Process Optimization of Microwave-assisted Extraction of Volatile Oil of Scindapsus aureus and Chemical analysis by GC-MS

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Abstract. To determine optimum extraction process of volatile oil from flower of Scindapsus aureus and analyze its chemical composition. The essential oils were extracted from S. aureus by microwave-assisted extraction (MAE), distillation time, solid-liquid ratio. Microwave Power were investigated with extraction ratio of volatile oil as index, extraction technology of volatile oil from flower of S. aureus was optimized by orthogonal test. Chemical composition of the volatile oil was analyzed by gas chromatography-mass spectrometry (GC-MS). The amount of the components from essential oil were determined by normalization method. The essential oils were identified with WileyNist05 and Nist05 mass spectrum atlas. Optimal extraction process of volatile oil was as follows: solid-liquid ratio 1:14, distillation time 4h and microwave power 700W. There were 45 components that composed of about 81.94% of the total essential from Scindapsus aureus by MAE oil and they were separated and identified. The study provided scientific basis for further exploration and utilization of S. aureus.

Introduction

Scindapsus aureus, a perennial evergreen vine plant of genus S. araceae, is mild-natured with sweet flavor and mainly grows in high altitude areas over one thousand kilometers in Sichuan province, Guizhou province and Guangxi province in China. S. aureus is bland and mild, with no poisons and the functions of decreasing internal heat, promoting flow of pneuma and blood circulation [1-4]. It is found through modern pharmacological researches that S. aureus has the effects of antidiabetic, hypotensive, softening artery blood vessel and increasing coronary artery flow. Long term use of drinks containing S. aureus also helps to delay cell senescence and improve immunity [5-6]. MAE has been shown to promote the extraction content of bioactive components from a wide variety of herbs [7]. Compared with the conventional extraction techniques, MAE is a simpler and cheaper method, which is suitable for processing wider varieties of raw materials with less limitation to the polarity of solvent [8]. At the same time, only a few minutes are required in MAE, while the conventional extraction techniques usually need several hours. So far, there was no report concerning the MAE of volatile oil from S. aureus. The aim of this study was to optimize the MAE of volatile oil and analyze its constituents by GC-MS from S. aureus.

Chemicals and Methods

Materials and Chemicals

S. aureus was purchased from Fukang traditional Chinese medicine Co.,Ltd (Zunyi, Guizhou province). The crushed powder was kept in a vacuum dryer until use. Diethyl ether and anhydrous sodium sulfate used in this experiments were as of analytical grade and purchased from Changzheng Chemical Reagent Co., Ltd (Shanghai, China).

Optimization of Microwave-assisted extraction

Technological process. XH-100A microwave-assisted extraction apparatus (2450MHz, Xianghao Microwave Instrument Co., Ltd.) equipped with a programmable heating power from 0 to 800 W with 0.1% increment was used. 9 specimens of S. aureus were prepared and each of specimens weighed exactly (100.0 g) and placed in a 2000 mL round bottom flask extraction
equipped with reflux system. All of the experiments were performed under a set of microwave power (between 100 W and 1000 W) for a certain period of time (between 1h and 3h) in a set of solvent volume (between 1:10 and 1:14). When the extraction completed, the distillate is extracted three times by using diethyl ether(20mL×3). And then 30 g of anhydrous sodium sulfate was added into the extractive to remove water. The extractive was filtrated and concentrated to acquire the volatile oil with RE-5000A rotary evaporator (Yarong Scientific and Technology Company, Shanghai, China) at 50 °C. The volatile oil was further dried in a vacuum dryer to remove the residual solvent. In this study, all of the experiments were performed in triplicates, and the results reported here were the means of the three trials.

Orthogonal Experimental Design. The yield of volatile oil from S. aureus is taken as an indicator in the study and a three-factor and three-level orthogonal experiment is designed, where the three factors include extraction time, liquid to solid ratio and microwave power. The experiment is scheduled in accordance with Table L_9 (3^4). Factors and levels of orthogonal test listed in Table 1.

<table>
<thead>
<tr>
<th>A (microwave power, w)</th>
<th>B (liquid to solid ratio, g/ml)</th>
<th>C (Extract time, h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>1:10</td>
<td>1.0</td>
</tr>
<tr>
<td>500</td>
<td>1:12</td>
<td>2.5</td>
</tr>
<tr>
<td>700</td>
<td>1:14</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Extraction yield determination. S. aureus was calculated using the following formula:

\[
EY = \frac{m_e}{m_t} \times 100\%
\]  

Where \(m_e\) is the mass of volatile oil extracted in the solution (g) and \(m_t\) is the mass of S. aureus (g).

GC-MS Analysis

The essential oil of S. aureus were analyzed by GC-MS 6890N-5973 insert with HP-5MS capillary column (Agilent, USA)\[a\]. Gas Chromatography Conditions was as follows: vaporizing chamber temperature was 260°C; high-purity hydrogen (99.999%) was used as carrier gas, carrier gas flow rate was 1.0mL/min, injection volume was 1μL and split ration was 20:1. The temperature programs were 70 °C (5 min) to 145°C (10min) at a rate of 3 °C /min, then to 200 °C (10 min) at a rate of 2 °C /min, then to 230 °C (10 min) at a rate of 7 °C /min. The injector temperature was 260 °C, the injection volume was 1.0μL, split ratio was 20:1, carrier gas was He with at a rate of 1 mL / min; temperature of MS transfer line was 280 °C.

Mass Spectrum Condition was as follows: El source was used as ion source; ion source temperature was 230°C; quadrupole rod temperature was 150°C; electron energy was 70 eV; emission current was 34.6μA; multiplier voltage was 1994 kV; interface temperature was 250°C; solvent delayed 4 min; mass ranged 10~550 amu. Scan range was10~550amu. The components in the essential oils of fresh materials were identified by comparison of their mass spectra with those of Winnist05. L and Nist05. L library data.

Results and discussion

Optimization results of extraction conditions

It could be found through intuitive analysis and variance analysis in the orthogonal experiment that the influence of various factors about the extraction conditions of essential oils was extraction time (C) > Microwave power (A) > liquid to solid ratio (B). As shown in variance analysis (See Table 3), microwave power has significant influence on yield of volatile oil from S. aureus while the other two factors do not have significant influences. The optimal process combination for microwave assisted extraction of volatile oil is A_3B_3C_3, which means the microwave power was 700W, the extraction time was 4.0h and the liquid to solid ratio was 1:14.
### Table 2 Experiment scheme and results

<table>
<thead>
<tr>
<th>No.</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D (Deviation)</th>
<th>Extraction Yield/100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.51</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0.43</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>0.59</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>0.65</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0.69</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>0.80</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0.73</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0.68</td>
</tr>
<tr>
<td>K1</td>
<td>0.530</td>
<td>0.633</td>
<td>0.643</td>
<td>0.613</td>
<td></td>
</tr>
<tr>
<td>K2</td>
<td>0.643</td>
<td>0.603</td>
<td>0.567</td>
<td>0.640</td>
<td></td>
</tr>
<tr>
<td>K3</td>
<td>0.737</td>
<td>0.673</td>
<td>0.700</td>
<td>0.657</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>0.207</td>
<td>0.070</td>
<td>0.133</td>
<td>0.044</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3 Variance analysis

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Deviation Square</th>
<th>F-ratio</th>
<th>Significant F Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwave power/A</td>
<td>2</td>
<td>0.064</td>
<td>21.333</td>
<td>*</td>
</tr>
<tr>
<td>Liquid to solid ratio / B</td>
<td>2</td>
<td>0.007</td>
<td>2.333</td>
<td></td>
</tr>
<tr>
<td>Extract time/C</td>
<td>2</td>
<td>0.027</td>
<td>9.000</td>
<td></td>
</tr>
<tr>
<td>Deviation/D</td>
<td>2</td>
<td>0.003</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

### Verification Experiment

Three batches of specimens are prepared in accordance with the optimal extraction conditions of volatile oil from *S. aureus*. The result is reliable and stable, as shown in Table 4.

### Table 4 Results of content found in specimens (n=3)

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Yield of Volatile Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>131101</td>
<td>0.87</td>
</tr>
<tr>
<td>131102</td>
<td>0.85</td>
</tr>
<tr>
<td>131103</td>
<td>0.83</td>
</tr>
</tbody>
</table>

### Chemical constituents of the essential oils

Essential oils from flower of *S. aureus* Hieron with microwave-assisted extraction were analyzed by GC/MS. The identified constituents and their related percentages were shown in Table 5 and the chromatogram was shown in Fig.1. 78 components are separated and 45 compounds are identified by using Nist05 and WILLEY standard mass spectrum database combined with related documents [7]. The relative content of each chemical component was obtained on basis of the peak area normalization method (See Table 5).

The essential oil constituents are classified into hydrocarbon (40.36%), terpene (24.30%), aldehyde (8.72%), fatty acid (6.92%) and others (1.94%). 78 components are obtained from the volatile oil, accounting for 81.94% of the total amount of volatile oil. Components with relatively high content include beta-pinene (5.65%), asarone (8.66 %), n-octadecane (19. 50%), β-ionone (1.77%), lauric acid (2.29%), farnesene (1.85%) and myristic acid (1.89%), and other components have relatively low content. In conclusion, this article provides a technique to analyze its volatile oil by using GC-MS, especially for separation of each chemical constituent.
Table 5 chemical constituent comparison of Volatile Oil from S. aureus

<table>
<thead>
<tr>
<th>No.</th>
<th>compounds</th>
<th>retain time /min</th>
<th>formula</th>
<th>content /%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethylbenzene</td>
<td>25.944</td>
<td>C_{10}H_{16}</td>
<td>0.37</td>
</tr>
<tr>
<td>2</td>
<td>p-Xylene</td>
<td>26.521</td>
<td>C_{10}H_{14}O</td>
<td>0.82</td>
</tr>
<tr>
<td>3</td>
<td>1,3-dimethyl- Benzene</td>
<td>26.894</td>
<td>C_{10}H_{14}O</td>
<td>0.61</td>
</tr>
<tr>
<td>4</td>
<td>1,2,3-trimethyl- Benzene</td>
<td>27.105</td>
<td>C_{9}H_{10}O_{2}</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>4-ethyl-1,2-dimethyl- Benzene</td>
<td>29.017</td>
<td>C_{10}H_{12}O_{2}</td>
<td>2.50</td>
</tr>
<tr>
<td>6</td>
<td>1-methyl-3-(1-methylethyl)- Benzene</td>
<td>29.509</td>
<td>C_{10}H_{12}O_{2}</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>beta-Pinene</td>
<td>31.956</td>
<td>C_{11}H_{14}O_{2}</td>
<td>5.65</td>
</tr>
<tr>
<td>8</td>
<td>Thymol</td>
<td>32.501</td>
<td>C_{9}H_{10}O_{3}</td>
<td>0.21</td>
</tr>
<tr>
<td>9</td>
<td>2-methyl-5-(1-methylethyl)- Phenol</td>
<td>33.895</td>
<td>C_{9}H_{10}O_{3}</td>
<td>6.10</td>
</tr>
<tr>
<td>10</td>
<td>1-(3-methoxyphenyl)- Ethanone</td>
<td>33.971</td>
<td>C_{11}H_{14}O_{2}</td>
<td>0.70</td>
</tr>
<tr>
<td>11</td>
<td>Eugenol</td>
<td>34.256</td>
<td>C_{10}H_{16}O</td>
<td>0.20</td>
</tr>
<tr>
<td>12</td>
<td>4-ethenyl-1,2-dimethoxy- Benzene</td>
<td>34.921</td>
<td>C_{14}H_{28}</td>
<td>0.18</td>
</tr>
<tr>
<td>13</td>
<td>1,2-dimethoxy-4-(2-propenyl)- Benzene</td>
<td>35.278</td>
<td>C_{12}H_{24}O_{2}</td>
<td>0.51</td>
</tr>
<tr>
<td>14</td>
<td>4-(2,6,6-trimethyl-2-cyclohexen)- Buten</td>
<td>35.413</td>
<td>C_{13}H_{24}O</td>
<td>1.77</td>
</tr>
<tr>
<td>15</td>
<td>1-(4-methoxyphenyl)-Ethanone</td>
<td>35.840</td>
<td>C_{16}H_{20}O</td>
<td>8.72</td>
</tr>
<tr>
<td>16</td>
<td>Methyl eugenol</td>
<td>37.482</td>
<td>C_{15}H_{24}</td>
<td>0.65</td>
</tr>
<tr>
<td>17</td>
<td>Octadecanal</td>
<td>38.794</td>
<td>C_{15}H_{24}</td>
<td>0.16</td>
</tr>
<tr>
<td>18</td>
<td>(E)-2-Tetradecene</td>
<td>39.772</td>
<td>C_{15}H_{28}O</td>
<td>1.79</td>
</tr>
<tr>
<td>19</td>
<td>Dodecanoic acid</td>
<td>39.886</td>
<td>C_{12}H_{16}O_{3}</td>
<td>2.29</td>
</tr>
<tr>
<td>20</td>
<td>2,6-Di-tert-butyl-4-methylphenol</td>
<td>40.177</td>
<td>C_{13}H_{24}O</td>
<td>0.71</td>
</tr>
<tr>
<td>21</td>
<td>Hexadecanal</td>
<td>40.388</td>
<td>C_{15}H_{22}</td>
<td>0.93</td>
</tr>
<tr>
<td>22</td>
<td>alpha-Cedrene</td>
<td>40.858</td>
<td>C_{16}H_{26}O</td>
<td>1.30</td>
</tr>
<tr>
<td>23</td>
<td>Farnesene</td>
<td>41.717</td>
<td>C_{12}H_{28}O</td>
<td>1.85</td>
</tr>
<tr>
<td>24</td>
<td>Cedrol</td>
<td>41.965</td>
<td>C_{15}H_{26}O</td>
<td>0.44</td>
</tr>
<tr>
<td>25</td>
<td>Asarone</td>
<td>43.073</td>
<td>C_{12}H_{18}O_{3}</td>
<td>8.66</td>
</tr>
<tr>
<td>26</td>
<td>Agarospirol</td>
<td>43.359</td>
<td>C_{17}H_{36}</td>
<td>0.17</td>
</tr>
<tr>
<td>27</td>
<td>4-(1,2,2-trimethylcyclopentyl)- Benzene</td>
<td>44.137</td>
<td>C_{16}H_{12}O</td>
<td>2.34</td>
</tr>
<tr>
<td>28</td>
<td>tau-Muurolol</td>
<td>46.060</td>
<td>C_{10}H_{12}O_{4}</td>
<td>0.19</td>
</tr>
<tr>
<td>29</td>
<td>alpha.-Cadinol</td>
<td>47.594</td>
<td>C_{14}H_{20}O_{2}</td>
<td>1.23</td>
</tr>
<tr>
<td>No.</td>
<td>Substance</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>----------------------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Octadecanal</td>
<td>C₁₈H₃₈O</td>
<td>48.794</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>Asarone</td>
<td>C₁₉H₄₀O</td>
<td>49.409</td>
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</tr>
<tr>
<td>32</td>
<td>Heptadecane</td>
<td>C₁₉H₄₀</td>
<td>49.977</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>Hexadecanl</td>
<td>C₁₉H₄₀</td>
<td>51.419</td>
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</tr>
<tr>
<td>34</td>
<td>2,4,5-trimethoxy- Benzaldehyde</td>
<td>C₆H₁₂Cl₈O</td>
<td>51.846</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Tetradecanoic acid</td>
<td>C₁₆H₂₂O₄</td>
<td>52.488</td>
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<tr>
<td>36</td>
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<td>C₁₈H₃₂O₂</td>
<td>52.618</td>
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</tr>
<tr>
<td>37</td>
<td>6,10,14-trimethyl-2-Pentadecanone</td>
<td>C₁₈H₃₂O₂</td>
<td>54.736</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>N-Hydroxy-N′-(2-phenyl)-niotinamide</td>
<td></td>
<td>55.216</td>
<td></td>
</tr>
<tr>
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<td>55.708</td>
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</tr>
<tr>
<td>40</td>
<td>1,1'-oxybis[2,3,3,3-tetrachloro]- Propane</td>
<td>C₁₅H₂₆O₂</td>
<td>56.929</td>
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<tr>
<td>41</td>
<td>Dibutyl phthalate</td>
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<td>57.134</td>
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<td>n-Hexadecanoic acid</td>
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<td>59.398</td>
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</tr>
<tr>
<td>43</td>
<td>Hexadecanoic acid, ethyl ester</td>
<td>C₁₈H₃₂O₂</td>
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<tr>
<td>44</td>
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<td>C₂₁H₴₄</td>
<td>68.651</td>
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<tr>
<td>45</td>
<td>1,3-diphenyl-1,3-Propanedione</td>
<td>C₁₂H₂₆</td>
<td>71.131</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

*S. aureus* contains a number of biologically active chemical components and its volatile oil is mainly composed of terpenes, fatty acids and alkanes. Terpene refers to hydrocarbons in nature of which the molecular formula is several times of isoprene and their oxygen-carrying derivatives. It has many physiological activities, such as expectorant, cough relief, carminative, sweating, anthelmintics and analgesia etc [10].

This experiment adopts GC-MS to analyze and study the components of volatile oil from *S. aureus* and has scientific significance to the exploration and utilization of Scindepus aureus resources.

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References


