Chemical Specification of *Gynostemma pentaphyllum* (Thunb.) Makino Extract

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**ABSTRACT** An aqueous extract of *Gynostemma pentaphyllum* (Thunb.) Makino aerial parts demonstrates its potential as an herbal extract for health products. Gypenosides, Dammarane-type saponins, are major constituents with various biological activities such as antitumor, antilipemic and anti-inflammatory activities. In this study, the evaluation of *G. pentaphyllum* aqueous extract was performed in 15 samples of crude drugs purchased from different cultivated sources in the northern part of Thailand. The chemical specification for the aqueous extract of *G. pentaphyllum* was established as following: total ash content was not more than 20.0%w/w, acid-insoluble ash content was not more than 1.0%w/w, moisture content was not more than 9.0%w/w and total crude saponins content was not less than 22.0%w/w. Moreover, the chemical identification of the extract both by color test and Thin Layer Chromatographic analysis was also reported.

**key words**: *Gynostemma pentaphyllum* extract, Chemical specification, Chemical identification, Total crude saponins.

**INTRODUCTION**

*Gynostemma pentaphyllum* (Thunb.) Makino or Panchakhan is a perennial creeping herb belonging to the family Cucurbitaceae. This plant is widely distributed in the South Shaanxi and the southern area of the Yangtze River of China, Japan, India, Indo-China, and Indonesia. In Thailand this plant is found growing wild on highlands in the northern region such as Doi Chiang Dao, Chiang Mai province where it is also cultivated for commercial purposes\(^1\text{–}^3\).

The aerial parts have been used in Chinese and Japanese folk medicine for the treatment of cough, chronic bronchitis, inflammation\(^4\), hyperlipidemia\(^5,6\) and health food supplement\(^7\). Phytochemical studies of *G. pentaphyllum* have reported about 90 dammarane-type saponins\(^8\text{–}^{16}\), mainly named gypenosides (Figure 1), which structurally related to ginsenosides from the Ginseng root. Indeed, gypenosides III, IV, VIII and XII and malonylgypenosides III and...
VIII were identical to ginsenosides $R_{1}$, $R_{3}$, Rd, $F_{2}$ and malonylginsenosides $R_{1}$ and Rd, respectively (8–11). Due to structural similarity, *G. pentaphyllum* tea is suggested to promote good health and alleviate the severity of many disorders.

Pharmacological studies of *G. pentaphyllum* extract and isolated saponins have shown a variety of interesting biological activities such as antilipemic (5,6), antitumor (17, 18), anti-peptic ulcer (19, 20), anti-inflammatory (21), anti-platelet aggregation (22), anti-atherosclerosis and anti-aging activities (23). Recently, gypenosides are found to induce apoptosis in human hepatoma cell (24), inhibit nitric oxide synthesis (25), be a LXR-$\alpha$ (Liver X receptor-$\alpha$) activator (26) and PPAR-$\alpha$ (Peroxisome proliferator-activated receptor-$\alpha$) activator (27, 28). In addition, the aqueous extract and gypenoside III and VIII possessed cardiovascular protective activity (29). The chronic toxicity study of *G. pentaphyllum* aqueous extract indicated that this plant extract did not produce any significant toxic effects (30). Although these findings convinced the utilization of this plant extract in herbal health products, there was no chemical identification and quantitation of active constituent of *G. pentaphyllum* extract available in literature. The aim of this investigation is to provide scientific information on quality control of *G. pentaphyllum* extract to facilitate its appropriate chemical specification in Thailand.

**MATERIALS AND METHODS**

**Materials**

Preparation of aqueous extracts: 15 crude drug samples of *Gynostemma pentaphyllum* (Thunb.) Makino were purchased from various cultivated areas in the northern part of Thailand (Chiang Mai, Chiang Rai, Lamphun, Mae Hong Son and Phetchabun province) during September 2005 to October 2006. All samples were chemically identified and determined the quality (31). The qualified crude drugs were extracted with water and dried by means of lyophilization (Labconco freeze dryer, Germany), then kept in the well-closed containers.

Antimony trichloride was obtained from E. Merck (Germany). Reference standard, ginsenoside $R_{1}$, was purchased from ChromaDex (USA). Concentrated sulfuric acid was obtained from Farmitalia Carlo Erba (Italy). Solvents and chemicals used in this investigation were all analytical grade and water was distilled water. TLC Plate was Silica gel 60, precoated, 0.25 mm thickness, Art. 1.05721 (E. Merck, Germany).

**Equipment**

Loss on drying was obtained from Memmert hot air oven model ULE–400 (Germany). Total ash and acid–insoluble ash content were carried out on Thermolyne Type 6000 furnace (USA).
Methods

I. Chemical Identification

1. The extract (0.2 g) was heated with 10 ml of water on a water-bath for 15 min, then transferred to a separatory funnel and extracted with an equal volume of butanol. If necessary, activated charcoal (0.1 g) was added to the butanol layer, stirred and filtered. The butanol extract was proceeded as following:

1.1 Two-ml of the butanol extract was evaporated to dryness in porcelain dish, added a dropwise of saturated solution of antimony trichloride in chloroform, evaporated to dryness again and the color was noted. 

Figure 1 Some chemical constituents in aerial parts of *G. pentaphyllum* (Thunb.) Makino.

### Methods

#### I. Chemical Identification

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1.1 Two-ml of the butanol extract was evaporated to dryness in porcelain dish, added a dropwise of saturated solution of antimony trichloride in chloroform, evaporated to dryness again and the color was noted.
1.2 Two-ml of butanol extract was evaporated to dryness in porcelain dish, added a few drop of conc. sulfuric acid and the color was noted.

2. Froth test: the extract (0.2 g) was heated with 10 ml of water on a water-bath for 15 min. One-ml of this solution was transferred to a test-tube and shaked for 15 seconds, a long lasting foam was observed\(^{(23)}\).

3. Thin Layer Chromatographic analysis. Test solution: The extract (0.5 g) was dissolved in 50 ml of water, then transferred to a 100-ml volumetric flask and adjusted to volume. Ten-ml portion of the solution was transferred to a separatory funnel, 10 ml of water was added and the mixture was extracted with three 15-ml portions of butanol. The butanol extract was evaporated to dryness and dissolved in 1 ml of methanol.

   Standard solution: Ginsenoside Rb\(_1\) (1 mg) was dissolved in 1 ml of methanol.

   Adsorbent: Silica gel 60, precoated 0.25 mm thickness, E. Merck

   Developing solvent: Chloroform: methanol: water (65:35:10, lower phase)

   Developing distance: 10 cm

   Spotting amount: 5 \(\mu\)l

   Detection: Spray with excess amount of 20% sulfuric acid and activating at 105\(^{\circ}\)C for 5 min.

II. Determination of Ash

The total ash method is designed to measure the total amount of material remaining after ignition. Acid-insoluble ash is the residue obtained after boiling total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. Total ash and acid-insoluble ash content were determined as described in Thai Pharmacopoeia\(^{(24)}\).

III. Determination of Loss on Drying

Loss on drying is the gravimetric method for determination of moisture content of the herbal drugs contained non-volatile substances by heating at 100–105 °C to constant weight. Loss on drying was carried out by method described in Thai Pharmacopoeia\(^{(35)}\).

IV. Determination of Total Crude Saponins Content by Modified Method

The analytical procedure for total crude saponins content determination of the extract was slightly modified from Jirawattanapong et al.\(^{(26)}\). G. pentaphyllum extract (0.4000 g) was accurately weighed, dissolved in 50 ml of water and transferred to 100-ml volumetric flask and adjusted to volume. Ten-ml portion of this solution was transferred to a separatory funnel, added 10 ml of water and then, extracted with three 15-ml portions of butanol. The butanol extracts were combined and washed with two 10-ml portions of water. After evaporating to dryness, the residue was dried to constant weight at 105°C. The total crude saponins content was calculated on the water free basis.

RESULTS

Chemical Identification

The chemical identification of 15 samples of G. pentaphyllum extract prepared from crude drugs which purchased from heterogenous cultivated areas in the northern part of Thailand was conducted by preliminary test and Thin-layer chromatographic analysis. These results are shown in Table 1, Figure 2 and Table 2, respectively.
Table 1 Chemical identification of *G. pentaphyllum* (Thunb.) Makino aqueous extract.

<table>
<thead>
<tr>
<th>Preliminary test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated antimony trichloride/chloroform conc. Sulfuric acid</td>
<td>All samples gave violet color</td>
</tr>
<tr>
<td>Froth test</td>
<td>All samples gave red color</td>
</tr>
<tr>
<td></td>
<td>All samples produced persisting foam for over 30 min.</td>
</tr>
</tbody>
</table>

Figure 2 TLC chromatograms of the aqueous extracts of aerial parts of *G. pentaphyllum* (Thunb.) Makino.

Developing solvent: chloroform:methanol:water (65:35:10, lower phase)

Detection: visible in daylight after spraying with 20% sulfuric acid and activating at 105°C for 5 min.

Remarks: 1–15 = test solutions of *G. pentaphyllum* aqueous extract

16 = standard solution, ginsenoside Rb₁
Table 2  hRf Values of components in aqueous extract of aerial parts of *G. pentaphyllum* (Thunb.) Makino.

<table>
<thead>
<tr>
<th>Spot</th>
<th>hRf Value</th>
<th>Detection with 20% sulfuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10 - 11</td>
<td>Violet</td>
</tr>
<tr>
<td>2</td>
<td>13 - 14</td>
<td>Violet</td>
</tr>
<tr>
<td>3</td>
<td>17 - 19</td>
<td>Deep violet</td>
</tr>
<tr>
<td>4a</td>
<td>21 - 23</td>
<td>Deep violet</td>
</tr>
<tr>
<td>5</td>
<td>23 - 24</td>
<td>Magenta</td>
</tr>
<tr>
<td>6</td>
<td>26 - 28</td>
<td>Magenta</td>
</tr>
<tr>
<td>7</td>
<td>29 - 31</td>
<td>Violet</td>
</tr>
<tr>
<td>8</td>
<td>34 - 36</td>
<td>Deep violet</td>
</tr>
<tr>
<td>9</td>
<td>36 - 37</td>
<td>Violet</td>
</tr>
<tr>
<td>10</td>
<td>37 - 39</td>
<td>Yellow</td>
</tr>
<tr>
<td>11</td>
<td>39 - 41</td>
<td>Violet</td>
</tr>
<tr>
<td>12</td>
<td>42 - 46</td>
<td>Deep violet</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>hRf Value</th>
<th>Detection with 20% sulfuric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>49 - 50</td>
<td>Magenta</td>
</tr>
<tr>
<td>14</td>
<td>50 - 52</td>
<td>Yellow</td>
</tr>
<tr>
<td>15</td>
<td>56 - 57</td>
<td>Violet</td>
</tr>
<tr>
<td>16</td>
<td>58 - 59</td>
<td>Violet</td>
</tr>
<tr>
<td>17</td>
<td>61 - 62</td>
<td>Violet</td>
</tr>
<tr>
<td>18</td>
<td>64 - 65</td>
<td>Violet</td>
</tr>
<tr>
<td>19</td>
<td>69 - 71</td>
<td>Violet</td>
</tr>
<tr>
<td>20</td>
<td>77 - 72</td>
<td>Violet</td>
</tr>
<tr>
<td>21</td>
<td>81 - 84</td>
<td>Violet</td>
</tr>
<tr>
<td>22</td>
<td>91 - 92</td>
<td>Violet</td>
</tr>
<tr>
<td>23</td>
<td>95 - 96</td>
<td>Violet</td>
</tr>
<tr>
<td>24</td>
<td>98 - 99</td>
<td>Green</td>
</tr>
</tbody>
</table>

\( a = \text{ginsenoside Rb}_1 \)

Quantitative analysis

To estimate the value of *G. pentaphyllum* aqueous extracts, the quantitative analysis was performed by determination of total ash content, acid-insoluble ash content, loss on drying and total crude saponins content. The results of quantitation of *G. pentaphyllum* extract are shown in Table 3.

Table 3  Quantitative analysis of *G. pentaphyllum* (Thunb.) Makino aqueous extract.

<table>
<thead>
<tr>
<th>Determination</th>
<th>Range (% w/w) (n = 15)</th>
<th>( \bar{x} \pm \text{S.D.} ) (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash content</td>
<td>12.89 - 20.64</td>
<td>18.08 ± 1.97</td>
</tr>
<tr>
<td>Acid-insoluble ash content</td>
<td>0.01 - 0.45</td>
<td>0.16 ± 0.13</td>
</tr>
<tr>
<td>Loss on drying</td>
<td>3.50 - 13.59</td>
<td>7.81 ± 2.88</td>
</tr>
<tr>
<td>Total saponins content</td>
<td>17.96 - 32.35</td>
<td>24.88 ± 4.02</td>
</tr>
</tbody>
</table>
DISCUSSION

Traditional plant–derived medicines are being increasingly utilized for treatment and prevention of many diseases. *G. pentaphyllum* extract is also one of the potential extracts using in the herbal health products. Because there is no report of distribution of this plant in Thailand and it is found both wild and cultivated areas in the northern part, all samples of *G. pentaphyllum* in this investigation are collected from these areas. In the current study, the chemical identification of 15 samples of *G. pentaphyllum* aqueous extract was performed by preliminary test and TLC analysis. Since dammarane-type saponins were the active compounds, the preliminary test was emphasized on detection of the total saponins by color reaction with saturated solution of antimony trichloride and concentrated sulfuric acid, and by froth test. Besides, Thin Layer Chromatographic fingerprint was particularly valuable for the qualitative determination. The TLC analysis of *G. pentaphyllum* extracts was carried out on silica gel using a mixture of chloroform, methanol and water (65:35:10, lower phase) as a developing solvent and 20% sulfuric acid as a spraying reagent. The TLC chromatogram illustrated that all of them contained ginsenoside Rb1, one of the active constituents found in Ginseng, and other unidentified saponins.

Ash residue consists of an inorganic mixture of metallic salts and silica. The contamination such as sand or earth can be detected by acid–insoluble ash value. The values of total ash and acid insoluble ash content are varied from 12.89–20.64 and 0.01–0.45% w/w, respectively. The increase of these values is possibly due to the presence of the high minerals content in *G. pentaphyllum* extract\(^{37}\). As the presence of excessive water in either crude drug or its extract will promote microbes, fungi and the hydrolysis of constituents leading to deterioration of drug, it is necessary to determine the water content of these extracts. The water content of *G. pentaphyllum* extracts was obtained from loss on drying method given the values of 3.50–13.59% w/w. To assess the standard requirement of phytopharmaceutical products, the determination of active constituents must be carried out. The total crude saponins are varied from 17.96 to 32.35 % w/w. Variations in the quality of extracts are probably due to many factors such as cultivars, cultivation, harvesting time, post–harvest handling and storage of plant materials.

From the results of quantitative analysis, the appropriate chemical specification of aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino extract could be set up. When $\bar{x}$ is the arithmetic mean of the results, the maximum limits $\bar{x} + 10\%$ (if the results are not integers, they will be rounded to the next higher integers) are stated for the limited amount of loss on drying or moisture content, total ash and acid–insoluble ash content and the term “not more than” are expressed for their specifications. Besides these, the required limit of active constituents or total crude saponins content are stated for the minimum limit $\bar{x}−10\%$ and the term “not less than” is used for their specifications. Thus, the
appropriate chemical specification of aerial parts of *Gynostemma pentaphyllum* (Thunb.) Makino extract are proposed as follows: total ash content should not more than 18.0% w/w, acid-insoluble ash content should not more than 1.0% w/w, moisture content should not more than 9.0% w/w and total crude saponins content should not less than 22.0% w/w

**CONCLUSION**

The results from the chemical identification showed that *G. pentaphyllum* aqueous extract contained dammarane-type saponins. The appropriate chemical specification of *G. pentaphyllum* aqueous extract could be established as follows:

- Total ash content
  - not more than 20.0% w/w
- Acid-insoluble ash content
  - not more than 1.0% w/w
- Moisture content
  - not more than 9.0% w/w
- Total crude saponins content
  - not less than 22.0% w/w

**ACKNOWLEDGEMENT**

It is great pleasure to acknowledge Mrs. Pranee Chavalittumrong, Senior Principle Medical Scientist 10, Department of Medical Sciences, for providing some crude drug samples. Authors would also like to express our appreciation and thanks to all of our colleagues at Herbal Quality Assurance Center, Medicinal Plant Research Institute, for their contributions during this study.

**REFERENCE**

Chemical Specification of *Gynostemma pentaphyllum*  
Warunee Jirawattanapong et al.


ข้อกำหนดทางเคมีของสารสกัดปัญชัน

วารุณี จิระวัฒนพงศ์ อภ 함수น์ ทองเจ้น นารีนัน เจดี และธีรัตน์ บุญยอด สถาบันวิจัยสมุนไพร กรมวิทยาศาสตร์การแพทย์ ถนนดิลินทร์ นนทบุรี 11000

บทความวิจัยสารสกัดด้วยน้ำจากส่วนเหนือติดของปัญชัน Gynostemma pentaphyllum (Thunb.) Makino เป็นสารสกัดจากสมุนไพรชนิดหนึ่งที่มีศักยภาพในการเตรียมเป็นผลิตภัณฑ์เสริมสุขภาพจากสมุนไพรสารออกฤทธิ์ประเภท dammarane-type saponins ที่ชื่อ gypenosides มีฤทธิ์ทางเภสัชวิทยามากมาย เช่น ฤทธิ์ยับยั้งการเจริญเติบโตของเนื้องอกตลอดไปในเลือด และฤทธิ์ด้านอักเสบ จึงได้ศึกษาเพื่อประเมินคุณภาพของสารสกัดด้วยน้ำของปัญชัน โดยใช้วิธีตีบจากแห้งปูนต่าง ๆ ทำภาคเหนือของประเทศไทยจำนวน 15 ตัวอย่าง เพื่อชี้ว่าข้อกำหนดทางเคมีของสารสกัดปัญชันดังนี้ ปริมาณแอลกอฮอล์ไม่เกินร้อยละ 20.8 โดยน้ำหนัก ปริมาณแก๊สที่ไม่ละลายในกรดไม่เกินร้อยละ 1.0 โดยน้ำหนัก ปริมาณความชื้นไม่เกินร้อยละ 9.0 โดยน้ำหนัก และปริมาณสารสกัดยาของชาไปนั้นรวมไม่น้อยกว่าร้อยละ 22.0 โดยน้ำหนัก นอกจากนี้ได้พิจารณาถึงหลักฐานทางเคมี ด้วยปฏิกิริยาการเกิดสี และระดับผิวบาง (Thin layer chromatography)