H10 | 95-47-6  | 106| 91/76          | -     | -     | 0.3   | 0.15  | 0.11  |
| 8   | 10.393 | 2,2-Dimethyl-1-butanol           | C8H10O | 1185-33-7 | 102| 71/43/27       | -     | -     | 1.38  | 1.30  | 1.13  |
| 9   | 10.578 | 1-Nitropentane                   | C8H14NO | 628-05-7 | 117| 71/43/29       | -     | -     | 5.35  | 5.39  | 4.82  |
| 10  | 11.791 | 2-Methyl-5-(1-methyl)pentanal    | C11H18 | 2867-05-2 | 136| 93/77         | -     | -     | 0.13  | 0.10  | 0.07  |
| 11  | 12.055 | 1R-α-Pinene                      | C9H14S | 7785-70-8 | 136| 121/93/77      | 0.17  | 0.29  | 0.15  | 0.12  | 0.11  | 0.11  |
| 12  | 13.451 | Benzaldehyde                     | C8H10O | 100-52-7 | 106| 77/51          | -     | -     | 0.10  | 0.08  | 0.07  |
| 13  | 14.158 | Sabine                        | C9H14S | 3387-41-5 | 136| 93/77/41       | 0.17  | 0.33  | 0.14  | 1.02  | 0.53  | 0.45  |
| 14  | 15.227 | β-Mycene                        | C9H13S | 123-35-3 | 136| 93/69/41/27    | -     | -     | 0.61  | 0.36  | 0.25  |
| 15  | 16.423 | (55)-4-Carene                   | C10H5O | 29050-33-7 | 136| 121/93/79      | -     | -     | 0.07  | 0.06  | 0.05  |
| 16  | 16.846 | p-Cymene                        | C10H5S | 535-77-3  | 134| 119/91        | -     | -     | 0.17  | 0.13  | 0.11  |
| 17  | 17.044 | β-Limonene                      | C10H16 | 5989-27-5 | 136| 121/107/93/68  | 1.64  | 1.95  | 1.19  | 2.20  | 2.95  | 2.10  |
| 18  | 17.258 | 2-Ethyl-1-hexanol                | C10H16O | 104-76-7 | 130| 70/57/41/29    | 1.16  | 2.86  | 1.61  | -     | -     | -     |
| 19  | 17.351 | Benzyl alcohol                   | C10H16O | 100-51-6 | 108| 91/79/77/51    | -     | -     | 1.17  | 0.81  | 0.86  |
| 20  | 17.695 | α-Pine                           | C10H16O | 80-56-8  | 136| 93/77/59/27    | -     | -     | 0.50  | 0.21  | 0.10  |
| 21  | 18.209 | β-Oxime                          | C10H16S | 13877-91-3 | 136| 93/79/53/39    | 1.19  | 0.56  | 0.79  | 5.69  | 5.22  | 5.58  |
| 22  | 18.638 | γ-Terpine                        | C10H16 | 99-85-4  | 136| 121/93/77/43   | 0.19  | 0.33  | 0.15  | 0.65  | 0.39  | 0.26  |
| 23  | 21.073 | Nonanal                          | C10H16O | 124-19-6 | 142| 98/70/57/41    | 0.15  | 0.39  | 0.15  | 0.15  | 0.14  |
| 24  | 22.362 | 2-Methyl-1-undecene              | C11H22O | 18516-37-5 | 168| 69/56/41       | -     | -     | 1.36  | 1.70  | 1.08  |
| 25  | 22.934 | 4-Oxoisophorone                  | C10H16O | 1125-21-9 | 152| 96/68/39       | -     | -     | 5.57  | 5.79  | 5.62  |
As a result, in total, 38 volatile compounds were identified from the female and male buds of *H. pedunculosum*, including 18 compounds in female buds and 32 compounds in male buds, covering alkanes, alkenes, alcohols, aldehydes, ketones, and oximes, etc. The female and male buds had 12 common compounds, the female buds had 6 unique compounds, and the male buds had 20 unique compounds.

A compound of the highest content in volatile components from both female and male buds of *H. pedunculosum* was isomyl alcohol whose content was 83.29%, 90.17%, and 90.19% in female buds, and was 43.23%, 57.90%, and 64.01% in male buds, and the average relative content in female buds was 1.60 times higher than that in male buds. Isoamyl alcohol has the aroma of apple brandy, which has the effect of increasing the aroma and obvious spicy taste, so its higher content may be the main reason for the weaker fragrance of female flowers and the stronger one of male flowers. Among other common compounds, the content of β-ocimene in male buds (5.58%, 5.22%, and 5.69%) was higher than that in female buds (0.56%, 0.79%, and 1.19%) and the average content in female buds was 6.47 times higher than that in male buds. The content of δ-limonene in male buds (2.10%, 2.95%, and 4.20%) was also higher than that in female buds (1.19%, 1.64%, and 1.95%), and the average content in male buds was 1.94 times higher than that in female buds.

The characteristic volatile compounds in female buds were (E)-4-hexenol, isomyl acetate, 2-ethylhexanol, *n*-pentadecane, *n*-hexadecane, and neophytadiene. The characteristic volatile compounds in male buds had 20 compounds, including 2 oximes, 3 terpenes (β-myrcene, α-pinene, and α-copaene), and 4 phenyl compounds (p-xylene, o-xylene, benzaldehyde, and benzyl alcohol).

### 2.2. Multivariate Statistical Analysis

In order to further explore the difference of volatile compounds between female and male buds, multivariate statistical analysis such as principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) were carried out. The PCA was performed on the female and male buds and the result was shown in Figure 3A. The contribution rates of principal component 1 (PC1) and principal component 2 (PC2) were 88.20% and 9.54%, respectively, and the total contribution rate was 97.74%. R²X and Q² were 0.977 and 0.917, respectively, where R²X represented the fitting degree and Q² represented the prediction degree of the model. Additionally, it was indicated that the model was stable and reliable from these data. The classification results of the female

| No. | Retention Time (min) | Compound Name | Molecular Formula | CI m/z | Relative Intensity | R² | Q² |
|-----|---------------------|---------------|-------------------|--------|-------------------|-----|-----|
| 26  | 24.110              | 2,2,6-Trimethyl-1,4-cyclohexanediol | C₉H₁₀O₂ | 20547-99-3 | 154 | 139/69/56/42 | -   | -   | 2.68 | 1.97 | 1.88 |
| 27  | 30.453              | *n*-Pentadecane | C₁₅H₃₂ | 629-62-9 | 212 | 85/71/57/43 | 0.19 | 0.27 | 0.19 | -   | -   |
| 28  | 33.704              | α-Copaene      | C₃₀H₴₀ | 3856-25-5 | 136 | 161/119/105/91 | -   | -   | 0.09 | 0.10 | 0.11 |
| 29  | 34.834              | *n*-Hexadecane | C₃₂H₆₄ | 629-73-2 | 198 | 85/71/57/43 | 0.35 | 0.51 | 0.22 | -   | -   |
| 30  | 35.068              | (+)-7-epi-Sesquithujene | C₃₀H₴₀ | 159407-35-9 | 204 | 119/93/77/69/41 | 0.13 | 0.13 | 0.13 | 0.50 | 0.88 | 0.97 |
| 31  | 35.435              | trans-α-Bergamotene | C₃₀H₄₀ | 13474-59-4 | 204 | 119/107/93/69/41 | 0.25 | 0.13 | 0.17 | 1.11 | 1.94 | 2.17 |
| 32  | 37.199              | (15,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)methylene[3.1]hexane | C₃₀H₴₀ | 58319-04-3 | 204 | 93/69/41 | -   | -   | 1.03 | 1.71 | 1.85 |
| 33  | 39.901              | (1R,5R)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)methylene[3.1]hexane | C₃₀H₄₀ | 58319-04-3 | 204 | 93/69/41 | 0.64 | 0.45 | 0.57 | 1.39 | 1.78 | 1.99 |
| 34  | 51.476              | Neophytadiene  | C₂₃H₃₆ | 504-96-1 | 278 | 123/95/82/68/55/41 | 0.27 | 0.76 | 0.35 | -   | -   |
| 35  | 54.139              | (E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene | C₃₂H₄₀ | 77898-97-6 | 272 | 93/81/69/55/41 | -   | -   | 0.10 | 0.10 | 0.10 |
| 36  | 56.232              | (E,E)-7,11,15-Trimethyl-3-methylhexene-1,6,10,14-hexadecatetraene | C₃₂H₄₀ | 70901-63-2 | 272 | 93/81/69/41 | 0.21 | 0.67 | 0.17 | 0.24 | 0.14 | 0.13 |
| 37  | 57.391              | (Z)-9-Tricosene | C₃₀H₄₀ | 27519-02-4 | 322 | 97/83/55 | -   | -   | 0.14 | 0.14 | 0.18 |
| 38  | 57.569              | α-Eicosane     | C₃₂H₄₀ | 112-95-8 | 282 | 85/71/57/43 | 0.21 | 0.13 | 0.23 | 0.12 | 0.18 | 0.26 |

F: formula; MW: molecular weight; -: not detected.
and male buds were ideal, the female buds were distributed on the right side of the Y axis, and the male buds were distributed on the left side of the Y axis, which suggested that the volatile compounds of different sexes were significantly different. The Loadings analysis was shown in Figure 3B. The volatile compounds with the largest contribution rate to distinguishing the female and male buds were screened with the coordinate axis 0.2 or -0.2 as the limit value. It was found that the five compounds had the largest contribution rate, i.e., isoamyl alcohol (1), 4-oxoisophorone (25), β-ocimene (21), (Z)-3-methylbutyraldehyde oxime (3), and 1-nitropentane (9).

**Figure 3.** PCA scores (A) and Loadings (B) for female and male buds of *H. pedunculosum*. The green numbers in figure B were consistent with the No. in Table 1.

As an unsupervised analysis method, PCA cannot ignore within-group and eliminate irrelevant random errors. Therefore, supervised OPLS-DA was used to further determine the difference of volatile compounds between female and male buds. However, overfitting was easy to occur while expanding the differences between groups, so it was necessary to arrange experiments with the help of external model validation methods to prove the validity of the model. Different random Q^2 values were obtained by changing the arrangement order of the categorical variable y randomly multiple times (*n* = 200). In this
In this experiment, the greater the slope of the regression line, the smaller the difference between $R^2$ and $Q^2$, indicating that there were more data with which to explain the model; the predictive ability of the model was better. The test result was shown in Figure 4A. Among them, the $R^2$ and $Q^2$ values generated by any random arrangement on the left end were smaller than those on the right, the slope of the regression line was large, and the lower regression line intersected the negative half-axis of the Y-axis, indicating that the model was effective, stable, and predictable, and could continue to screen the difference compounds. From the OPLS-DA scatter plot of female and male buds ($R^2$X: 0.977, $R^2$Y: 0.998, and $Q^2$: 0.994) (Figure 4B), it could be seen that the two types of samples were clearly distinguished. According to the corresponding VIP value (Figure 4C), these variables whose VIP value was greater than 1 and whose confidence interval did not contain 0 were screened, and it was determined that there were nine differential volatile compounds between the female and male buds (Table 2). Among them were section A for isoamyl alcohol of higher levels in female buds and section B for (Z)-3-methylbutyraldehyde oxime, 1-nitropentane, β-ocimene, 4-oxoisophorone, 2-methyl-1-undecene, 2,2,6-trimethyl-1,4-cyclohexanedione, p-xylene, and (E)-3-methylbutyraldehyde oxime of higher levels in male buds.
Figure 4. OPLS-DA arranges the model verification diagram (A), scores (B), and VIP scores (C) between female and male buds of *H. pedunculosum*. The numbers of Var ID in Figure C were consistent with the No. in Table 1.

Table 2. Nine differential volatile compounds between female and male buds of *H. pedunculosum*.

| Section | No. | Compound                                      | VIP  |
|---------|-----|-----------------------------------------------|------|
| A       | 1   | Isoamyl alcohol                               | 4.00 |
| B       | 3   | (Z)-3-Methylbutanal oxime                     | 1.83 |
| B       | 9   | 1-Nitropentane                                | 1.59 |
| B       | 21  | β-Octimene                                    | 1.48 |
| B       | 25  | 4-Oxoisophorone                               | 1.26 |
| B       | 24  | 2-Methyl-1-undecene                           | 1.07 |
| B       | 26  | 2,2,6-Trimethyl-1,4-cyclohexanedione          | 1.03 |
| B       | 4   | p-Xylene                                      | 1.03 |
| B       | 6   | (E)-3-Methylbutanal oxime                     | 1.00 |

A: higher in females than in males; B: higher in males than in females.

3. Discussion

There were a lot of differences in the morphological structure between female and male flowers and buds of the dioecious plant *H. pedunculosum*, but the difference in volatile compounds has not been reported in the literature. Buds are the state when flowers are about to bloom; their chemical components have not been lost and polluted by foreign substances, thus the research on chemical components of buds has a more realistic effect. Therefore, there were many studies which took buds as research objects, such as *Eugenia caryophyllus* (Myrtaceae) [18–20], *Capparis spinosa* (Capparaceae) [21], *Magnolia biondii* and *M. sirindhorniae* (Magnoliaceae) [22,23], *Populus tomentosa* (Salicaceae) [24], and *Pyrus pyrifolia* (Rosaceae) [25]. In this study, it was found that there was significant difference in the volatile compounds between female and male buds of *H. pedunculosum*.

Isoamyl alcohol is a fragrance ingredient used in cosmetics, detergents, fine fragrances, household cleaners, shampoos, toilet soaps, and other toiletries [26]. Because of its advantages of high energy density, low hygroscopicity, and compatibility with the current infrastructure, isoamyl alcohol has attracted considerable attention as one of the biofuels [27]. As a result, it was showed that the content (up to 90.19%) of isoamyl alcohol was the highest, which provided a new perspective for improving the economic value of *H. pedunculosum*. 
Some unique compounds in female or male buds made this study more meaningful. Two kinds of oximes and phenyl compounds were only present in male buds. For (Z)-3-methylbutyraldehyde oxime and (E)-3-methylbutyraldehyde oxime, the reports on plant volatile compounds were extremely rare: only the night-flowering plants of Gaura drummondii (Onagraceae) and Trichosanthes kirilowii (Cucurbitaceae) [28,29]. For phenyl compounds, benzaldehyde was only detected in male flowers and the content of benzyl alcohol in male flowers was significantly higher than that in female flowers of T. kirilowii [29]. Similarly, 4-o xoisorphonone was detected only in male buds in this study, which was one of nine differential compounds filtered by multivariate statistical analysis. It was shown that 4-oxoisophorone may be a key compound for honeybees’ perceptions of flower odor, which was likely to be implicated in bee foraging behavior [30].

(E)-4-Hexenol, isoamyl acetate, and 2-ethylhexanol were only detected in female buds. It was found that the volatile compounds such as isoamyl alcohol and isoamyl acetate were more attractive to pollinators in the field behavior measurements [31]. High contents of alcohol and ester compounds were beneficial to better pollination of the female flowers of H. pedunculosum, which could promote the yield of fruits and improve the economic value of female plants.

In addition, seven terpenes were detected in female or male buds, including β-myrcene, α-pinene, α-copaene, sabinene, β-ocimene, δ-limonene, and γ-terpinene. It was shown that terpenes had the function of attracting pollinating insects, which caused the plant to attract a variety of flower visiting insects, and could promote the natural pollination of flowers [32].

There were few reports on the difference in chemical composition of dioecious plants. For example, the contents of 2-cyclohexenone and methyl benzoate in female buds were significantly higher than those in male buds, and the content of ethyl benzoate in male buds was significantly higher than that in female buds of P. tomentosa [23]. The content of linalool in female flowers was significantly higher than that in male flowers, whereas the content of benzyl alcohol in male flowers was significantly higher than that in female flowers of T. kirilowii [29]. The comparative study on the volatile compounds of the buds revealed the characteristic difference between female and male buds of H. pedunculosum, pointed out the preliminary relationship between the volatile compounds and plant physiology, and enriched the basic research of dioecious plants.

Based on the differences of the volatile compounds from female and male buds of H. pedunculosum, the synthesis mechanism of these differential compounds and their specific effects on plant growth should be studied in the future. In addition, the differences in non-volatile compounds and biological activities between female and male plants are also worthy of further study.

4. Materials and Methods

4.1. Apparatus

The SPME fiber 50 mm carboxen/polydimethylsiloxane/divinylbenzene (CAR/PDMS/DVB) was purchased from Supelco (Bellefonte, PA, USA). An AOC-5000 auto injector solid-phase microextraction device and a SHIMADZU QP 2010 gas chromatography–mass spectrometer were purchased from Shimadzu (Tokyo, Japan). An ME204/02 electronic balance was purchased from Mettler Toledo Instruments Co., Ltd. in Shanghai in China.

4.2. Sample Collection

Because H. pedunculosum was a night-flowering plant, the buds of both female and male plants were collected at 11:00 pm just before they bloomed. The collection date was August 31, 2021, and the collection site was located in the Shangri-La Alpine Botanical Garden, Diqing Tibetan Autonomous Prefecture, Yunnan Province (27°54’17.07” N, 99°38’15.02” E, and 3269 m altitude). The female and male buds were obtained by pinching
off the flower stalk with tweezers, which were identified as fresh female and male buds of *Herpetospermum pedunculosum* (Ser.) C. B. Clarke (Family Cucurbitaceae) by Prof. Zhimao Chao (Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences) according to the description in Flora of China (Editorial Board of Flora of China, 1984). The voucher specimens (HPF1-3 and HPM1-3) were deposited at 1022 laboratory of Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China.

4.3. Sample Preparation

A fresh bud sample was placed in a 15 mL headspace vial. A SPME fiber 50 mm CAR/PDMS/DVB was extended through the needle and exposed into the headspace vial to adsorb volatile compounds at 40 °C for 30 min and then immediately injected into the gas chromatography injection port at 250 °C for 3 min to desorb volatile compounds.

4.4. Chromatographic Condition

The volatile compounds from the buds were analyzed by GC-MS method. A GC-MS QP 2010 HP-5MS (SHIMADZU, Tokyo, Japan) was used coupled to UI elastic quartz capillary column (0.25 μm × 0.25 mm × 30 m). The splitless injection mode was used. The carrier gas was high purity helium which was used at a constant flow rate of 1 mL/min. The temperature of injection port was 250 °C. The heating program was as follows: the initial temperature was 40 °C and held for 5 min; the temperature was increased to 190 °C at a rate of 3 °C/min; then increased to 300 °C at a rate of 20 °C/min and held for 1 min.

4.5. MS Condition

The ion source temperature was 200 °C. The interface temperature was 230 °C. The solvent delay time was 2 min. The scanning range was m/z 45–500. The mass spectra would be obtained at 70 eV with electron ionization (EI) mode.

4.6. Data Processing

The mass spectral identification of volatile compounds was obtained by comparing with the National Institute of Standards and Technology (NIST) 14. The qualitative analysis of mass spectral data was verified by comparing the retention time and mass spectra. The quantitative analysis of volatile compounds was determined by peak area normalization of total ion chromatography.

4.7. Statistical Analysis

All multivariate analysis and calculations were performed on SIMCA-P software (version 14.1, Umetrics, Malmö, Sweden). The data were imported into the software and scaled by Pareto scaling method to reduce the relative importance of large values and to keep the data structure partially intact. Then, the data were submitted to PCA and OPLS-DA analysis. The differential compounds between female and male buds were filtered and used for the subsequent in-depth analysis.

5. Conclusions

In this study, HS-SPME-GC-MS technology coupled to multivariate statistics analysis was used to explore some difference in volatile compounds of *H. pedunculosum*. It was found that there was a significant difference between female and male buds. In total, 38 volatile compounds were identified by GC-MS, and 18 and 20 volatile compounds were detected in female and male buds, respectively. Among them, 6 unique compounds were detected, such as (E)-4-hexenol and isoamyl acetate in female buds, 20 unique compounds were detected such as (Z)-3-methylbutyraldehyde oxime and benzyl alcohol in male buds. In total, 9 differential volatile compounds of female and male buds were screened out,
including isoamyl alcohol, (Z)-3-methylbutanal oxide, and 1-nitropentane based on multivariate statistical analysis. This is the first time that the differences in volatile compounds of the dioecious plant *H. pedunculosum* with HS-SPME-GC-MS have been reported, which reveals the essential differences in female and male buds.

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**References**

1. Ma, L.; Yang, L.; Zhao, J.; Wei, J.; Kong, X.; Wang, C.; Zhang, X.; Yang, Y.; Hu, X. Comparative proteomic analysis reveals the role of hydrogen sulfide in the adaptation of the alpine plant *Lamiophlomis rotata* to altitude gradient in the northern Tibetan plateau. *Planta* 2015, **281**, 887–906.

2. Xu, B.; Liu, S.; Fan, X.D.; Deng, L.Q.; Ma, W.H.; Chen, M. Two new coumarin glycosides from *Herpetospermum caudigerum*. *J. Asian Nat. Prod. Res.* 2015, **17**, 738–743. https://doi.org/10.1080/20866020.2014.996137.

3. Yang, L.; Suo, Y.; Zhou, C.; Li, Y.; Wang, H. Study on trace elements in seeds of *Herpetospermum penduculosum* (Ser) Baill. *Trace Elem. Sci.* 2003, **10**, 45–47.

4. Zhao, X.; Suo, Y.; Wang, L.; You, J.; Ding, C. Analysis of carbohydrates in a Tibetan medicine using new labeling reagent, 1-(2-naphthyl)-3-methyl-5-pyrazolone, by HPLC with DAD detection and ESI-MS identification. *J. Liq. Chromatogr. Relat. Technol.* 2008, **31**, 2375–2400.

5. Li, Q.; Li, H.J.; Xu, T.; Du, H.; Huan, G.C.; Fan, G.; Zhang, Y. Natural medicines used in the traditional Tibetan medical system for the treatment of liver diseases. *Front. Pharmacol.* 2018, **9**, 1–16. https://doi.org/10.3389/fphar.2018.00029.

6. Li, G.; Wang, X.Y.; Suo, Y.R.; Wang, H.L. Protective effect of seed oil of *Herpetospermum penduculosum* against carbon tetrachloride-induced liver injury in rats. *Saudi Med. J.* 2014, **35**, 981–987.

7. Hang, L.Y.; Shen, B.D.; Shen, C.Y.; Yang, K.; Yuan, H.L. Advances in modern research on anti-liver disease of Tibetan medicine Semen Hertospermii. *Chin. Trad Her. Drug.* 2020, **51**, 549–556.

8. Huang, D.; Ma, Y.X.; Wei, L.; Sun, Y.; Zeng, Q.H.; Lan, X.Z.; Liao, Z.H.; Chen, M. One new coumarin from seeds of *Herpetospermum penduculosum*. *China J. Chin. Mater Med.* 2021, **46**, 2514–2518. https://doi.org/10.19540/cnki.jcmm.20210125.601.

9. Liu, J.; Chen, X.; Zhang, Y.; Zhang, M. Chemical constituents of the ethyl acetate extract from Semen Herpetospermi. *Pharm Clin. Chin. Mater Med.* 2010, **1**, 15–18.

10. Wang, C.; Wang, X.; Tseringand, T.; Song, Y.; Zhu, R. The plastid genome of *Herpetospermum pedunculosum* (Cucurbitaceae), an endangered traditional Tibetan medicinal herbs. *Mitochondrial DNA B Resour.* 2020, **5**, 495–497. https://doi.org/10.1080/23802359.2019.1703603.

11. Xie, K. Molecular Sex Identification in *Herpetospermum pedunculosum* (Ser.) C.B. Clarke via RAPD and SCAR Markers. Master’s Thesis, Chengdu University, Chengdu, China, 2021.

12. Feng, X.; Dengba, D.J.; Kong, S.X.; Li, H.K.; Shuya, K.; Zhong, G.J. Forecasting the seed yield of Tibetan herb medicine *Herpetospermum pedunculosum* Baill and analyzing for its influencing factors based on linear regression analysis model. *Med. Chin. Med.* 2020, **22**, 409–411+426. https://doi.org/10.13313/j.issn.1673-4890.20190413003.

13. Zhao, Y.; Xu, F.; Liu, J.; Guan, F.; Quan, H.; Meng, F. The adaptation strategies of *Herpetospermum pedunculosum* (Ser.) Baill at altitude gradient of the Tibetan plateau by physiological and metabolomic methods. *BMC Genom.* 2019, **20**, 451. https://doi.org/10.1186/s12864-019-5778-y.

14. Feng, Z.Y.; Ma, Y.X.; Wang, H.; Chen, M. Studies on chemical constituents of stems of *Herpetospermum pedunculosum*. *China J. Chin. Mater Med.* 2020, **45**, 2571–2577. https://doi.org/10.19540/j.cnki.cjcmm.20200227.201.
15. Liu, R.; Tang, D.; Liu, Q. Experimental study on the extraction process of water-soluble polysaccharide from Tibetan medicine *Herpetospermum pedunculosum* flower. *J. Southwest Nat. Univ. (Nat. Sci. Ed.)* 2019, 45, 57–578. https://doi.org/10.11920/xnmdzk.2019.06.004.

16. Zhang, Y.X.; Li, C.Y.; Liu, C.; Zhang, Y.; De, L. Preliminary study of quality standards of Tibetan medicine *Herpetospermum pedunculosum* flower. *J. Southwest Nat. Univ. (Nat. Sci. Ed.)* 2015, 41, 432–435. https://doi.org/10.11920/xnmdzk.2015.04.007.

17. Chen, Z.Q.; Zhou, Z.L.; Wang, L.L.; Meng, L.H.; Duan, Y.W. Development of microsatellite markers for a dioecious *Herpetospermum pedunculosum* (Cucurbitaceae). *Evol. Bioinform. Online* 2020, 16, 1176934320908261. https://doi.org/10.1177/1176934320908261.

18. Chaieb, K.; Hajlauj, H.; Zmantar, T.; Khala-Nakbi, A.B.; Rouabha, M.; Mahdouani, K.; Bakhrouf, A. The chemical composition and biological activity of clove essential oil, *Eugenia carpyphyllata* (Syzygium aromaticum L. Myrtaceae): A short review. *Phytother. Res.* 2007, 21, 501–506. https://doi.org/10.1002/ptr.2124.

19. Hemalatha, R.; Nivetha, P.; Mohanapriya, C.; Sharmila, G.; Muthukumaran, C.; Gopinath, M. Phytochemical composition, GC-MS analysis, in vitro antioxidant and antibacterial potential of clove flower bud (*Eugenia carpyphyllus*) methanolic extract. *J. Food Sci. Technol.* 2016, 53, 1189–1198. https://doi.org/10.1007/s13197-015-2108-5.

20. Bitterling, H.; Schäfer, U.; Krammer, C.; Brückner, S.I.; Hartmann, B.; Ongouta, J.; Carle, R.; Steinag, C.B. Investigations into the natural occurrence of 1-phenylethanol (styrallyl acetate). *J. Agric. Food Chem.* 2020, 68, 8613–8620. https://doi.org/10.1021/acs.jafc.0c02758.

21. Sonmezdog, A.S.; Kelebek, H.; Selli, S. Characterization of aroma-active compounds, phenolics, and antioxidant properties in fresh and fermented capers (*Capparis spinosa*) by GC-MS-olfactometry and LC-DAD-ESI-MS/MS. *J. Food Sci.* 2019, 84, 2449–2457. https://doi.org/10.1111/1750-3841.14777.

22. Hu, M.; Bai, M.; Ye, W.; Wang, Y.; Wu, H. Variations in volatile oil yield and composition of “Xin-yi” (*Magnolia biondii* Pamp. Flower Buds) at different growth stages. *J. Oleo Sci.* 2018, 67, 779–787. https://doi.org/10.5650/jos.ess17229.

23. Ghosh, D.; Chaudhary, N.; Uma Kumari, K.; Singh, J.; Tripathi, P.; Meena, A.; Luqman, S.; Yadav, A.; Chanotiya, C.S.; Pandey, G.; et al. Diversity of essential oil-secretory cells and oil composition in flowers and buds of magnolia sirindhorniae and its biological activities. *Chem. Biodivers.* 2021, 18, e2000750. https://doi.org/10.1002/cbdv.202000750.

24. Xu, L.; Liu, H.P.; Ma, Y.C.; Wu, C.; Li, R.Q.; Chao, Z.M. Comparative study of volatile components from male and female flower buds of *Populus tomentosa* by HS-SPME-GC-MS. *J. Nat. Prod. Res.* 2019, 33, 2105–2108. https://doi.org/10.1080/14786419.2018.1483929.

25. Horikoshi, H.M.; Sekozawa, Y.; Kobayashi, M.; Saito, K.; Kusano, M.; Sugaya, S. Metabolomics analysis of ‘Housui’ Japanese pear flower buds during endodormancy reveals metabolic suppression by thermal fluctuation. *Plant Physiol. Biochem.* 2018, 126, 134–141. https://doi.org/10.1016/j.plaphy.2018.02.028.

26. McGinty, D.; Lapczynski, A.; Scognamiglio, J.; Letizia, C.S.; Api, A.M. Fragrance materials review on isoamyl alcohol. *Food Chem. Toxicol.* 2010, 48, S102–S109. https://doi.org/10.1016/j.fct.2010.05.040.

27. Tu, R.; Lv, T.; Sun, L.; He, R.; Wang, Q. Development of a simple colorimetric assay for determination of the isoamyl alcohol-producing strain. *Appl. Biochem. Biotechnol.* 2020, 192, 632–642. https://doi.org/10.1007/s12010-020-03553-3.

28. Shaver, T.N.; Lingren, P.D.; Marshall, H.F. Nighttime variation in volatile content of bud of the night blooming plant *Gaura drummondii*. *J. Chem. Ecol.* 1997, 23, 2673–2682.

29. Sun, W.; Chao, Z.M.; Wang, C.; Wu, X.Y.; Tan, Z.G. Research on the difference of volatile compounds in female and male buds of *Trichosanthes kirilowii* Maxim based on HS-SPME-GC-MS. *China J. Chin. Mater. Med.* 2012, 37, 1570–1574.

30. Twidale, A.M.; Mas, F.; Harper, A.R.; Horner, R.M.; Welsh, T.J.; Suckling, D.M. Kiwifruit flower odor perception and recognition by honey bees, *Apis mellifera*. *J. Agric. Food Chem.* 2015, 63, 5597–5602. https://doi.org/10.1021/acs.jafc.5b01165.

31. Tang, Y.C.; Zhou, C.L.; Chen, X.M.; Zheng, H. Visual and olfactory responses during butterfly foraging. *J. Insect. Behav.* 2013, 26, 387–401.

32. Zhu, X.Z.; Lu, Q.B.; Hu, X.H.; Deng, T.; Duan, Y.B.; Fang, Z.M.; Huang, S.X. Leaf volatile components and foraging insects of dioecious *Siraitia grosvenorii*: Sexual differences and its ecological effects. *Guilinuia* 2020, 40, 1259–1268.