HPLC determination of aloperine in branches and leaves of *Sophora alopecuroides* L.

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To establish a HPLC method for the determination of aloperine in branches and leaves of *Sophora alopecuroides* L., and improve the utilization of *Sophora alopecuroides* L. branch and leaf resources have been studied. HPLC is performed using Diamonsil C₁₈ (4.6 mm × 250 mm, 5 µm) column, with acetonitrile-anhydrous ethanol-3% aqueous phosphoric acid (90:9:1) as the mobile phase, at detection wavelength of 205 nm, column temperature of 25°C, and flow rate of 1.0 mL/min. Linear range of *Sophora alopecuroides* L. is 12.00~240.00 mg·L⁻¹ (r =0.9999), and average recovery is (n =6) 100.13% (RSD 2.24%). Aloperine content in *Sophora alopecuroides* L. branches and leaves is 1.20%. The method established is simple, accurate and reproducible, which can be used for quantitative determination of aloperine in *Sophora alopecuroides* L. branches and leaves. Aloperine content is rather high in leaves, which provides the basis for comprehensive utilization of *Sophora alopecuroides* L. branch and leaf resources, particularly aloperine.

**Keywords:** *Sophora alopecuroides* L, Branches and leaves, Aloperine, HPLC, Colorectal cancer

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*Sophora alopecuroides* L. is a perennial herb belongs to genus *Sophora*, family Leguminosae. It is widely distributed in China's desert regions. The herb is bitter in taste and cold, and has heat-clearing, damp-drying, analgesic and insecticidal properties. Modern pharmacological studies have shown that it has antioxidant, anti-inflammatory, immunity enhancing and anti-tumor effects. Currently, studies have found that main chemical constituents of *Sophora alopecuroides* L. include alkaloids, flavonoids, organic acids, amino acids, proteins, polysaccharides, as well as fatty acids and inorganic elements. Among them, quinolizidine type alkaloids are its major active constituents. Such alkaloids are all nitrogen-containing organic alkali compounds, which are the focus of anticancer research in recent years with their distinct anti-cancer effects. Aloperine, a main constituent of *Sophora alopecuroides* L., also has a good effect on colorectal cancer. This paper finds by studying aloperine in *Sophora alopecuroides* L. branches and leaves that branches and leaves, which are generally neglected, contain rather high aloperine resources, thus providing the basis for further development and utilization of aloperine resources.

**Materials**

Agilent 1260 HPLC system, Mettler LE104E ten thousandth electronic analytical balance (readability 0.1mg/0.01 mg), Desen DSA600-SK2 high power single slot ultrasonic cleaner, Merck Pharo 300 UV-Vis spectrophotometer. Aloperine reference substance was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, whose purity was determined by HPLC area normalization method to be >98.0%. Methanol and acetonitrile were of HPLC grade, water was deionized water, and other reagents were of analytical grade. *Sophora alopecuroides* L. branches and leaves were collected from Zhongwei County, Ningxia in August 2014, which was identified by researcher Wang Xiangkun of Ningxia Institute of Botany as the
branches and leaves of *Sophora alopecuroides* L. in the genus *Sophora* of family Leguminosae.

**Methodology and Results**

**Chromatographic conditions**

Diamonsil C$_{18}$ column (4.6 mm×250 mm, 5µm), mobile phase: acetonitrile- methanol-5% aqueous phosphoric acid (90:9:1), detection wavelength: 205 nm, column temperature: 25°C, flow rate: 1.0 mL/min, injection volume: 10µL. Theoretical plate number is not less than 4,000 (Fig. 1).

**Preparation of reference solution**

2.4 mg of aloperine reference substance was accurately weighed, placed in a 10 mL volumetric flask, dissolved in methanol and diluted to 10mL, and shaken well to prepare the reference solution with an aloperine concentration of 0.24 mg/mL.

**Preparation of test solution**

Appropriate amounts of *Sophora alopecuroides* L. branches and leaves were crushed, and passed through a 40 mesh sieve. 0.5 gm of the resulting fine *Sophora alopecuroides* L. branch and leaf powder was accurately weighed, placed in a 50 mL volumetric flask, added with 1 mL of ammonium hydroxide, and soaked for 30 min, then added with 50 mL of methanol, weighed precisely, ultrasonicated (power 250 W, frequency 33 kHz) for 30 min, and placed at room temperature. After replenishing lost weight with methanol, the solution was shaken well, and filtered through a 0.45µm membrane to give the test solution.

**Investigation of linear relationship**

A series of reference solutions with mass concentrations of 12, 60, 120, 180 and 240 µg/mL were prepared as per the reference solution preparation method under ”Preparation of reference solution”. Each 10 µL of the above solutions were aspirated and injected into HPLC, followed by determination of peak areas according to the above chromatographic conditions. Linear regression was performed with mass concentration as abscissa (X) and peak area as ordinate (Y), and the resulting regression equation of aloperine was Y=10.0126X-0.6239 (r=0.9998). The results showed that aloperine was in a good linear relationship with peak area within a range of 12.00~24.00 µg/mL.

**Accuracy test**

Reference solution under ”Preparation of reference solution” was injected continuously six times according to the chromatographic conditions under ”Chromatographic conditions”, and the RSD of aloperine peak area was determined to be 0.69%.

**Reproducibility test**

Six aliquots of *Sophora alopecuroides* L. branch and leaf test solutions were prepared as per the test solution preparation method under ”Preparation of test solution”, and injected according to the chromatographic conditions under ”Chromatographic conditions”. The RSD of aloperine mass fraction was determined to be 2.32%.

**Stability test**

*Sophora alopecuroides* L. branch and leaf test solution prepared under ”Preparation of test solution” was precisely drawn, injected at room temperature according to the chromatographic conditions under ”Chromatographic conditions” at 0, 2, 4, 8, 16 and 24 hrs, respectively. RSD of aloperine peak area

![Fig. 1- HPLC of Sophora alopecuroides L. branch and leaf test substance and aloperine reference substance. (a) Reference substance and (b) Sophora alopecuroides L. branches and leaves 1. Aloperine](image-url)
was determined to be 1.89%; the results demonstrated that the test solution was stable within 24 hrs.

**Recovery test**

Six aliquots of 0.25 gm of fine *Sophora alopecuroides* L. branch and leaf powders were accurately weighed, placed in 50 mL volumetric flasks, added with 1 mL of ammonium hydroxide, and soaked for 30 min, then added with 12.5 mL of reference solution (equivalent to 3 mg of reference substance) under "Preparation of reference solution", respectively, diluted to 50 mL with methanol, stoppered, weighed precisely, ultrasonicated for 30 min, and placed at room temperature. After replenishing lost weight with methanol, the solutions were shaken well, and filtered through a 0.45 μm membrane to give the recovery test samples. The samples were injected and determined according to the above chromatographic conditions, and the average recovery of aloperine was calculated to be 100.13%, with a RSD of 2.46%. The results are shown in Table 1.

**Sample content determination**

Two aliquots of *Sophora alopecuroides* L. branch and leaf sample solutions were prepared as per the method under "Preparation of test solution", and injected for analysis according to the chromatographic conditions under "Chromatographic conditions" (Fig. 1), followed by calculation of aloperine content by external standard method (Table 2).

**Discussion**

Colorectal cancer (CRC) is one of the common malignancies worldwide, which ranks third in morbidity and fourth in mortality among world's malignancies. Its incidence is about 7/100000; every year there are about 5,000 new CRC cases and 3,200 deaths by CRC. At present, surgery plus postoperative chemotherapy remains an effective treatment for CRC. But its side effects lead to a serious decline in quality of life. Compared with chemotherapeutic drugs, traditional Chinese medicines and their active constituents have many advantages in terms of tumor prevention and treatment. Anti-tumor effects of traditional Chinese medicines and their active constituents has become a hot research topic around the world. The search for efficient, low-toxic drugs that can prevent CRC recurrence and metastasis is an issue urgently needs to be addressed in CRC treatment currently. This study clarifies the content of aloperine in *Sophora alopecuroides* L. branches and leaves, thereby providing a new source for future CRC therapeutic drugs. To further increase aloperine content in *Sophora alopecuroides* L., its extraction process needs further in-depth study.

### Table 1—Aloperine recovery test results (n = 6)

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample content (mg)</th>
<th>Sample addition (mg)</th>
<th>Measured amount (mg)</th>
<th>Recovery (%)</th>
<th>Average (%)</th>
<th>RSD (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3.0095</td>
<td>3.0000</td>
<td>6.1203</td>
<td>1.0369</td>
<td>100.13</td>
<td>2.46</td>
</tr>
<tr>
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<td>6.1121</td>
<td>1.0027</td>
<td>1.0013</td>
<td>1.37</td>
</tr>
<tr>
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<td>3.0000</td>
<td>5.9989</td>
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<td>100.13</td>
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<td>5.9991</td>
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<tr>
<td>6</td>
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<td>3.0000</td>
<td>6.0123</td>
<td>1.0033</td>
<td>100.13</td>
<td>2.46</td>
</tr>
</tbody>
</table>

### Table 2—Aloperine content in *Sophora alopecuroides* L. branches and leaves (n = 3)

<table>
<thead>
<tr>
<th>No.</th>
<th>Aloperine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.201</td>
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<tr>
<td>2</td>
<td>1.196</td>
</tr>
<tr>
<td>3</td>
<td>1.203</td>
</tr>
<tr>
<td>Mean</td>
<td>1.200</td>
</tr>
</tbody>
</table>
acetonitrile-phosphoric acid system not only has a relatively strong aloperine retention property, but can also effectively overcome the tailing phenomenon. Under the present experimental conditions, aloperine is baseline separated from impurities, and the baseline is stable.

As alkaloids are soluble in dilute acid water, alcohols and lipophilic organic solvents, extractions are carried out with 0.1% hydrochloric acid solution, chloroform and methanol as solvents, respectively. The results show that methanol has higher extraction efficiency and less interference from impurities. Extraction efficiency will be better if the herbal powder is soaked in an appropriate amount of alkaline water for 30 min before extraction. Extraction methods such as cold soak, ultrasonic and reflux extractions are compared, which reveals that ultrasonic extraction contributes to higher aloperine content. Ultrasonication time (20, 30 and 40 min) is also investigated, which finds that a duration of 30 min has already been able to completely extract aloperine. Therefore, extraction method is adopted to be ultrasonic extraction with methanol for 30 min (soaking of herbal powder in an appropriate amount of alkaline water for 30 min before extraction) is adopted as the extraction method. The step of herbal powder soaking in an appropriate amount of alkaline water before extraction can indeed effectively improve the extraction rate of aloperine, but whether ammonium hydroxide reacts with other constituents to generate aloperine still needs to be confirmed.

Alkaloids like matrine, oxymatrine and sophoridine extracted from *Sophora alopecuroides* L. have already been industrialized in China, and corresponding preparations have been used clinically\(^1\). But the development of aloperine, which has good pharmacological activities in terms of anti-inflammation, anti-tumor, anti-virus, etc., is still in its infancy. The results of this study demonstrate that aloperine content is rather high in branches and leaves. In the future, during aloperine development process, branches and leaves can be taken as the best harvest parts, in order to achieve efficient, scientific and rational utilization of herbal medicinal parts.

**References**