Reduction of Colchicine Content from Radix Gloriosae Superbae Preparata

Sasithorn Tandhavadhana, Chayan Picheansoonthon*

ABSTRACT

Introduction: Gloriosae Superbae Radix is a crude drug employed in Thai herbal remedies for several ailments. Colchicine is known as an active constituent in the roots. It was reported that 7-11 mg of colchicine may cause lethal effect in human. In Thai traditional medical practice, the roots must be treated prior use to prepare Thai herbal preparations. However, pre-treated method has not yet been well established in any literature. This study aimed to document the pre-treated method and to quantitatively compare the amount of colchicine both before and after pre-treated. Methods: Firstly, interviewing with Thai traditional medicine experts and document the pre-treated methods of Gloriosae Superbae Radix to conclude the 2 commonly used methods. Two pre-treated methods (roasting and burning) were chosen for further study. Colchicine in Gloriosae Superbae Radix from 8 sources were analyzed before and after pre-treat by High Performance Liquid Chromatography (HPLC). Results: After pre-treat by roasting and burning, amount of colchicine in root were significantly decreased by 40.61±9.55% (p=0.000) and 26.79±10.89% (p=0.001), respectively. Comparison of colchicine contents of samples after roasting and burning, the amount of colchicine decreased non statistically insignificantly (p=0.110). Conclusion: Pre-treats of Gloriosae Superbae Radix by roasting and burning had significantly reduce colchicine content. Both methods have been proven to be the effective ways in preparing certain potentially toxic crude drugs before using in compounding into Thai herbal remedies.

Key words: Gloriosae Superbae Radix, Colchicine, Pre-treat method, Thai herbal remedy, HPLC.

INTRODUCTION

Gloriosae Superbae Radix is a materia medica used in traditional medicine in South Africa, India and Southeast Asia. It is wildly used in remedies for skin diseases, snake bite, murder, leprosy, wound-healing, to mention a few.1 Gloriosae Superbae Radix has been used in several ailments, such as body’s degeneration diseases,2 particularly nasal diseases. Sixty-six percent of Thai remedies for nasal treatment placed Gloriosae Superbae Radix as the major ingredients.3 In Thai traditional medical practice, Gloriosae Superbae Radix must be pretreated prior using in compounding Thai herbal remedies. Pre-treatment methods are individual knowledge of each Thai traditional medicine healers. There is no written record on pre-treatment methods in any Thai literature. One of the active chemical constituents in Gloriosae Superbae Radix is colchicine (Figure 1). However, 7-11 mg of colchicine may cause lethal effect in human. Side effects of colchicine are nausea, vomiting, diarrhea and abdominal pain approximately 2-6 h after administration.4 The research aimed to identify pretreatment methods from Thai traditional medical experts and analyze the amount of colchicine in Gloriosae Superbae Radix before and after each pretreatment methods.

MATERIALS AND METHODS

Part I, Thai traditional medicine experts signed in an informed consent form, screening by inclusion and exclusion criteria then interview about pre-treatment methods. This study approved by the Institutional Ethics Committee of Mahasarakham University number 038/2560.

Inclusion/exclusion Criteria

The inclusion criteria were any gender of Thai traditional medicine experts who have knowledge passed through by teachers or ancestors or study by texts, still healing patients and aged over 18 years. All participants signed the consent form and understood the details of the project before study enrollment. The exclusion criteria were those who never been a sub-committee or committee in Thai Traditional Medicine Council or Department of Thai Traditional Medicine and Alternative medicine or Thai FDA, Ministry of Public Health or who never been a specialist for Thai traditional medicine license examination. Experience in Thai traditional medicine field less than 5 years.

Part II, provide Gloriosae Superbae Radix from various locations, 8 samples and verify the authenticity of all the samples by comparing the...
macroscopic characteristics of Gloriosae Superbae Radix with The Ayurvedic Pharmacopoeia of India part I volume III and approved by a board member of the Thai Herbal Pharmacopoeia. Each authentic sample divided into 3 groups. First group would be treated as a control, second and third group was pre-treated by methods which Thai traditional medicine experts recommend from part I Then colchicine extraction and quantitative analysis by High Performance Liquid Chromatography technique.

**Chemicals**
Colchicine was purchased from Sigma Aldrich co., Ltd Thailand, methanol and acetonitrile (HPLC grade) from Merck, methanol (AR grade) from Labscan.

**Plant Materials**
Total eight roots of Gloriosae Superbae Radix samples, one sample collected from wild population in Khon Kaen province and seven samples purchased from herbal shops in Bangkok and Suphanburi provinces, Thailand Clean and dried of each sample at 75°C for 2 h.

**Pretreatment Methods**

**Method 1, Roasting:** The Gloriosae Superbae Radix 300 g were ground and pass through 45 µm sieve. Roasted in clay pot Ø 10 inches until powder’s temperature was 130ºC the samples were taken out of the heat and cooled.

**Method 2, Burning:** The Gloriosae Superbae Radix 120 g were put into a clay pot Ø 10 cm and covered with 120 g of rice husks giving a 1-inch coverage. Burning the pot and rice husks continued until the temperature at the center of the pot reached 75ºC. Then the heat was stopped but the samples continued being baked in the hot pot until the temperature at the center of the pot reached 90ºC.

**Extraction of Gloriosae Superbae Radix**
Both before and after pretreatment 2 methods were extracted in the same manner. The root of Gloriosae Superbae Radix was ground and pass through 45 µm sieve. Take 20 g. (accurately weighted) of each powder sample into thimble (33 x 80 mm) for soxhlet extractor 200 ml. Extraction process was continued for six hours with methanol at 60-65ºC. The solution was evaporated to dryness to get crude extract.

**HPLC Analysis**
Each crude extract 0.250 g. (accurately weighed) dissolved in 10 ml HPLC grade methanol in volumetric flask after that 1 ml of stock solution was further diluted in another 10 ml HPLC grade methanol in volumetric flask then subjected to HPLC for qualitative and quantitative analysis of colchicine and other compounds. The HPLC system consist of Shimadzu LC-20AD which was equipped with photodiode array detector (Shimadzu SPD-M 20 A), Phenomenex Column (RP, Kromasil 5u 100 A C-18, 150x4.60 mm), Guard column, (Kromasil 5u 100 A C-18, 2.1 mm) and data were integrated by Shimadzu Class VP series software. Separation was achieved with a two pump isocratic program for pump A and B (acetonitrile: H₂O, 38:62). The flow rate was 1 ml/min, runtime 10 min and determined at wave length 350 nm. Results were obtained by comparison of peak areas of the samples with the calibration curve of referent standard. Every process was repeated 3 times.

**Validation Method Linearity**
The following concentration of colchicine 0.031, 0.063, 0.125, 0.375, 0.500 and 0.750 mg/ml were prepared and analysis by HPLC. Graph between concentration of standard colchicine and area under curve was plotted and calculated for linear regression.

**Accuracy and Precision**
The concentration of colchicine 0.031, 0.125 and 0.500 mg/ml was analysis by HPLC for only intraday precision, because running time of each sample was 10 min. Overall analysis was for less than 12 h. The percent recovery and relative standard deviation were calculated.

**Statistical Analysis**
For both parts, all categorical data were analyzed using percentage. The continuous data which were comparisons before and after pretreatment as well as those between the different pretreatment methods were analyzed using dependent t-test and independent t-test, respectively. Both of these tests were based on 2-sided t-tests where α = 0.05 was considered as statistically significant. All the statistical analyses were conducted using SPSS. version 11.5

**RESULTS**

**Part I**
**Participant Demographics**
Five Thai traditional medicine experts were enrolled. The average age and working time of the study participants were 71.4±4.2 and 41.0±15.6 years, respectively. All participants were male and got knowledge passed through ancestors. The interview results, every Thai traditional medicine experts using Thai traditional medicine license examination. Every participant has at least 1 Thai traditional medicine license. Demographics and qualification data showed property of all participants comply inclusion and exclusion criteria. (Table 1)

**Result of Interview about Pretreatment**
The interview results, every Thai traditional medicine experts using Thai traditional medicine knowledge from ancestors. The basic pre-treat
methods were roasting and burning. Hence, we decided to set the roasting and burning methods for the study. (Table 2)

**Part II**

Color of Gloriosae Superbae Radix after pre-treated by roasting and burning were darker than control sample. The moisture of pre-treated by roasting and burning groups compare with control group were significantly decreased 52.13±3.81% (\( p=0.000 \)) and 19.30±3.06% (\( p=0.000 \)), respectively. The control group extract gave dark brown oil as same as the pre-treated by roasting and burning groups extract. The average amount of extract on control group was 6.79±1.45% w/w dried root less than the extract of roasting and burning groups were 7.77±1.46% and 6.93±1.45%, respectively.

This HPLC analysis method was accepted for determination of colchicine in Gloriosae Superbae Radix which it validation method showed the coefficient of determination (\( R^2 \)) 0.993 (Figure 2) and the percent recovery between 97-107%. The HPLC chromatogram of referent standard colchicine was showed in the Figure 3. The HPLC chromatogram of Gloriosae Superbae Radix extract before and after pre-treated by roasting and burning methods were showed in the Figure 4-6. The retention time of colchicine appeared at 3.429 min. The position of colchicine in HPLC chromatogram of extract was identified by comparison of retention time. The retention time of control extract, pre-treated by roasting and burning extracts appeared at 3.430, 3.431 and 3.429 min, respectively.

The amount of colchicine in crude extract determined by comparison of the area under curve with calibration curve of colchicine. The average amount of colchicine in Gloriosae Superbae Radix extract before pre-treated was 14.065±2.942 mg/g and after pre-treated by roasting and burning were 8.371±2.214 and 10.319±2.548 mg/g, respectively. Comparison between before-after pretreatment, average amount of colchicine after pre-treated by roasting and burning were significantly decreased by 40.61±9.55% (\( p=0.000 \)) and 26.79±10.89 (\( p=0.001 \)), respectively. Comparison between roasting and burning method, amount of colchicine after pre-treated by roasting were less than burning averagely 17.61±15.83%. It doesn’t statistic significantly difference (\( p=0.110 \)). (Table 3)

**DISCUSSION**

Result of this study demonstrated that roasting and burning methods recommended by 5 Thai traditional medical experts. Both methods, roasting and burning, significantly reduced colchicine content in Gloriosae Superbae Radix: 40.61±9.55% (\( p=0.000 \)) and 26.79±10.89

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**Table 2: Results of interview about pretreatment.**

<table>
<thead>
<tr>
<th>Details</th>
<th>Participants, n (%)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment objective</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxin reduction, n (%)</td>
<td>3 (60%)</td>
<td></td>
</tr>
<tr>
<td>Toxin and property reduction, n (%)</td>
<td>2 (40%)</td>
<td></td>
</tr>
<tr>
<td>Property doesn’t change after pretreatment, yes, n (%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Basic pre-treat method was roasting, n (%)</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Basic pre-treat method was burning, n (%)</td>
<td>5 (100%)</td>
<td>3 persons of experts recommend that it’s more complicated method than roasting method</td>
</tr>
</tbody>
</table>

**Figure 1: The chemical structure of colchicine.**

**Figure 2: Calibration curve of colchicine.**

**Figure 3: HPLC chromatogram of standard colchicine.**
Table 3. Amount of colchicine in extract.

<table>
<thead>
<tr>
<th>No</th>
<th>Sources</th>
<th>Amount of colchicine in extract (mg/extract 1 g) (mean ± SD, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group 1 (control group)</td>
</tr>
<tr>
<td>1</td>
<td>Thai herbal shop 1</td>
<td>11.617±0.236</td>
</tr>
<tr>
<td></td>
<td>(Aug 27, 2014)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Thai herbal shop 1</td>
<td>16.452±0.025</td>
</tr>
<tr>
<td></td>
<td>(Apr 1, 2015)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Thai herbal shop 2</td>
<td>10.966±0.194</td>
</tr>
<tr>
<td></td>
<td>(Apr 1, 2015)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Thai herbal shop 3</td>
<td>14.829±0.268</td>
</tr>
<tr>
<td></td>
<td>(Apr 1, 2015)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Thai herbal shop 4</td>
<td>9.855±0.027</td>
</tr>
<tr>
<td></td>
<td>(Apr 1, 2015)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Thai herbal shop 5</td>
<td>14.197±0.243</td>
</tr>
<tr>
<td></td>
<td>(Apr 1, 2015)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>wild collection (Feb 24, 2015)</td>
<td>17.161±0.350</td>
</tr>
<tr>
<td>8</td>
<td>Thai herbal shop 6</td>
<td>17.442±0.245</td>
</tr>
<tr>
<td></td>
<td>(Apr 23, 2015)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ave (mean± SD)</td>
<td>14.065±2.942</td>
</tr>
<tr>
<td>% Decreasing compare with control group.</td>
<td>40.61±9.55 (p=0.000)</td>
<td>26.79±10.89 (p=0.001)</td>
</tr>
<tr>
<td>% Decreasing compare between roasting and burning group.</td>
<td>40.61±9.55</td>
<td>26.79±10.89</td>
</tr>
</tbody>
</table>
can’t detect this factor in burning method because the burning method using the temperature lower than 130°C. 4) The Percentage reduction of 6 samples in burning groups average 21.5% but there are 2 samples which reduce colchicine content 42.7% and 42.9%. Because 2 samples using more thin and smaller pieces of Gloriosae Superbae Radix than other samples. Burning method using the heat penetrate into the roots for reduce colchicine. It’s based on root’s size. However, this factor cannot be observed in roasting method because of using powder of Gloriosae Superbae Radix.

In general, the percent variation in reduction of colchicine contents by roasting method may be the combination of many factors, as from 1), 2) and 3) and burning method may be combination factor from 1), 2) and 4). Therefore, further research should be studied, particularly on the factors affected the reduction of colchicine content, such as moisture content, cooling temperature, sample’s size and method of temperature assessment.

CONCLUSION

Roasting and burning significantly reduces the colchicine content in Gloriosae Superbae Radix to the safer level for compounding into Thai traditional medical formula. Roasting method exhibited higher reduction of colchicine than burning method, but not statistically difference. Therefore, both pretreatment methods, roasting and burning, have been proven to be one of the effective ways in pretreatment of certain crude drugs prior using in compounding Thai traditional herbal remedies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

HPLC: High-Performance Liquid Chromatography.

REFERENCES


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