

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 causes 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.

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INTRODUCTION

Gloriosae Superbae Radix is a materia medica used in traditional medicine in South Africa, India and Southeast Asia. It is widely 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 herbal 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

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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 130°C. 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 75°C. 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 90°C.

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 60-65°C. 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 100A C-18, 150x4.60 mm), Guard column, (Kromasil 5u 100A 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 $\alpha = 0.05$ 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. Amount of participants have been working as committees in Department of Thai Traditional Medicine, Ministry of Public Health and Thai Traditional Medicine Council were 80% and 40%, respectively. Forty percent of participants ever been specialists for 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 heating for pretreatment Gloriosae Superbae Radix. The basic pre-treat

Table 1: Participant demographics.

Details	Participants, n (%)
Age, years (mean ± SD)	71.4±4.2
Gender, male, n (%)	5 (100%)
Status, married, n (%)	5 (100%)
Education, secondary education, n (%)	1 (20%)
• Bachelor's Degree, n (%)	2 (40%)
• Over bachelor's Degree, n (%)	2 (40%)
Thai traditional medicine knowledge from ancestors, n (%)	5 (100%)
Amount of Thai traditional medicine license	
• 1 license, n (%)	3 (60%)
• 2 license, n (%)	1 (20%)
• 3 license, n (%)	1 (20%)
Thai traditional medicine experience years (mean ± SD)	41.0±15.6
Amount of patients, 10-20 patients/month, n (%)	2 (40%)
> 100 patients/month, n (%)	3 (60%)
Ever been committee of Thai Traditional Medicine Council, n (%)	2 (40%)
Ever been a specialist for Thai traditional medicine license Examination, n (%)	2 (40%)

Table 2: Results of interview about pretreatment.

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

methods were roasting and burning. Hence, we decided to set the roasting and burning methods for the study. (Table 2)

Part II

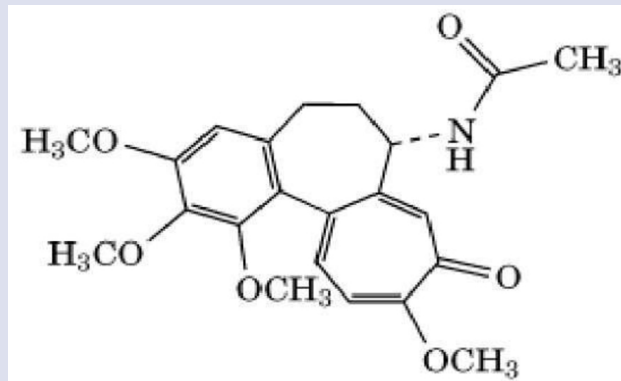
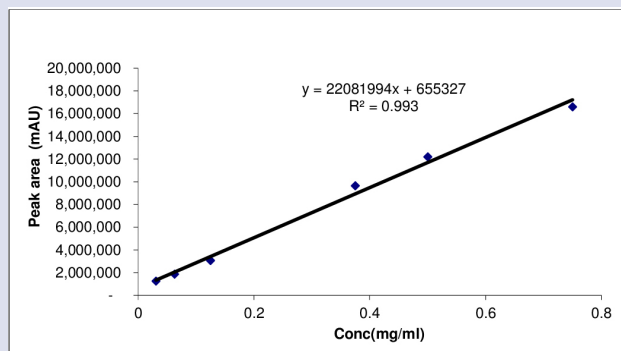
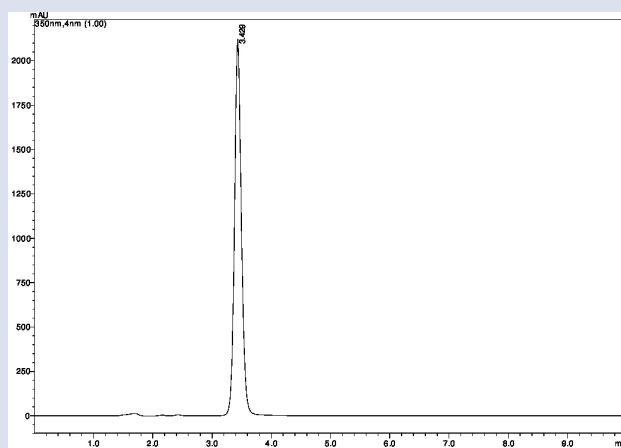
Color of *Gloriosae Superbae Radix* after pre-treated by roasting and burning were darker than control sample. The moisture of pre-treated by roasting and burning groups compare with control group were significantly decreased $52.13 \pm 3.81\%$ ($p=0.000$) and $19.30 \pm 3.06\%$ ($p=0.000$), respectively. 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 $6.79 \pm 1.45\%$ w/w dried root less than the extract of roasting and burning groups were $7.77 \pm 1.46\%$ and $6.93 \pm 1.45\%$, respectively.

This HPLC analysis method was accepted for determination of colchicine in *Gloriosae Superbae Radix* which its 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 $40.61 \pm 9.55\%$ ($p=0.000$) and $26.79 \pm 10.89\%$ ($p=0.001$), respectively. Comparison between roasting and burning method, amount of colchicine after pre-treated by roasting were less than burning averagely $17.61 \pm 15.83\%$. 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*: $40.61 \pm 9.55\%$ ($p=0.000$) and $26.79 \pm 10.89\%$

**Figure 1:** The chemical structure of colchicine.**Figure 2:** Calibration curve of colchicine.**Figure 3:** HPLC chromatogram of standard colchicine.

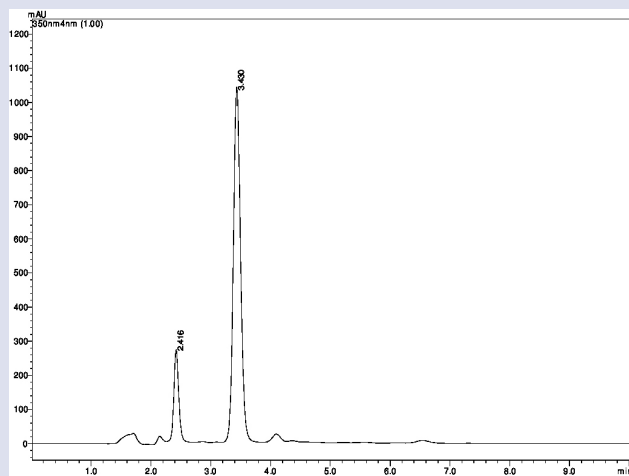


Figure 4: HPLC chromatogram of group 1 extract (control group).

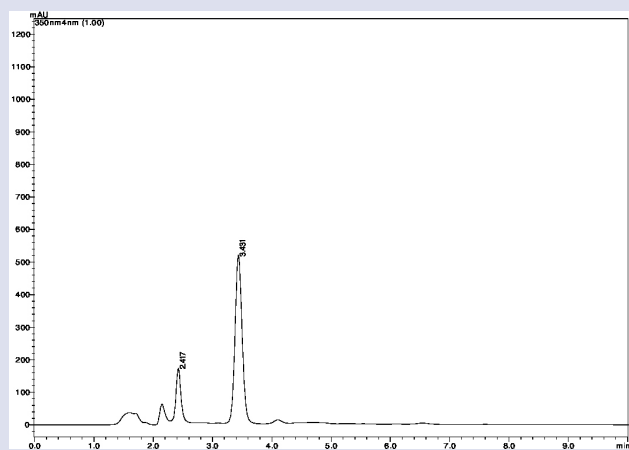


Figure 5: HPLC chromatogram of group 2 extract (pre-treated by roasting).

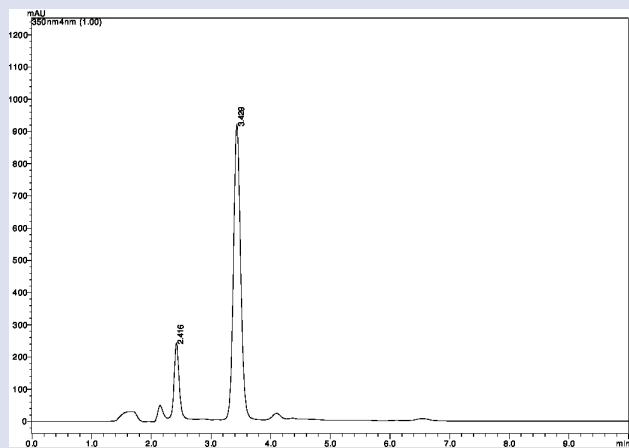


Figure 6: HPLC chromatogram of group 3 extract (pre-treated by burning).

Table 3: Amount of colchicine in extract.

No	Sources	Amount of colchicine in extract (mg/extract 1 g) (mean \pm SD, n=3)		
		Group 1 (control group)	Group 2 (pre-treated by roasting)	Group 3 (pre-treated by burning)
1	Thai herbal shop 1 (Aug 27, 2014)	11.617 \pm 0.236	7.428 \pm 0.047	9.447 \pm 0.231
2	Thai herbal shop 1 (Apr 1, 2015)	16.452 \pm 0.025	11.230 \pm 0.033	12.743 \pm 0.300
3	Thai herbal shop 2 (Apr 1, 2015)	10.966 \pm 0.194	6.050 \pm 0.013	6.281 \pm 0.020
4	Thai herbal shop 3 (Apr 1, 2015)	14.829 \pm 0.268	6.847 \pm 0.175	13.015 \pm 0.256
5	Thai herbal shop 4 (Apr 1, 2015)	9.855 \pm 0.027	6.966 \pm 0.204	7.459 \pm 0.310
6	Thai herbal shop 5 (Apr 1, 2015)	14.197 \pm 0.243	7.145 \pm 0.047	10.966 \pm 0.194
7	wild collection (Feb 24, 2015)	17.161 \pm 0.350	9.225 \pm 0.311	9.798 \pm 0.266
8	Thai herbal shop 6 (Apr 23, 2015)	17.442 \pm 0.245	11.652 \pm 0.234	12.845 \pm 0.306
Ave (mean\pm SD)		14.065\pm2.942	8.371\pm2.214	10.319\pm2.548
% Decreasing compare with control group.			40.61 \pm 9.55 (<i>p</i> =0.000)	26.79 \pm 10.89 (<i>p</i> =0.001)
% Decreasing compare between roasting and burning group.				17.61 \pm 15.83 (<i>p</i> =0.110)

(*p*=0.001), respectively. However, both pretreatment method did not demolish all colchicine content from the crude drug.

The range of colchicine content in each sample of the control group was 9.855 \pm 0.027 to 17.442 \pm 0.245 mg/1-g-extract. The results may be associated with the plant age and the plant locations and, also, the harvesting season. The colchicine contents of *Gloriosae Superbae Radix* were varied from herbal shops or sources, hence the variation of colchicine content in control group. Therefore, a certain plant age, specific place of cultivation and collecting season of the samples are factors required for further study.

After roasting and burning, the colchicine content was found to be reduced, compared with the control group, 40.61 \pm 9.55% and 26.79 \pm 10.89%, respectively. That mean variable of minimum to maximum value in each method around 20%. The result may be due to the combination of several factors, such as 1) Percent reductions of moisture content in samples compared with control group of each sample were comparably difference. We found that percent reduction of moisture content positively correlated with percent reduction of colchicine content. 2) Method of temperature assessment during pre-treat and cooling times waiting were carried on using the manual method and the open systems. The environmental temperatures affected the temperature assessment. After the pretreatment processes kept the *Gloriosae Superbae Radix* samples in tight containers at room temperature. 3) While waiting for samples to cool down after roasting, the temperature of some samples were found decreasing to the room temperature only but some samples the temperature increase 6°C before decrease to the room temperature. The subgroup which the temperature decrease to the room temperature only can reduced colchicine content around 45% to 50% but the other subgroup reduced colchicine content around 33% to 40%. However, we

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.

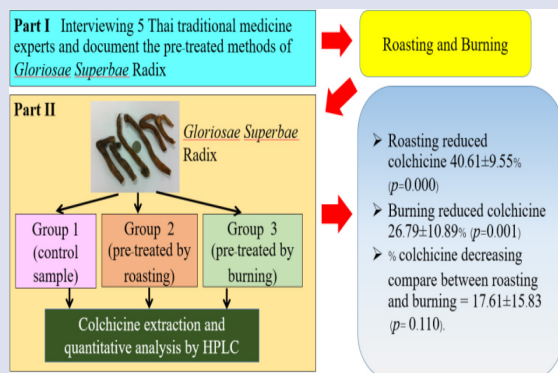
ABBREVIATIONS

HPLC: High-Performance Liquid Chromatography.

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GRAPHICAL ABSTRACT



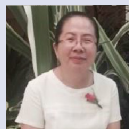
SUMMARY

- Interviewing 5 Thai traditional medicine experts and document the pre-treated methods of *Gloriosae Superbae Radix*.
- The basic pre-treat methods were roasting and burning.
- *Gloriosae Superbae Radix* samples, divided into 3 groups. First group would be a control, second and third group were pre-treated by roasting and burning methods. Colchicine extraction and quantitative analysis by HPLC.
- Roasting and burning reduced colchicine 40.61±9.55% ($p=0.000$) and 26.79±10.89% ($p=0.001$). % colchicine decreasing Compare between roasting and burning = 17.61±15.83 ($p=0.110$).

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