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# PROFILING OF THE VOLATILE COMPONENTS IN *SELAGINELLA* BASED ON HS-SPME AND GC-MS

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## Abstract

Difference of the volatile components of four herbs of *Selaginella* genus, i.e. *Selaginella remotifolia, S. bodinieri, S. biformis, S. trichoclada* were compared for the first time based on headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). SPME extraction parameters (SPME fibres, extraction temperatures and extraction time) were optimized by single factor evaluation. In terms of maximum signal recorded and number of isolated volatilemetabolites, the best results were obtained as follows: a 65 μm DVB/PDMS coating fiber at 90ºC for 10 min. A total of 53 components of the essential oils were identified, which included ketones (6), sesquiterpenes (4), monoterpenes (11), esterscompounds (2), higher alcohols (7), aldehydes (8), furan derivatives (2), alkanes (4), aliphatic hydrocarbons (7), sulphur compounds (2). Compared with different volatility of components, monoterpenes (38.14%) were the highest in the four species of Selaginellaceae, followed by alcohols (18.95%), alkanes (12.87%), and furans (12.85%). Thus, from the obtained results it may be suggested that four *Selaginella* plants can be used as possible source for comprehensive utility and development of *Selaginella* herbs.

## Introduction

*Selaginella remotifolia* (Sr), *S. bodinieri* (Sbo), *S. biformis* (Sbi), *S. trichoclada* (St) are commonly used in traditional Chinese medicines (Jin *et al.* 2007). They generally belong to Selaginellaceae (Jiang *et al.* 2017). Of all these Selaginellas, 700 species are widely distributed throughout the world and about 60-70 species are native to China (Wang *et al.* 2015, Li *et al.* 2017). Traditional Chinese Medical (TCM) Science thinks that the Selaginellaceae was beneficial for detoxifying heat and detoxifying (Wang *et al.* 2013, Song *et al.* 2014), activating blood circulation and removing blood stasis (Jin *et al.* 2015), removing wind and dehumidifying (Wang *et al.* 2017) etc. Modern pharmacological andclinical research (Zheng *et al.* 2014) had shown that the main active ingredients in Selaginellas are volatile oils and biflavonoids. The latest studies have reported that these species of *Selaginella* are mainly used for the treatment of laryngopharyngeal swelling, rheumatism, nasopharyngeal carcinoma, chorionic epithelial carcinoma (Valdespino *et al.* 2015), cervical cancer, and traumatic bleeding (Sawaya *et al.* 2015).

Considering the limitations of the traditional sampling techniques, headspace solid phase microextraction (HS-SPME) has attracted more and more attention in recent years (Araújo *et al.* 2015, Wang *et al.* 2015). HS-SPME has many advantages, such as efficient extraction preconcentration methods and reliable alternatives to traditional sample preparation techniques because of their simplicity, low cost, selectivity, and sensitivity when combined with appropriate detection modes (García *et al.*2006, Singh *et al.* 2017).

At present, HS-SPME technology has not been applied to the study of *Selaginella* plants. In this experiment, the volatile oils of four *Selaginella* species have been identified and analyzed via headspace solid-phase microextraction combined with gas chromatography-mass spectrometry

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(HS-SPME-GC-MS). It is also the first time that the volatile metabolomic composition of the *Selaginella* plants has been investigated, which could provide useful information for the development and utilization of *Selaginella* herbs.

## Material and Methods

The mixture of standard n-alkanes containing $C_8$-$C_{20}$ straight-chain alkanes in hexane was purchased from Ruizhx Technology (Beijing, China). SPME holders were purchased from Supelco (Bellefonte, USA) and coated with the following polymers: polydimethylsiloxane (PDMS, 100um), carboxen/polydime-thylsiloxane (CAR/ PDMS, 75um), divinylbenzene/carboxen on polydi-methylsiloxane (DBV/CAR/ PDMS, 50/30 μm), and polydimethylsiloxane/divinylbenzene (PDMS/DVB, 65 μm).

Several herbs from*selaginella* were collected in south of the Yangtze River, China, in January 2018 (Table 1). Four *Selaginella* species were identified by Yang Jianwen, Deputy Director Pharmacist of Zunyi Medical University.

**Table 1.Collection sites of different *selaginella.***

| No. | A. n. | Collection site | Longitude | Latitude | Altitude(masl) |
|---|---|---|---|---|---|
| 1 | Sr | Liu Zhou, Guang Xi Province, China | N109° 4 ' | E24° 33 ' | 101 |
| 2 | Sbo | Zun Yi, Gui Zhou Province, China | N114° 32 ' | E29°85 ' | 28.5 |
| 3 | Sbi | Guang Yuan, Si Chuan Province, China | N105° 46 ' | E32° 24 ' | 1200 |
| 4 | St | An Ning, Yun Nan Province, China | N102° 44 ' | E24° 95 ' | 1862 |

The SPME device included a fused silica fibre coating partially cross-linked with 65 μm DVB/PDMS. Before use, the SPME fibre was maintained at 250ºC for 120 min in the GC injector. Before sampling, blank runs were completed to ensure no carryover of analytes from previous extractions. For the HS-SPME assay, aliquots of 1.0 ± 0.001 g broken herbs passed through a 20 mesh were placed into a 4 ml glass vial which was closed and placed in a thermostatic.

The temperature of HS-SPME apparatus was adsorpted at 90°C, SPME fibre was manually inserted into the sample vial headspace for 5 min. After extraction, the fibre was retracted to remove from the sample vial and immediately inserted into the GC injection port for desorption at 250ºC for 4 min in splitless mode. For headspace extraction of the volatile oils from *Selaginella* plants, aliquots of 1g were placed into a 4 ml glass vial and subjected to the same procedure to determine the volatile metabolites.

To evaluate the effect on HS-SPME of the volatile oils from the *Selaginella* plants, SPME fibres (CAR/PDMS, PDMS/DVB, PDMS, DVB/CAR/PDMS), extraction temperatures (50, 60, 70, 80, 90 and 100ºC), extraction time (5, 10, 15, 20, and 25 min) were investigated as single factor variables.

GC-MS analysis of the SPME-collected volatile metabolites were performed on an Agilent Technologies 6890N GC equippednetwork gas chromatograph with a HP-5MS capillary column (30m×0.25mm×0.25 μm) and connected to an Agilent quadrupole mass-selective detector. Helium was used as the carrier gas at a flow rate of 1.0 ml/min (column head pressure of 12 psi). The injections were performed in the splitless mode (4 min). The GC temperature program was from 50ºC (held for 5 min) up to 150ºC at a rate of 10/min (held for 1.0 min) and further up to180ºC at a rate of 1/min (held for 1 min),10/min ramp until 250ºC and then held isothermally at 250ºC for 5min.

For the MS system, the temperatures of the transfer line, quadrupole and ionization source were 250, 180 and 230ºC, respectively; electron impact mass spectra were recorded at 70 eV and the ionization current was about 20UA. The acquisitions were performed in full scan mode (50 - 550 m/z). Reproducibility was expressed as relative standard deviation (RSD). Volatile metabolites were identified by comparison of GC retention times and mass spectra with those obtained for pure standard compounds (when available) including Wiley07Nist, NIST05 database and Kovat's retention indices values (RI) calculated according to Van Den Dool and Kratz (1963):

$$RI=100\times(A - C(n)/C(n+1) - C(n)+100n)$$

where A is the retention time of analyte, C(n) is the retention time of n-alkane with n carbon atoms eluting before A, C(n + 1) is the retention time of the next n-alkane with (n + 1) carbon atoms eluting after A, and n is the number of carbon atoms in the n-alkanes.

In order to verify the statistical significance, all analyses were carried out in triplicate and results were expressed as mean ± SD.

**Results and Discussion**

The HS-SPME experimental parameters were established and optimized by the number of volatile organic metabolites and total peak area. As previously described, four commercially available SPME fibres, namely CAR/PDMS, PDMS/DVB, PDMS, DVB/CAR/PDMS, were tested for extraction efficiency. The present results showed that 65 μm DVB/PDMS was the best fibre to extract the volatile metabolites from SR (Fig. 1a). In addition, Fig.1b illustrates that DVB/PDMS fiber had a larger affinity for monoterpenes (Mon), higher alcohol (HA) and aldehydes (Ald).

The next experimental parameter analysed was temperature. Using the best extracting fibre, DVB/PDMS, the temperatures of 50, 60, 70, 80, 90 and 100ºC were assayed (Fig. 1d). The highest total peak area was achieved at 90ºC (Fig. 1d). Finally, using the best extracting fibre, DVB/PDMS, and the optimal extraction temperature 90ºC, five extraction times, 5, 10, 15, 20 and 25 min, were assayed (Fig. 1c). In all five assays, the number of volatiles identified at 10 min was the most and therefore total peak area was the highest.

A total of 53 volatile metabolites,25 in Sr, 31 in Sbo, 32 in Sbi, and 36 in St were identified based on optimized HS-SPME experimental parameters combined with MS database and retention indices ($RI_{calc}$) calculated for HP-5MS capillary column (Table 2). The retention indices of the experimental data were in good agreement with those reported in the literature. The average relative standard deviations (RSD, 1.7%) for the retention indices ranged from 0.8 to 2.2%. Total ion chromatogram (TIC) of volatile metabolites from four *Selaginell* plants by HS-SPME-GC–MS has been shown in Fig. 2.

The metabolites identified were from different chemical classes including ketones (6), sesquiterpenes (4), monoterpenes (11), esterscompounds (2), higher alcohols (7), aldehydes (8), furan derivatives (2), alkanes (4), aliphatic hydrocarbons (7), sulphur compounds (2). It was worth to note that the amount of monoterpenes in Sbowas significantly higher than those found in the other *Selaginella* plants. However, the amount of sesquiterpenes in St was the highest.

According to the experimental results, 25 metabolites from *S. remotifolia* were identified by GC-MS and furan compounds ($5.57 \times 10^7 \pm 1.03\%$) were the major component (Table 2). Monoterpenes ($5.46 \times 10^7 \pm 8.17\%$) werethe second most abundant metabolite followed by aldehydes ($5.94\times10^6 \pm 5.93\%$),higher alcohol ($5.59 \times 10^6 \pm 7.00\%$), aliphatic hydrocarbons ($3.42 \times 10^6 \pm 2.18\%$), ketones ($1.98\times10^6 \pm 1.55\%$), sesquiterpenes ($1.69 \times 10^6 \pm 2.21\%$) and alkanes ($1.43 \times 10^6 \pm 3.35\%$). In the individual metabolites (Table 2), 2-n-butyl furan (22;$5.57 \times 10^7 \pm 6.48\%$) was found to be the main component found followed by dl-limonene (29; $2.90 \times 10^7 \pm 3.40\%$) and acetaldehyde (1; $1.18 \times 10^7 \pm 2.95\%$).

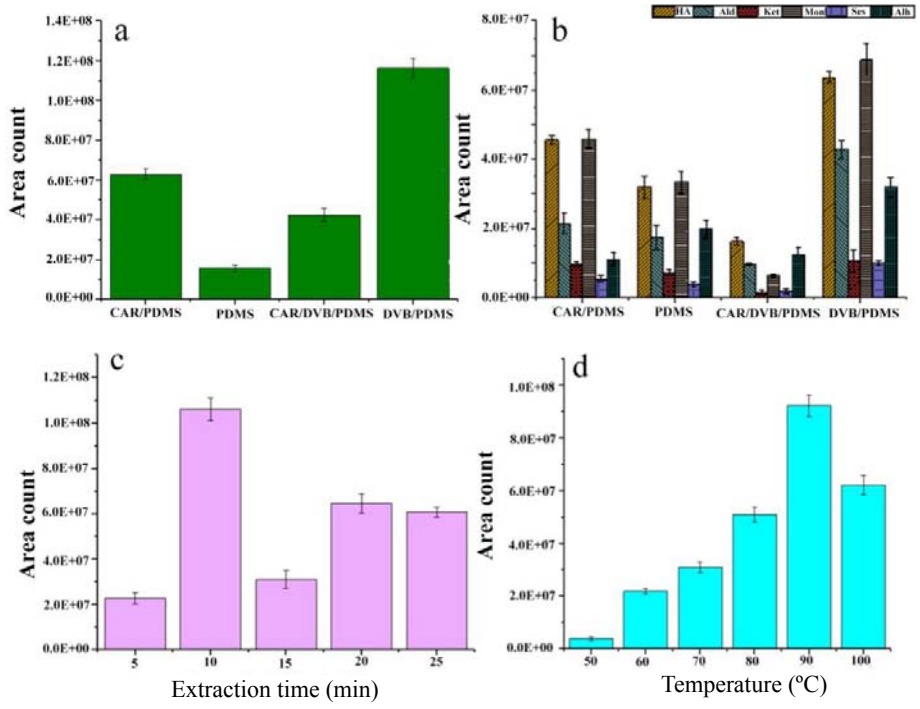


Fig. 1.Optimisation of the extraction-influencing factors which affect the efficiency of HS-SPME: (a) effect of SPME fibre coating; (b) performance of SPME coatings on the extraction efficiency of higher alcohol(HA), aldehydes (Ald), ketones (Ket), monoterpenes (Mon), sesquiterpenes (Ses),and aliphatic hydrocarbons (Alh); (c) effect of the extraction temperature; and (d) influence of the extraction time on the extraction efficiency of *Selaginella.* Error bars represent standard error of the mean (n = 4 for each data point).

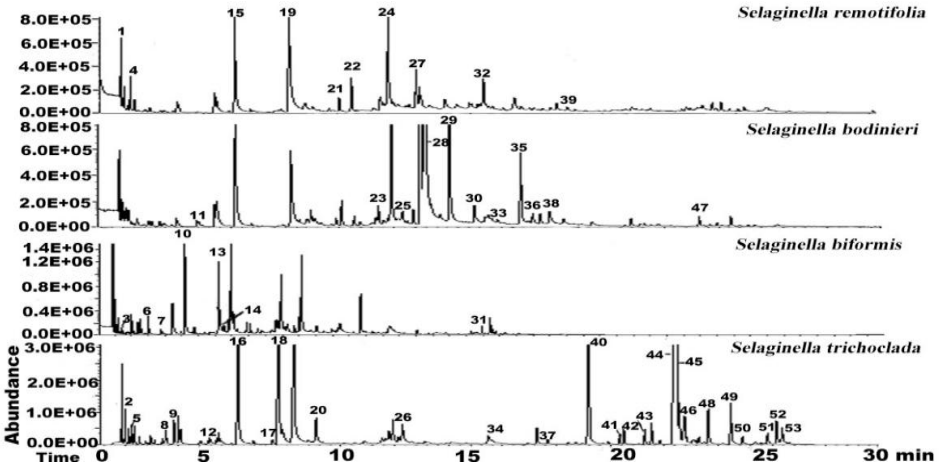


Fig. 2.Typical chromatograms (GC–MS) of volatile metabolites from *S. remotifolia, S. bodinieri, S. biformis. S. trichoclada* extract, extracted via HS-SPMEDVB/PDMS (peak assignments and identification, see Table 1).

**Table 2. Volatile metabolites identified in four species of *Selaginella* by HS-SPME DVB/PDMS /GC-MS (extraction temperature 90ºC for 10 min), with the corresponding retention indices, retention time, compound name and total peak area.**

| No. | RT | $RI^{a}_{cal}$ | $ID^{b}$ | Compound | Family | Total peak area ($\times 10^{5}\sigma$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Sr | Sbo | Sbi | St |
| 1 | 1.888 | 418 | Wiley7Nist05 | Acetaldehyde | Aldehyde | 118.0689 ± 30.22 | 132.223 ± 66.38 | 241.262 ± 78.54 | 520.397 ± 164.53 |
| 2 | 2.001 | 435 | NIST05 | 2-Propanone | Ketone | 32.266 ± 3.57 | 34.521 ± 10.98 | 85.170 ± 28.96 | 186.304 ± 64.84 |
| 3 | 2.124 | 446 | NIST05 | Ethyl alcohol | Higher alcohol | - | 27.348 ± 7.42 | 24.828 ± 6.86 | 80.093 ± 34.63 |
| 4 | 2.227 | 486 | Wiley7Nist05 | 1-Pentene | Aliphatic hydrocarbon | 44.408 ± 12.71 | 35.751 ± 14.32 | 46.270 ± 15.52 | 102.396 ± 40.98 |
| 5 | 2.529 | 526 | Wiley7Nist05 | Dimethyl sulfide | Sulfur | - | 18.934 ± 2.86 | - | 55.067 ± 19.02 |
| 6 | 2.953 | 561 | NIST05 | Butanal | Aldehyde | - | - | 59.748 ± 22.42 | 29.905 ± 7.35 |
| 7 | 3.386 | 581 | Wiley7Nist05 | 1,5-Hexadiene | Aliphatic hydrocarbon | - | - | 39.457 ± 9.43 | - |
| 8 | 3.509 | 612 | NIST05 | Essigeste | Ester | - | - | 98.402 ± 34.72 | 141.231 ± 55.82 |
| 9 | 3.82 | 622 | NIST05 | 2-Methyl-1-Propanol | Higher alcohol | - | - | | 26.588 ± 9.32 |
| 10 | 3.99 | 657 | Wiley7Nist05 | 3-Methyl-Butanal | Aldehyde | 25.576 ± 6.86 | 11.399 ± 3.73 | 58.907 ± 13.43 | 199.184 ± 89.48 |
| 11 | 5.149 | 711 | Wiley7Nist05 | 1-Heptene | Aliphatic hydrocarbon | 43.225 ± 16.36 | - | - | - |
| 12 | 5.413 | 745 | Wiley7Nist05 | Tiglaldehyde | Aldehyde | 75.289 ± 20.53 | 49.451 ± 14.84 | 116.092 ± 43.82 | |
| 13 | 5.516 | 766 | Wiley7Nist05 | Cyclopentanone | Ketone | 24.790 ± 2.34 | 119.057 ± 50.45 | 22.624 ± 5.73 | 82.074 ± 35.52 |
| 14 | 5.959 | 779 | NIST05 | Toluene | Alkane | 14.381 ± 1.31 | - | - | - |
| 15 | 6.176 | 781 | NIST05 | Cyclopentanol | Higher alcohols | 55.999 ± 10.02 | - | 1121.186 ± 403.62 | 3671.559 ± 890.97 |
| 16 | 7.703 | 866 | Wiley7Nist05 | 5-Hepten-2-one | Ketone | - | - | - | 320.7562 ± 111.84 |
| 17 | 7.844 | 871 | NIST05 | Ethylbenzene | Alkane | - | - | - | 325.547 ± 103.63 |
| 18 | 8.268 | 883 | NIST05 | 2-n-Butyl furan | Furan | 557.956 ± 103.38 | 285.863 ± 105.32 | 534.725 ± 208.46 | 2416.739 ± 639.93 |

(Contd.)

(Contd.)

| No. | RT | $RI^{a}_{cal}$ | $ID^{b}$ | Compound | Family | Total peak area ($\times 10^{5}\sigma$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Sr | Sbo | Sbi | St |
| 19 | 8.796 | 932 | Wiley7Nist05 | Alpha.-thujene | Monoterpene | 27.014 ± 2.39 | - | 119.886 ± 33.73 | - |
| 20 | 9.088 | 943 | NIST05 | 1-Ethyl-4-methyl-benzene | Alkane | - | - | 145.412 ± 39.48 | 293.037 ± 120.64 |
| 21 | 10.087 | 969 | Wiley7Nist05 | Sabinene | Monoterpene | 30.009 ± 3.98 | 58.257 ± 15.92 | 48.668 ± 14.74 | - |
| 22 | 11.359 | 993 | NIST05 | 2-Pentylfuran | Furan | - | 22.803 ± 4.39 | - | - |
| 23 | 11.661 | 1017 | Wiley7Nist05 | 2-Isobutylthiazole | Sulfur | 53.513 ± 8.94 | - | 110.193 ± 33.94 | - |
| 24 | 11.934 | 1031 | Wiley7Nist05 | Dl-limonene | Monoterpene | 290.391 ± 89.20 | 397.097 ± 96.19 | 401.912 ± 158.92 | 280.277 ± 89.53 |
| 25 | 12.339 | 1044 | NIST05 | 2,2,6-Trimethyl-cyclohexanon | Ketone | - | 64.708 ± 16.46 | 105.232 ± 43.73 | 318.202 ± 106.54 |
| 26 | 12.726 | 1052 | NIST05 | 2-Octenal, (E)- | Aldehyde | - | 41.385 ± 9.02 | 38.768 ± 15.82 | - |
| 27 | 12.999 | 1057 | Wiley7Nist05 | 3,5-Octadien-2-ol | Monoterpene | 104.523 ± 29.30 | 762.596 ± 204.87 | 81.203 ± 38.87 | - |
| 28 | 13.122 | 1064 | NIST05 | Terpinolene | Monoterpene | 56.423 ± 9.76 | 6301.175 ± 598.27 | 560.015 ± 209.63 | - |
| 29 | 13.725 | 1069 | NIST05 | Benzeneacetaldehyde | Monoterpene | 37.593 ± 3.39 | 14.978 ± 4.91 | - | - |
| 30 | 14.064 | 1076 | Wiley7Nist05 | Decahydronaphthalene | Aliphatic hydrocarbon | 50.519 ± 9.78 | 445.065 ± 140.79 | - | - |
| 31 | 14.978 | 1105 | NIST05 | 2-Methyl-decane | Aliphatic hydrocarbon | 11.612 ± 0.98 | 65.759 ± 19.03 | - | - |
| 32 | 15.468 | 1142 | Wiley7Nist05 | 3,5-Octadien-2-one | Ketone | 2.200 ± 0.03 | | 56.308 ± 4.51 | 90.637 ± 40.98 |
| 33 | 15.61 | 1143 | NIST05 | Linalool | Higher alcohol | - | - | - | - |
| 34 | 16.694 | 1147 | Wiley7Nist05 | Thujone | Monoterpene | 1.019 ± 0.01 | 213.494 ± 89.06 | 229.198 ± 99.43 | |
| 35 | 16.92 | 1155 | Wiley7Nist05 | Endo-Borneol | Monoterpene | - | 40.977 ± 16.06 | | 151.057 ± 74.53 |
| 36 | 17.419 | 1162 | Wiley7Nist05 | 1-Nonanol | Higher alcohol | - | 32.936 ± 8.93 | - | - |

(Contd.)

(Contd.)

| No. | RT | $RI^{a}_{cal}$ | $ID^{b}$ | Compound | Family | Total peak area ($\times 10^5\sigma$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Sr | Sbo | Sbi | St |
| 37 | 17.674 | 1171 | NIST05 | 2-Nonenal | Aldehyde | - | 60.681 ± 17.29 | - | - |
| 38 | 18.267 | 1201 | Wiley7Nist05 | 2-methyl-Undecane | Aliphatic hydrocarbon | 21.327 ± 1.56 | - | - | - |
| 39 | 18.437 | 1207 | Wiley7Nist05 | Decanal | Aldehyde | - | - | 161.326 ± 54.84 | |
| 40 | 19.201 | 1225 | NIST05 | 1,2,4-triethyl-Benzene, | Alkane | - | - | - | 3045.057 ± 1212.54 |
| 41 | 20.341 | 1272 | NIST05 | 1-Decanol | Higher alcohol | - | - | - | 68.493 ± 27.63 |
| 42 | 21.274 | 1301 | Wiley7Nist05 | 2,4-Decadienal | Aldehyde | - | - | - | 141.205 ± 67.53 |
| 43 | 21.528 | 1325 | NIST05 | 2-Undecanone | Ketones | - | - | - | 202.134 ± 90.87 |
| 44 | 22.433 | 1360 | NIST05 | Pentalenene | Monoterpene | - | - | - | 820.522 ± 108.87 |
| 45 | 22.518 | 1415 | Wiley7Nist05 | Alpha-Amorphene | Sesquiterpene | - | - | - | 440.81 ± 105.39 |
| 46 | 22.744 | 1370 | Wiley7Nist05 | 1-Undecanol | Higher alcohol | - | - | - | 282.341 ± 99.83 |
| 47 | 23.291 | 1381 | NIST05 | 5-Tetradecene | Aliphatic hydrocarbon | - | 24.215 ± 4.33 | | |
| 48 | 23.63 | 1396 | NIST05 | Ethyl caprate | Ester | - | - | 41.195±3.32 | 372.552 ± 115.64 |
| 49 | 24.459 | 1439 | NIST05 | Caryophyllene | Sesquiterpene | 27.507 ± 5.47 | | 97.781±46.72 | 458.006 ± 201.53 |
| 50 | 24.902 | 1445 | Wiley7Nist05 | Aromadendrene | Sesquiterpene | - | - | - | 76.412 ± 22.73 |
| 51 | 25.826 | 1488 | Wiley7Nist05 | Beta-Ionone | Monoterpene | - | - | - | 125.297 ± 64.74 |
| 52 | 26.156 | 1493 | NIST05 | Delta.-Selinene | Sesquiterpene | - | - | - | 259.908 ± 107.64 |
| 53 | 26.363 | 1513 | NIST05 | Elemol | Monoterpene | - | - | - | 175.727 ± 102.42 |

[a] ID methods: NIST05 and Wiley7Nist05, [b] Kovat's retention index relative n-alkanes ($C_8$ –$C_{20}$ ) on a HS-5MS capillary column.

A total of 31 volatile metabolites from *S. bodinieri* belonging to distinct chemical groups, mainly included monoterpenes (7.79 × $10^8$ ± 9.02%), furan compounds (3.08 × $10^7$ ± 1.96%), aliphatic hydrocarbons (1.42 × $10^7$ ± 2.04%) and ketones (7.27 × $10^6$ ± 5.17%) (Fig. 3). The distribution of different volatile metabolites has been presented in Fig 3. Considering the individual metabolites (Table 2), alpha-terpinolene (28; 6.30 × $10^8$ ± 18.48%) was the main component found followed by octadien-2-ol (27; 7.62 × $10^7$ ± 2.16%),dl-limonene(24; 3.97 × $10^7$ ± 2.74%) and acetaldehyde (1;1.32 × $10^7$ ± 5.70%).

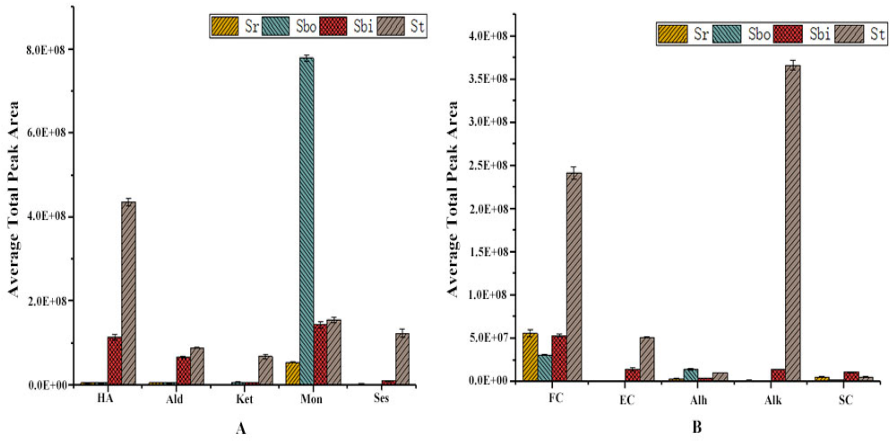


Fig. 3.Distribution of chemical classe of volatile metabolites in *Selaginellas*. Chemical class code: HA : higher alcohols; Ald: aldehyde; Ket: ketone; Mon: monoterpenes; Ses: sesquiterpenes: FC: furan compounds; EC: estercompounds; Alh: aliphatic hydrocarbons; Alk: alkanes; SC: sulphur compounds.

*S. biformis* for TCM has a good function of eliminating wind and scattering frigidity. As shown in Table 2, 32 metabolites were identified with monoterpenes (1.44 × $10^8$ ± 4.32%), higher alcohols (1.15 × $10^8$ ± 9.51%), aldehydes (6.76 × $10^7$ ± 4.97%), alkanes (1.45 × $10^7$ ± 9.02%) and esters compounds (1.39 × $10^7$ ± 2.87%). According to the experimental results, cyclopentano (15; 1.12 × $10^8$ ± 15.32%) was the major component. alpha-pinene (19; 7.86 × $10^7$ ± 3.12%) was the second most abundant metabolite followed by alpha-terpinolene (28; 5.60 × $10^7$ ± 3.97%), 2-n-butyl furan (18; 5.34 × $10^7$ ± 3.84%), dl-limonene (24; 4.01 × $10^7$ ± 3.39%), and acetaldehyde (1; 2.41 × $10^7$ ± 3.72%).

As depicted in Fig 2, there were 36 volatile metabolites from *S. trichoclada*. They mainly contain higher alcohols (4.37 × $10^8$ ± 18.52%), alkanes (3.66 × $10^8$ ± 9.27%), furan compounds (2.42 × $10^8$ ± 11.73%). Based on peak area (Table 2), cyclopentanol (15; 3.67×$10^8$ ± 17.41%)was found to be the main component followed by 1,2,4-triethyl-benzene (40; 3.04 × $10^8$ ± 16.96%), 2-n-butyl furan (18; 2.42 ×$10^8$ ±16.64%), pentalenene (44; 8.20 × $10^7$ ± 8.96%) and acetaldehyde (1; 5.20 × $10^7$ ± 3.61%).

HS-SPME firstly provided a suitable, rapid and selective method to obtain many volatile oils of four *Selaginella* species by GC-MS. According to peak area and the quantity of volatile metabolites identified, the results showed that DVB/PDMS fiber was the most suitable to separate the volatile oils using HS-SPME technique. The optimum conditions for the influential extraction parameters were 10 min and 90ºC for extraction time and extraction temperature, respectively. Besides, the volatile oils of several herbs were mainly composed of ketones (6), sesquiterpenes (4), monoterpenes (11), higher alcohols (7), aldehydes (8), furan derivatives (2), alkanes (4), aliphatic hydrocarbons (7). The major chemical groups occurring in the essential oils were monoterpenes

(38.14%), alcohols (18.95%), alkanes (12.87%) and furans (12.85%). Therefore, the data presented here showed the potential of a solvent-free and high-throughput extraction technique to establish the volatile metabolomic pattern of different *selaginella* plants. The findings of the essential oils from the related four species of *Selaginella* could be a valuable tool to distinguish the difference of their volatile chemical groups.

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**References**

Araújo WA, Lacerda CA and Cappelaro EA 2015. Modified headspace-SPME-GC devicefor sampling volatile compounds in acidic medium.J. Separ. Sci. **26**(6-7): 624-628.

García-González DL, Nicole B and Michael R 2006. A fuzzy filter to study the selectivity and sensitivity of a SPME enhanced SAW sensor system characterizing virgin olive oil aroma.Sens. Actuators B Chem. **116**(1-2): 49-54.

Jiang YM, Liang LZ, TQ Gan, Li D and Wang G 2017. Phytochemistry and Bioactivities of biflavonoids: a review. Biol. Envir. Engin. Soc.**25**(3): 105-108.

Jin Y, Khadka DB and Cho WJ 2015. Pharmacological effects of berberine and its derivatives: A patent update.Expert Opin. Therap. Patents **26**(2): 229.

Jin Z, Matsumoto Y and Tange T 2007.Structural characteristics of lignin in primitive pteridophytes: *Selaginella* species. J.Wood Sci. **53**(5): 412-418.

Li D, Qian Y, Tian YJ and Wang G 2017.Optimization of ionic liquid assisted extraction of biflavonoids from *Selaginella doederleinii* and evaluation of its antioxidant and antitumor activity. Molecules **22**(5): 586-602.

Sawaya GF, Kulasingam S and Denberg TD 2015. Cervical cancer screening in average-risk women: best practice advice from the clinical guidelines committee of the American College of Physicians.Annals Internal Med. **162**(12): 851-859.

Singh V, Kumar V and Yadav U 2017. Sensitive and selective detection of copper ions using low cost nitrogen doped carbon quantum dots as a fluorescent sensing plateform. Isss J. Micro.Smart Systems. **6**(2):1-9.

Song GH, Aisikaertulahong and Geng F 2014. Experimental study on activating blood circulation and removing blood stasis herbs to therapy on rats with chronic nonbacterial prostatitis.China J. Trad. Chin. Medi. Phar. **29**(7): 2341-2343.

Valdespino IA, Heringer G and Salino A 2015.Seven new species of Selaginellas. Stachygynandrum (Selaginellaceae) from Brazil and new synonyms for the genus. Phytokeys. **50**(50): 61-99.

Wang G, Yao S, Cheng L, Luo YF and Song H 2015.Antioxidant and anticancer effection of the volatile oil from various habitats of *Selaginella doederleinii* Hieron.Tech. Health Care 2015, 29-36.

Wang G, Yao S, Zhang XX and Song H 2015. Rapid screening and structural characterization of antioxidants from the extract of *Selaginella doederleinii* Hieron with DPPH-UPLC-Q-TOF/MS method. Int. J. Anal. Chem. 2015: 1-9.

Wang G, Song H, Yao S and Zhang ZR 2013.Analysis of volatile oil in *Selaginell doederleinii* Hieron from various habitats by GC-MS.Adv. Mater. Res. **641-642**: 862-866.

Wang G, Li SH, Zhou HL and Jiang YM 2017. Phytochemical screening, antioxidant, antibacterial and cytotoxic activities of different extracts of *Selaginella doederleinii.* Bangladesh J. Bot. **46**(3): 1193-1201.

Zheng PC, Chen X and Zhu HW 2014. Capn4 is a marker of poor clinical outcomes and promotes nasopharyngeal carcinoma metastasis via nuclear factor-κB-induced matrix metalloproteinase 2 expression. Cancer Sci. **105**(6): 630-638.

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