การศึกษาการควบคุมคุณภาพของลำต้นชั้นในเครือ

Quality Control Study of Arcangelisia flava Stem

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บทเพิ่มเติม

งานวิจัยเป็นการศึกษาการควบคุมคุณภาพของลำต้นชั้นในเครือ จำนวน 20 ต้น นำมาถูกล้างด้วยน้ำจากสายพันธุ์
แผนที่รายการคุณภาพและแหล่งเรียนรู้จากเล่ห์ทราย 2532-2534 โดยศึกษาปรับเปลี่ยนกับตัวเรียนจากสาสนุนไพร
การปรับรูปแบบการตีตัก ขั้นตอน แบบเรียน (belter) เป็นผลต่อช่องทางสัมผัสที่มีอยู่ในบุคคลหนึ่งที่มีการชั้นในเครือ
วัสดุและเป็นที่มีอยู่ซึ่งได้ด้วยการวิเคราะห์ปัจจัยที่มีในน้ำอ่างศักดิ์สิทธิ์โดยใช้เทคนิคการเหมาะสมและที่ใน
สารประกอบโดยวิธีคั่นเล็บ (column chromatography) โดยใช้ทับทิมเล็บหรือตัวผง (gel column) ที่ทำจากพืชที่มีผลไม้
(alumina) เพื่อ 2.5 กรัม ตามนี้นั้นจะเกิดของสารระเหยที่เจริญได้ใช้สารประกอบเจริญระเบียบส้มโดยผ่านเครื่องซักผ้า
(ultracentrifuge) ซึ่งเป็นวิธีที่ดี รวดเร็วและมีความถูกต้องเมื่อเทียบกับสารประกอบเครื่องซักผ้าที่มีอยู่
สมุนไพรให้กลับ

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ABSTRACT

Quality control study of 20 samples of Khamin-krua, Arcangelisia flava (L.) Merr. stems, purchased from different traditional drugstores in Bangkok and Nonthaburi during January 1989-1991 was performed compared to the authentic samples from Chanthaburi botanical garden. The principal alkaloid of Khamin-krua, berberine, has been used as antidiarrheal and stomachic. The assay was carried out by column chromatography using only 2.5 g of 

Key words: Khamin-krua, Arcangelisia flava, Quality control, Berberine, Spectrophotometry.

Introduction

Khamin-krua is a local name for several Thai medicinal plants belonging to different botanical origins i.e. Anamira cocculus Wight & Am. (Menispermaceae)[11], Arcangelisia flava (L.) Merr. (Menispermaceae)[12-14], Combrutum acuminatum Roxb. (Combretaceae)[15] and Fibraurea tinctoria Lour. (Menispermaceae).[16-18]. Traditionally, the stem of Khamin-krua has been used as stomachic, blood tonic, aminenagogue and astringent; flowers are used as antidiagnostic; roots are used to treat orchitis, red eyes, blepharitis and to improve lymph quality[19-20]. Panwiseasv et al.[21] reported that Khamin-krua from old-styled drugstores in Bangkok, Chanthaburi botanical garden and Songkhla province were derived from different plants. Khamin-krua from old-styled drugstores in Bangkok was different from the one from Chanthaburi botanical garden, but the rhizomes of both plants contained berberine and palmatine alkaloids while the one from Songkhla province contained palmatine and jatrorrhine alkaloids with no berberine. Amatayakul and Pecharaphi[22] reported that Khamin-krua from Chanthaburi botanical garden was botanically identified as Arcangelisia flava (L.) Merr (Menispermaceae). Boonyakarn et al.[23] isolated palmatine and jatrorrhine alkaloids from the stems of kramin-krua derived from Fibraurea tinctoria Lour. (Menispermaceae).

Khamin-krua used in this study was derived from Arcangelisia flava (L.) Merr. It is a climber with bright yellow wood (Figure 1)[24], widely distributed in south eastern and southern parts of Thailand[25]. The stems contain berberine[26-28], columbamine[28], palmatine[13-15], jatrorrhine[14-19], pycnenrueine, dehydrocorydamine, thalifendine, 3-hydroxyberberine, limacine, homoaromoline[19], 6-hydroxyarcangelisine, 2-dehydroarcangelisine, stilphyto, 6-hydroxyliberline, 6-hydroxyliberauline and fulecin[19] (Figure 2).

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Figure 1: The sketches of *Arcangelisia flava* (L.) Merr.

1. The stems, leaves and fruits.
2. The female flower.
3. The ovaries.
Figure 2: Structural formula of some alkaloids in *Arcegetis flava* (L.) Merr. stems.
Berberine has been shown to be the principal alkaloid in this plant\(^{13}\). Generally, plants containing berberine have been used as antidiarrheal agent and stomachic in China, Japan, and Korea in medicinal preparations\(^{14}\).

Since Khāmin-krūa (Arcangelis flava (L.) Merr.) contains berberine, an effective antidiarrheal agent. Thus for the purpose of the promotion and mobilization of this medicinal plant application, the authors have considered making a systematic study of its quality, then setting up its standard specifications. Moreover, a satisfactory assay procedure for berberine content in the crude drug was also investigated.

Materials and Methods

Materials

2. Microsphlet extracting apparatus consisting of a 35-ml flat-bottom flask with ground joint cone NS 12/19 and a 10-ml microsphlet extractor with condenser socket NS 24/25 and flask socket NS 12/18.
3. Column: Glass column, about 9 mm in internal diameter and about 15 cm long.
4. Extraction thimbles: Cellulose, single thickness, internal diameter X external length 10 mm X 50 mm, external diameter X external length 12 mm X 50 mm from Whatman Ltd., England.
5. Double beam spectrophotometer: JASCO, Model UV/DEC-650, 1-cm cells.

6. Authentic samples of crude drugs: Two fresh samples of Khāmin-krūa stems were collected from Chanthaburi botanical garden in April 1989 and October 1991. Its botanical origin was identified as Arcangelis flava (L.) Merr. by Botanical Section, Division of Medicinal Plant Research and Development, Department of Medical Sciences, DMS Herbarium no. 875. The stems were washed thoroughly, then cut into small pieces and dried in an oven at 45-50°C for 15 hrs. The dried samples were ground to powder, then passed through sieve no. 180 and kept in well-closed containers.

7. Crude drug samples: Twenty samples of Khāmin-krūa stems were purchased from different traditional drugstores in Bangkok and Nonthaburi during January 1989-1991. Other foreign substances were removed from the samples and cut into small pieces. Its pharmacognostical characteristics were studied by comparison with the authentic samples and were identified by Pharmacognostical Section, Division of Medicinal Plant Research and Development, Department of Medical Sciences. Each crude drug sample was ground to powder, passed through sieve no. 180 and kept in well-closed containers.

8. Standard substances: Berberine chloride was purchased from Ruka AG, CH-9470 Buchs, Switzerland. Palmatine iodide was isolated and identified by Phytochemical Section, Division of Medicinal Plant Research and Development, Department of Medical Sciences.
9. Neutral aluminum oxide and silica gel 60 TLC plates, precoated 20 X 20 cm, layer thickness 0.25 mm, Art. 5721 from E. Merck, Germany.

10. Solvents and chemicals were of analytical grade.

Methods

1. Chemical identification

A. Preliminary test

Test solution: Sample 1 g was refluxed with 25 ml of methanol for 10 min and filtered.

Reagent:
1. Conc. nitric acid.
2. Ether.
3. Hydrogen peroxide TS... A 3 per cent w/v of hydrogen peroxide in water.
4. Conc. hydrochloric acid.
5. Dragendorff TS.... Bismuth subnitrate 0.85 g was dissolved in a mixture of 40 ml of water and 10 ml of glacial acetic acid. A 40 percent w/v solution of potassium iodide (50 ml) was added and mixed. This stock solution was refrigerated for prolonged storage. For use, this stock solution (10 ml) was mixed with 20 ml of glacial acetic acid and diluted with water to 100 ml.

6.3 N Sulfuric acid ...... Sulfuric acid (84 ml) was carefully added to water, and diluted to 1,000 ml with water.

7. 0.1 N Potassium permanganate...... About 3.3 g of potassium permanganate were dissolved in 1,000 ml of water in a flask, and the solution was boiled for about 15 min. The stopper was inserted in the flask, and it was allowed to stand for at least 2 days, and filtered.

Procedure:
1. Test solution 2 ml were observed under UV at 365 nm, and the color was noted.

2. Conc. nitric acid 0.5 ml was added to 2 ml of test solution, mixed well and the color of the solution was noted.

3. Test solution 2 ml were evaporated until dryness, the residue was dissolved with 2 ml of ether, then 0.5 ml of hydrogen peroxide TS was added, mixed well and conc. hydrochloric acid 1 ml were carefully added. The color of the ring in the zone of contact was noted.

4. Test solution 1 ml was evaporated until dryness, then a few drops of Dragendorff TS were added and the color of the precipitate was noted.

5. Test solution 5 ml were evaporated until dryness, the residue was dissolved with 2 ml of 3 N sulfuric acid, then a few drops of 0.1 N potassium permanganate were added and the color of potassium permanganate on warming was noted.

6. Confirmatory test (Thin-layer chromatographic analysis)

Standard condition: Normal saturation, room temperature

Test solution: Sample 0.5 g was refluxed with 20 ml of methanol for 5 min and filtered. The filtrate was evaporated under reduced pressure to 5 ml.

Standard solution: Separately dissolved 1 mg
each of berberine chloride and palmatine iodide in 1 ml of water.
Layer: TLC plate silica gel 60.
Developing solvent: Butanol-acetic acid-water 7:1:2
Developing distance: 12 cm.
Spotting amount: 2 µl each.
Spray reagent: Dragendorff TS.
2. Fluorescence under UV.
3. Visible with Dragendorff TS.

II. Determination of ash

Total ash and acid-insoluble ash contents were carried out using the methods in Thai Pharmacopoeia.

III. Determination of extractives

Water-soluble and ethanol-soluble extractives were carried out using the methods in British Pharmacopoeia. Chloroform-soluble extractive was determined as described in the United States Pharmacopoeia, using chloroform instead of hexane.

IV. Determination of loss on drying

Loss on drying was determined as described in Thai Pharmacopoeia.

V. Determination of berberine in crude drug
1. Preparation of standard solutions

About 4, 8, 12, 16 and 20 mg of berberine chloride, previously dried at 110°C for 4 hr, were accurately weighed and placed in separate 50-ml volumetric flasks, dissolved with 25 ml of methanol, then diluted to volume with hydrochloric acid-methanol 1:100 and mixed well. The concentrations of standard solutions obtained were 0.08, 0.16, 0.24, 0.32 and 0.40 mg/ml, respectively.

2. Selection of a proper wavelength

Two and a half ml of standard solution at a concentration of 0.24 mg/ml were transferred to a 25-ml volumetric flask, then diluted to volume with ethanol and mixed well. Ten ml of this solution were transferred to a 25-ml volumetric flask, diluted to volume with 0.5 M sulfuric acid and mixed well.

The ultraviolet absorption spectrum of the solution in 1-cm cells scanned through a wavelength range from 200-500 nm was recorded.

3. Preparation of aluminum oxide column

Aluminum oxide column was prepared by liting the mini column with 2.5 g of neutral aluminum oxide using wet method, then washed with about 15 ml of ethanol.

4. Preparation of the calibration curve for berberine chloride

Two and a half ml of each standard solution was accurately applied to separate pretreated aluminum oxide columns, eluted with 15 ml of ethanol in portions, the eluate was combined into 25-ml volumetric flasks, then diluted to volume with ethanol and mixed well. Ten ml of each solution was accurately transferred to separate 25-ml volumetric flasks, diluted to volume with 0.5 M
sulfuric acid and mixed well. The absorbances of standard solutions in 1-cm cells were measured at 345 nm, using 0.5 M sulfuric acid as a blank.

The calibration curve between absorbances and concentrations of the standard solutions was plotted.

5. Assay of berberine in crude drug

The sample 0.5 g was accurately weighed, placed in a microsoxllet extracting apparatus, added with 25 ml of hydrochloric acid-methanol 1:100, then refluxed to colorless and allowed to cool. The extract was transferred to a 50-ml volumetric flask and the extractor was washed with hydrochloric acid-methanol 1:100 in portions. The washings and the extract were combined and made up to volume. This solution 2.5 ml were accurately applied to a pretreated aluminum oxide column, eluted with 15 ml of ethanol in portions, the eluate was combined into a 25-ml volumetric flask, then diluted to volume with ethanol and mixed well. This solution 10.0 ml were transferred to a 25-ml volumetric flask, diluted to volume with 0.5 M sulfuric acid and mixed well. The absorbance of the sample solution in 1-cm cells was measured at 345 nm, using 0.5 M sulfuric acid as a blank. The concentration of berberine chloride from the calibration curve was read. The percentage of berberine on the water-free basis sample was calculated as berberine chloride from the following formula:

\[
\text{Berberine chloride content} = \frac{C \times 125}{w \times 1000}
\]

Where

- \( C \) - Concentration of berberine chloride in the sample solution read from the calibration curve (µg/ml).
- \( w \) - Weight of sample on the water-free basis (g).

6. Recovery of the assay procedure

Known amount of 3.7 and 10 mg of berberine chloride, previously dried at 110°C for 4 hr, were separately added to three equal portions of the authentic sample of crude drug and the berberine contents were determined as described in the above assay procedure.

7. Sample analysis

The berberine content, calculated as berberine chloride, in 20 crude drug samples were determined as described in the assay procedure.

Results

I. Chemical identification

The chemical identification of 20 samples of Khamin-Kruea purchased from different traditional drugstores in Bangkok and Nonthaburi was performed compared to the authentic sample of the stems of \textit{Arcangelisia flava} (L.) Mer. collected from Chanthaburi botanical garden by preliminary test and thin-layer chromatographic analysis and the results are shown in Table 1 and Figure 3.
Table 1: Chemical identification of *Arcangelisia flava* (L.) Merr. stems.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Preliminary test</th>
<th>Confirmatory test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV&lt;sub&gt;254&lt;/sub&gt;</td>
<td>conc. HNO&lt;sub&gt;3&lt;/sub&gt;</td>
</tr>
<tr>
<td>Authentic</td>
<td>Both samples</td>
<td>Both samples</td>
</tr>
<tr>
<td></td>
<td>gave yellow</td>
<td>gave orange</td>
</tr>
<tr>
<td></td>
<td>fluorescence</td>
<td>color</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude</td>
<td>All the samples</td>
<td>All the samples</td>
</tr>
<tr>
<td>crude</td>
<td>gave yellow</td>
<td>gave orange</td>
</tr>
<tr>
<td></td>
<td>fluorescence</td>
<td>color</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: TLC chromatograms of the methanolic extract of *Arcangelisia flava* (L.) Merr. stems.
II. Assay of berberine in crude drug

Because berberine is rather unstable, it dissolves slowly in water with alkaline reaction and behaves as a quarternary base, forming salts by replacement of the hydroxy group\(^{\text{109}}\). Therefore berberine chloride was chosen as a standard substance for the assay. The result of the ultraviolet absorption spectrum of berberine chloride solution at a concentration (C) of 9.6 μg/ml in 1-cm cells (b) scanned through a wavelength range from 200-500 nm are shown in Figure 4.
1: Ultraviolet absorption spectrum of berberine chloride in a mixture of ethanol and 0.5 M sulfuric acid; b = 1 cm, C = 9.8 µg/ml.
Berberine chloride exhibited absorption maxima at the wavelength 227 nm, 283 nm, 345 nm and 424 nm. A wavelength at which the absorptivity was relatively large and the sensitivity of the assay to small changes in wavelength was 345 nm. Thus a wavelength in the vicinity of 345 nm would probably be the best for the assay of berberine, calculated as berberine chloride, in crude drug.

When the absorbances of five standard solutions of berberine chloride as a function of its concentrations were plotted, the resulting calibration curve obeyed Beer's law at a concentration range from 0 to 20 μg/ml (as shown in Figure 5).

![Absorbance vs Concentration Graph](image)

**Figure 5**: Calibration curve for berberine chloride prepared by plotting the absorbances of five standard solutions as a function of its concentrations.

The determination of berberine, calculated as berberine chloride, in authentic sample of crude drug by the recommended method gave the average recovery of 98.91% of berberine chloride from three determinations with a standard deviation of 0.69 (as shown in Table 2).
Table 2: Recovery of the assay procedure

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dry weight of sample (g)</th>
<th>Amount of standard added (g)</th>
<th>Berberine chloride content (%)</th>
<th>Amount of standard recovered (g)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4798</td>
<td>-</td>
<td>1.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0.4654</td>
<td>0.00357</td>
<td>1.98</td>
<td>0.00354</td>
<td>99.16</td>
</tr>
<tr>
<td>3</td>
<td>0.4703</td>
<td>0.00696</td>
<td>2.70</td>
<td>0.00682</td>
<td>97.97</td>
</tr>
<tr>
<td>4</td>
<td>0.4746</td>
<td>0.01015</td>
<td>3.38</td>
<td>0.01011</td>
<td>99.39</td>
</tr>
</tbody>
</table>

\[ \bar{x} = 99.91 \]

\[ SD = 0.59 \]

III. Quality evaluation of crude drug

Crude drugs were derived from heterogenous sources, it could well be that faulty collection or possible deterioration due to incorrect or extended storage might have altered the content of constituents in crude drugs. To assess the value of crude drugs, their quality evaluations were performed in four main categories; i.e. determination of ash, extractives, loss on drying, and berberine content. The results are shown in Table 3.
<table>
<thead>
<tr>
<th>Sample no</th>
<th>Ash content (%)</th>
<th>Extractive content (%)</th>
<th>Loss on drying (%)</th>
<th>Berberine chloride content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total acid-insol.</td>
<td>water ethanol chloroform</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.39 0.16</td>
<td>5.67 3.76 1.87</td>
<td>6.09</td>
<td>3.43</td>
</tr>
<tr>
<td>2</td>
<td>2.23 0.19</td>
<td>7.98 5.54 1.48</td>
<td>9.47</td>
<td>2.59</td>
</tr>
<tr>
<td>3</td>
<td>2.01 0.00</td>
<td>9.62 5.62 1.52</td>
<td>9.54</td>
<td>2.73</td>
</tr>
<tr>
<td>4</td>
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<td>10.61 5.75 1.39</td>
<td>7.29</td>
<td>2.83</td>
</tr>
<tr>
<td>5</td>
<td>2.68 0.37</td>
<td>10.27 5.30 1.53</td>
<td>8.53</td>
<td>3.83</td>
</tr>
<tr>
<td>6</td>
<td>2.50 0.19</td>
<td>6.34 3.88 1.27</td>
<td>8.06</td>
<td>2.60</td>
</tr>
<tr>
<td>7</td>
<td>2.65 0.49</td>
<td>8.62 4.90 1.02</td>
<td>7.04</td>
<td>3.02</td>
</tr>
<tr>
<td>8</td>
<td>2.74 0.40</td>
<td>8.33 4.61 1.07</td>
<td>8.29</td>
<td>2.84</td>
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<tr>
<td>9</td>
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<tr>
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<td>3.12 0.51</td>
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</tr>
<tr>
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<td>8.17</td>
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<tr>
<td>13</td>
<td>3.04 0.26</td>
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<td>14</td>
<td>2.64 0.19</td>
<td>8.11 4.86 0.92</td>
<td>7.78</td>
<td>2.54</td>
</tr>
<tr>
<td>15</td>
<td>2.71 0.28</td>
<td>7.38 5.33 0.95</td>
<td>7.69</td>
<td>2.69</td>
</tr>
<tr>
<td>16</td>
<td>2.69 0.41</td>
<td>6.05 4.39 0.95</td>
<td>8.34</td>
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</tr>
<tr>
<td>17</td>
<td>2.67 0.46</td>
<td>6.52 4.54 1.04</td>
<td>8.61</td>
<td>3.45</td>
</tr>
<tr>
<td>18</td>
<td>2.18 0.14</td>
<td>6.92 6.78 1.36</td>
<td>6.88</td>
<td>3.09</td>
</tr>
<tr>
<td>19</td>
<td>3.12 0.58</td>
<td>6.28 4.77 1.14</td>
<td>9.24</td>
<td>2.70</td>
</tr>
<tr>
<td>20</td>
<td>3.08 0.51</td>
<td>6.06 4.85 1.15</td>
<td>8.87</td>
<td>3.24</td>
</tr>
</tbody>
</table>

$\bar{x}$ 2.67 0.33 7.80 5.02 1.21 8.23 3.02
SD 0.31 0.15 1.30 0.71 0.25 0.95 0.42
Discussion

The function of quality control and drug evaluation is to assess the value of raw materials and to ensure that the final product is of the required standard. In this study, the chemical identification of various samples of Khamin-kruea from traditional drugstores was performed compared to the authentic samples by preliminary test and thin-layer chromatographic analysis. Since berberine, the major alkaloid, was present with other minor isoquinoline alkaloids in this crude drug, the preliminary test was emphasized on the detection of isoquinoline alkaloids by precipitation with Dragendorff TS and by color reaction with conc. nitric acid. Other reactions were also investigated as the characteristics of this crude drug. Thin-layer chromatographic separation was carried out on silica gel G using a mixture of butanol-acetic acid-water (7:1:2) as a mobile phase. This system gave a good spread of Rf values and had high reproducibility. The results of chemical identification showed that Khamin-kruea contained berberine and palmatine alkaloids.

Alkaloids are basic nitrogenous substances, physiologically active and usually obtained from natural resources. There are many methods for the extraction of alkaloids from crude drugs. The choice of the method will depend on the nature of the desirable alkaloids as well as other constituents present in crude drug. Ethanol is a good extracting solvent for both alkaloidal salts and free alkaloids. For industrial purpose, a commonly used solvent is acid ethanol which will yield the alkaloidal salts, and this method is low cost.

For these reasons, the authors extracted Khamin-kruea with different acid alcohol mixtures and found that the best extraction method was to extract the sample with a mixture of hydrochloric acid-methanol (1:100) in a microsoxhlet extracting apparatus. An alumina column afforded an elution of pure berberine chloride, confirmed by thin-layer chromatographic analysis and ultraviolet spectrophotometry with authentic sample of berberine chloride, other alkaloids are undetectable.

The results of the study showed that berberine chloride in a mixture of ethanol and 0.5 M sulfuric acid exhibited absorption maxima at the wavelength 227 nm, 263 nm, 345 nm and 424 nm. A wavelength in the vicinity of 345 nm would probably be the best for the assay of this crude drug. The assay of berberine, calculated as berberine chloride, in the crude drug by column chromatography followed by spectrophotometry gave a linearity at a concentration range from 0 to 20 μg/ml. This method gave percentage recovery of 97.97–99.59% for three determinations with a standard deviation of 0.69. The method was simple, rapid and sensitive for routine analysis.

Ash residue consists of an inorganic mixture of metallic salts and silica. In certain crude drugs the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates a change in quality. Unwanted parts of drugs sometimes possess a character which will raise the ash value. More direct contaminant such as sand or earth is immediately detected by ash value. The extraction of any drug with a solvent yields a solution of different compounds. The composition of this solution will
depend upon the drug and upon the solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of a particular drug sample. Because the presence of excessive water in the crude drugs will promote the growth of microbes, fungi or insects and the hydrolysis of constituents leading to deterioration of drug, it is necessary to determine the water content of this crude drug, the pharmacopoeial limits of water for vegetable drugs are usually 8-14% with few exception. Hence the appropriate specification to control the quality of this crude drug was established as shown in the following conclusion. This specification derived from the experimental results, i.e. ash content, acid-insoluble ash content, water-soluble extractive, ethanol-soluble extractive, chloroform-soluble extractive, loss on drying and berberine chloride content were 2.01-3.12%, 0.38-1.87%, 6.05-10.27%, 3.76-6.78%, 0.92-1.87%, 6.09-9.70%, and 2.21-3.83%, respectively, with the standard deviations of 0.31, 0.15, 1.30, 0.71, 0.25, 0.95, and 0.42, respectively.

Conclusion

From the results of the study, the appropriate chemical specifications of Arcangelia flava (L.) Merr. stems are proposed from the results of sample analysis. When $\bar{X}$ is the arithmetic mean of the results, the maximum limits ($\bar{X} \pm 1\sigma$) are stated for the limited amount of total ash, acid-insoluble ash, and loss on drying and the term "not more than" are expressed for their specifications. Besides these, the limits of active or major constituents and extractives are stated the minimum limit ($\bar{X} \pm 1\sigma$) and the term "not less than" is used for their specifications.

- Total ash content not more than 3%.
- Acid-insoluble ash content not more than 1%.
- Loss on drying not more than 10%.
- Water-soluble extractive not less than 7%.
- Ethanol-soluble extractive not less than 4%.
- Chloroform-soluble extractive not less than 1%.
- Berberine chloride content not less than 2.5%.

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