

## 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 *Scindepus 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 analized 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 *Scindepus 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<sup>[1-4]</sup>. 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<sup>[5-6]</sup>. MAE has been shown to promote the extraction content of bioactive components from a wide variety of herbs<sup>[7]</sup>. 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<sup>[8]</sup>. 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<sub>9</sub> (3<sup>4</sup>). Factors and levels of orthogonal test listed in Table 1.

Table 1 Factors and Levels		
A (microwave power, w)	B (liquid to solid ratio, g/ml)	C (Extract time, h)
300	1:10	1.0
500	1:12	2.5
700	1:14	4.0

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

$$EY = \frac{m_e}{m_t} \times 100\% \quad (1)$$

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)<sup>[4]</sup>. 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: EI 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 was 10~550 amu. 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<sub>3</sub>B<sub>3</sub>C<sub>3</sub>, 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

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

Table 3 Variance analysis

 $F_{0.05}(2,2)=19.00$ 

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

### 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)

Batch No.	Yield of Volatile Oil
131101	0.87
131102	0.85
131103	0.83

### 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%),  $\beta$ -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.

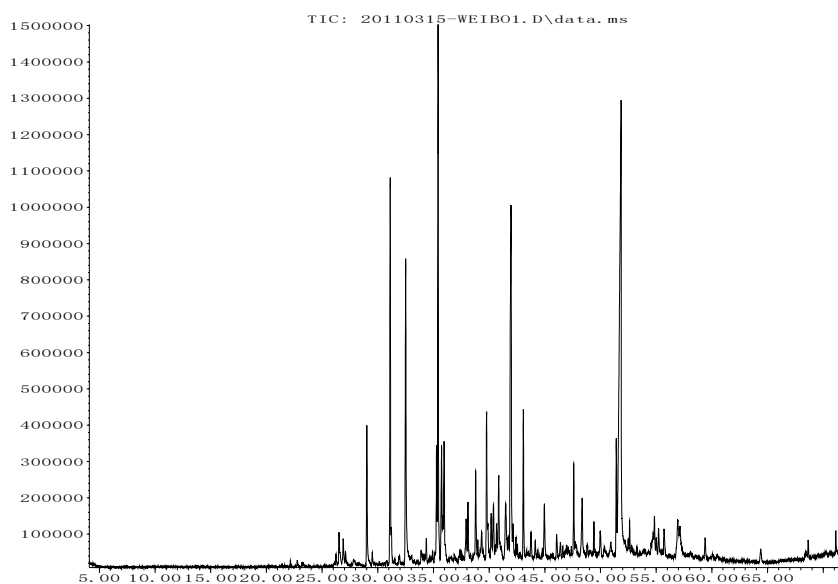


Fig. 1 Total ion current of *S. aureus*

Table 5 chemical constituent comparison of Volatile Oil from *S. aureus*

No.	compounds	retain time /min	formula	content /%
1	Ethylbenzene	25.944	C <sub>10</sub> H <sub>16</sub>	0.37
2	p-Xylene	26.521	C <sub>10</sub> H <sub>14</sub> O	0.82
3	1,3-dimethyl- Benzene	26.894	C <sub>10</sub> H <sub>14</sub> O	0.61
4	1,2,3-trimethyl- Benzene	27.105	C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>	0.30
5	4-ethyl-1,2-dimethyl- Benzene	29.017	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	2.50
6	1-methyl-3-(1-methylethyl)- Benzene	29.509	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	0.18
7	beta-Pinene	31.956	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	5.65
8	Thymol	32.501	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	0.21
9	2-methyl-5-(1-methylethyl)- Phenol	33.895	C <sub>9</sub> H <sub>10</sub> O <sub>3</sub>	6.10
10	1-(3-methoxyphenyl)- Ethanone	33.971	C <sub>11</sub> H <sub>14</sub> O <sub>2</sub>	0.70
11	Eugenol	34.256	C <sub>18</sub> H <sub>36</sub> O	0.20
12	4-ethenyl-1,2-dimethoxy- Benzene	34.921	C <sub>14</sub> H <sub>28</sub>	0.18
13	1,2-dimethoxy-4-(2-propenyl)- Benzene	35.278	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	0.51
14	4-(2,6,6-trimethyl-2-cyclohexen)- Buten	35.413	C <sub>15</sub> H <sub>24</sub> O	1.77
15	1-(4-methoxyphenyl)-Ethanone	35.840	C <sub>16</sub> H <sub>32</sub> O	8.72
16	Methyl eugenol	37.482	C <sub>15</sub> H <sub>24</sub>	0.65
17	Octadecanal	38.794	C <sub>15</sub> H <sub>24</sub>	0.16
18	(E)-2-Tetradecene	39.772	C <sub>15</sub> H <sub>26</sub> O	1.79
19	Dodecanoic acid	39.886	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>	2.29
20	2,6-Di-tert-butyl-4-methylphenol	40.177	C <sub>15</sub> H <sub>24</sub> O	0.71
21	Hexadecanal	40.388	C <sub>15</sub> H <sub>22</sub>	0.93
22	alpha-Cedrene	40.858	C <sub>15</sub> H <sub>26</sub> O	1.30
23	farnesene	41.717	C <sub>15</sub> H <sub>26</sub> O	1.85
24	Cedrol	41.965	C <sub>18</sub> H <sub>36</sub> O	0.44
25	Asarone	43.073	C <sub>12</sub> H <sub>16</sub> O <sub>3</sub>	8.66
26	Agarospirol	43.359	C <sub>17</sub> H <sub>36</sub>	0.17
27	4-(1,2,2-trimethylcyclopentyl)- Benzene	44.137	C <sub>16</sub> H <sub>32</sub> O	2.34
28	tau-Muurolol	46.060	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>	0.19
29	alpha.-Cadinol	47.594	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	1.23

30	Octadecanal	48.794	C <sub>18</sub> H <sub>38</sub>	0.50
31	Asarone	49.409	C <sub>18</sub> H <sub>36</sub> O	1.27
32	Heptadecane	49.977		0.24
33	Hexadecanal	51.419	C <sub>19</sub> H <sub>40</sub>	0.44
34	2,4,5-trimethoxy- Benzaldehyde	51.846	C <sub>6</sub> H <sub>6</sub> Cl <sub>8</sub> O	0.59
35	Tetradecanoic acid	52.488	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	1.89
36	Octadecane	52.618	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	19.50
37	6,10,14-trimethyl-2-Pentadecanone	54.736	C <sub>18</sub> H <sub>22</sub> O <sub>2</sub>	0.25
38	N-Hydroxy-N'-(2-phenyl)-niotinamidine	55.216	C <sub>20</sub> H <sub>42</sub>	0.45
39	Nonadecane	55.708	C <sub>15</sub> H <sub>12</sub> O <sub>2</sub>	0.64
40	1,1'-oxybis[2,3,3,3-tetrachloro]- Propane	56.929	C <sub>21</sub> H <sub>42</sub>	0.47
41	Dibutyl phthalate	57.134	C <sub>21</sub> H <sub>44</sub>	0.55
42	n-Hexadecanoic acid	59.398	C <sub>14</sub> H <sub>26</sub> O <sub>2</sub>	1.48
43	Hexadecanoic acid, ethyl ester	64.389	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	1.26
44	Eicosane	68.651	C <sub>20</sub> H <sub>42</sub>	0.49
45	1,3-diphenyl-1,3-Propanedione	71.131	C <sub>12</sub> H <sub>26</sub>	0.39

## 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 <sup>[10]</sup>.

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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