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Determination of Anthraquinone Glycoside Content in *Cassia fistula* Leaf Extracts for Alternative Source of Laxative Drug

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ABSTRACT

The pod of *Cassia fistula* Linn. has been used as a laxative drug in Thai traditional medicine for a long time. The pods and leaves contain anthraquinone aglycones and anthraquinone glycosides which are the active laxative form, while rhein is a major component. The degree of laxative potency is depended on the content of anthraquinone glycosides. This study determined the content of total anthraquinone glycosides in leaves of *C. fistula* collected in early summer (February-March, 2008) from 10 provinces in the North, North-East, Central, and South of Thailand. The leaves were extracted by decoction which was found to be a suitable method for extraction of anthraquinone glycosides from *C. fistula*. All extracts were analyzed using a UV-visible spectrophotometric method. The contents of total anthraquinone glycosides in the decoction leaf extracts were 0.62-2.01% dry weight (average 1.52% dry weight) while in the dried leaves were 0.09-0.63% w/w (average 0.36% w/w) calculated as rhein. The leaves collected from the Central and the North-East area, where the weather is warm in summer, contained high amounts (average 0.46 and 0.45% dry weight of total anthraquinones glycosides, respectively) while the samples from the South, where the weather is cool and raining throughout the year, contained a lower amount (average 0.13% dry weight). According to the standard of ASEAN Herbal Medicine, the central and north-eastern leaf samples of *C. fistula*, which contained about 0.5% of total anthraquinone glycosides, might be used as a source of laxatives just as the ripe pods. The decoction extract of the leaves containing an average total anthraquinone glycosides 1.52% w/w might be used as an alternative source of raw material for various laxative preparations.

Keywords: Indian laburnum, golden shower, rhein, decoction, UV-vis spectrophotometry

INTRODUCTION

Anthraquinone compounds are famous for their laxative property. The laxative effect of anthraquinones is caused by two independent mechanisms (Special Expert Committee of the German Federal Institute for Drugs and Medical Devices 1998; Van Gorkom *et al*. 1999; Shi *et al*. 2006). The first is the changing in colonic motility which leads to an accelerated large intestinal transit. Motility changes are caused indirectly by epithelial cell demand. The second one is alteration in colonic absorption and secretion, resulting in fluid accumulation which causes diarrhea (Van Gorkom *et al*. 1999). Glycosides of anthraquinones, which are hydrolyzed by β-glucosides of the intestinal flora to free anthraquinones and further reduced to anthrones, are the active form of the laxative effect (Bennett 1975; Bruneton 1995). Anthraquinone glycosides possess stronger activity than free aglycones (Thomson 1971; Moreau *et al*. 1985; De Wite *et al*. 1990). The studies of physiological disposition of sennosides A and B, sennidin A and B, rhein and anthraquinone aglycones, indicate that anthraquinone glycosides are less likely to enter the systemic circulation and, thus, are able to exert their laxative effects at lower doses than aglycones (Moreau *et al*. 1985; De Wite *et al*. 1990). Thus, the quantity of total anthraquinone glycosides in the plants indicates the strength of laxative or purgative activities.

Cassia fistula Linn. (Fabaceae) is locally called "Khun" and can be found everywhere in Thailand (The Forest Herbarium 1984). The flowering period of this plant is during the end of March to the beginning of May, while the ripe pods are found during January to April (Department of National Parks, Wildlife and Plant Conservation 2005). The ripe pods and leaves contain several anthraquinones both in aglycone and glycoside forms such as rhein, aloe-emodin,

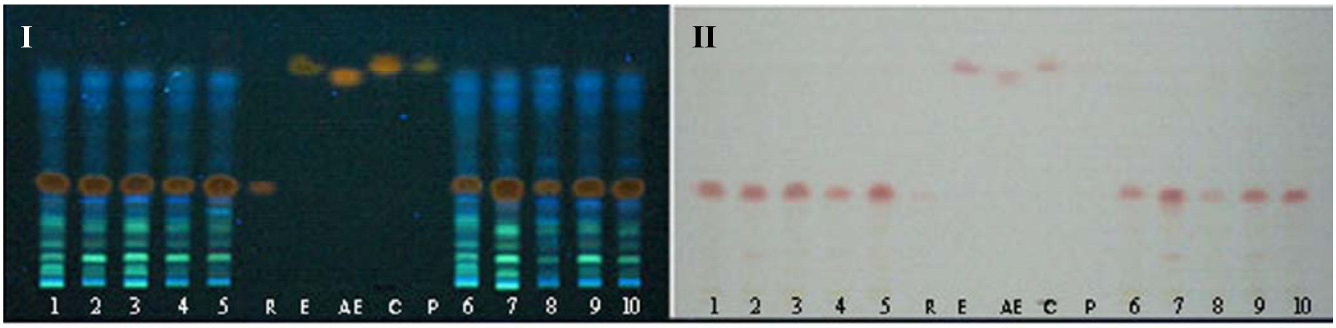

Fig. 1 TLC fingerprints of the decoction extracts of *C. fistula* leaves from 10 locations. I detected under UV 365 nm, II sprayed with 10% KOH. 1=Sukhothai, 2=Nan, 3=Udon Thani, 4=Roi Et, 5=Kalasin, 6=Phuket, 7=Nakhon Si Thammarat, 8=Bangkok, 9=Saraburi, 10=Ubon Ratchathani, R=Rhein, E=Emodin, AE=Aloe-emodin, C=Chrysophanol, P=Physcione.

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chrysophanic acid and sennosides (Chowdhury *et al*. 1996; Misra *et al*. 1996; Rani *et al*. 1998; Dutta and De 2001; Bahorun *et al*. 2005; Rizvi *et al*. 2009). In our work, the TLC fingerprints of the decoction leaf extract showed rhein as a major anthraquinone component (**Fig. 1**), as same as the ripe pod extract (Sakulpanich and Gritsanapan 2008). In Thai traditional medicine, the ripe pods have been used as a laxative drug for a long time by boiling with water and filtered through muslin cloth. The filtrate is then evaporated and the soft extract is made as small pills (Pongbunrod 1949). The laxative activity is depended on the degree of content of total anthraquinone glycosides which the Standard of ASEAN Herbal Medicine recommended should not be less than 0.5% of dried leaf raw materials. The recommended collecting time for leaf drugs used in Thai traditional medicine is the period before flowering while most of the leaf samples containing a high content of anthraquinone glycosides are samples collected in summer and winter (Gritsanapan *et al*. 2005). Thus, it is interesting to investigate the content of total anthraquinone glycosides in the leaves of *C. fistula* collected during the recommended period to indicate a good/bad source of laxative drug. In the case of a good source, the leaves of *C. fistula* could be used as an alternative source of the pods which are available only once a year. Since anthraquinones glycosides are soluble in water and decoction is the appropriate extraction method (Sakulpanich and Gritsanapan 2008). The decoction extract was prepared for this study.

MATERIALS AND METHODS

Plant materials

The mature leaves of *C. fistula* were collected from 10 different provinces in four regions of Thailand: northern, southern, central and north-eastern parts in early summer (February-March), 2008. They were identified by comparison with the herbariums (BKF. No. 118493, BKF. No. 114900) at The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (WCFL001-WCFL010) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University.

The leaves were cleaned and dried at 50°C for 6 hrs. Each leaf sample was powdered and passed through a sieve (mesh#40). The powdered samples were kept in air-tight containers protectd from light, at room temperature (28-30°C). The containers were kept in a desiccator over dried silica gel until used.

Extraction method

The powdered leaves of each sample of *C. fistula* (20.0 g) were boiled with distilled water (200 ml) for one hour at 95-98°C. The extract was filtered and the marc was re-boiled with distilled water 6-7 times until exhausted, monitored by Borntrager's reaction. The extracts of each sample were combined, filtered and the filtrate was evaporated to dryness on a boiling water bath to yield a decoction crude extract.

TLC analysis of decoction leaf extracts of *C. fistula* from 10 locations

TLC fingerprints were performed on a precoated aluminium plate of silica gel $60F_{254}$ (10 × 20 cm) using ethyl acetate: methanol: water (100: 17: 13) as the mobile phase. The developing distance was 8.0 cm. After removing the plate from the chamber, the plate was dried using an air dryer and spray with 10% alcoholic potassium hydroxide solution. Anthraquinones show pink spots. The hRf values of main components were determined by comparing with the hRf value of rhein standard.

Validation method for quantitative analysis of total anthraquinone glycosides by UV-vis spectrophotometric method

Linearity

A major component, rhein, was used as a standard for quantitative analysis of total anthraquinone glycosides. The calibration curve of rhein standard was made from 5 concentrations (1.92×10^{-6} to 9.60×10^{-6} g/ml). They were then added with 0.5% w/v of magnesium acetate and adjusted to volume with methanol. All these concentrations were measured by UV-vis spectrophotometric method at 515 nm (Lambda 35 UV/VIS spectrophotometer, Perkin Elmer, USA). A 1.0 cm quartz cell and software UV Winlab were used. The measurement was done in triplicate. The relation between concentration and absorbance was plotted. The linearity was evaluated by regression analysis and residual sum of squares and a correlation coefficient (r^2) was calculated.

Repeatability and reproducibility

The precision of the method was determined by repeatability and reproducibility. The repeatability was evaluated by assaying the samples 6 times at the same concentration, twice a day. The reproducibility was evaluated by comparing the assays on three different days. The percentage of relative standard deviation (%R.S.D.) was calculated. The values should be less than 5%.

Quantitative analysis of total anthraquinone glycosides in the decoction leaf extracts of *C. fistula* from various locations by UV-vis spectrophotometric method

The procedure for analysis of total anthraquinone glycosides was modified from the method for analysis of hydroxyanthracene derivatives of *Senna alata* (L.) Roxb. described in Standard of ASEAN Herbal Medicine. The procedure is shown in **Scheme 1**.

RESULTS

TLC fingerprints of the decoction extracts of *C. fistula* leaves collected from 10 different locations showed a similar pattern while rhein was found to be a major constituent at hRf value 36 (**Fig. 1**).

The UV-vis spectrophotometric method was validated for its linearity and precision. The relationship was obtained within the concentration range of 1.92-9.60 μg/ml of rhein with a correlation coefficient (r^2) of 0.9992. The representative linear equation was $y = 49{,}209.3254x - 0.0049$ (**Table 1**). The precision (% R.S.D. of repeatability and reproducibility) of rhein is shown in **Table 2**. The precision of the method is acceptable as revealed by %R.S.D. less than 2%.

Ten samples of *C. fistula* leaves were collected during the recommended collecting period for leaf drugs used in Thai traditional medicine. The contents of total anthraquinone glycosides, calculated as rhein, in all of the decoction extracts ranged from 0.6212 ± 0.0006 to 2.0077 ± 0.0016% w/w (average 1.5162 ± 0.4642% w/w), while in the dried leaves were 0.0877 ± 0.0006 to 0.6340 ± 0.0016% w/w (average 0.3632 ± 0.1618% w/w) (**Table 3**).

The highest average contents of total anthraquinone glycosides in the decoction leaf extract and dried leaves were found in the central samples (1.7768 ± 0.1667% w/w and 0.4627 ± 0.0586% w/w, respectively) while the lowest average contents (0.8374 ± 0.3058% w/w and 0.1304 ± 0.0604% w/w, respectively) were found in the southern samples (**Table 3**).

DISCUSSION

The important sources of anthraquinones are *Cassia*/*Senna* genera such as *Senna alata*, *Cassia angustifolia*, and *Cassia fistula*. These plants contain anthraquinones as both aglycones and glycosides and have been used as laxative drugs. Anthraquinone glycosides are in an active laxative form

Sample (0.3 g)

↓ Add 30 ml H_2O, mix, weigh and reflux for 15 min

Aqueous mixture

↓ Allow to cool, weigh, adjust to the original weight with water and centrifuge at 4000 rpm for 10 min

Residue | Supernatant

Supernatant

↓ 1. 20 ml supernatant + 2 M HCl 0.1 ml
2. Extract with chloroform 15 ml × 3 times

Aqueous layer | Chloroform layer (discard)

Aqueous layer

↓ 1. Add 0.1 g $NaHCO_3$, shake for 3 min
2. Centrifuge 4000 rpm for 20 min

Glycosides fraction

↓ 1. 10 ml supernatant + 20 ml 10.5% (w/v) $FeCl_3 \cdot 6H_2O$, reflux for 20 min
2. Add 1 ml conc. HCl, reflux for 20 min

Aglycone

↓ Extract with ether 25 ml × 3 times

Ether layer | Aqueous layer (discard)

Ether layer

↓ 1. Wash with 15 ml H_2O × 2 times
2. Adjust to 100 ml with ether

Ether layer (100 ml)

↓ Divide to 25 ml and evaporate to dryness

Residue

↓ Dissolve with 10 ml 0.5% (w/v) magnesium acetate in methanol

Solution containing anthraquinone aglycones

↓ Measure absorbance at 515 nm

% of total anthraquinone glycosides (calculated as rhein)

Scheme 1 Quantitative analysis of total anthraquinone glycosides in the decoction leaf extract.

Table 1 Data of the calibration curve of rhein reference standard in 0.5% magnesium acetate in methanol.

No.	Concentration of Rhein (g/ml)	Absorbance 1	Absorbance 2	Absorbance 3	Mean ± SD
1	1.92×10^{-6}	0.0810	0.0835	0.0846	0.0830 ± 0.00
2	3.84×10^{-6}	0.1864	0.1856	0.1872	0.1864 ± 0.00
3	5.76×10^{-6}	0.2853	0.2763	0.2744	0.2787 ± 0.01
4	7.68×10^{-6}	0.3695	0.3673	0.3654	0.3674 ± 0.00
5	9.60×10^{-6}	0.4702	0.4726	0.4741	0.4723 ± 0.00

Table 2 Method validation parameters for quantitative analysis of total anthraquinone glycosides in term of rhein by the proposed UV-spectrophotometric method.

Parameters	Results
Range of linearity	1.92-9.60 µg/ml
Regression equation[a]	y = 49,209.3254x – 0.0049
Correlation coefficient (r^2)	0.9992
% R.S.D. of repeatability	1.21
% R.S.D. of reproducibility	1.21

[a] x is the concentration of rhein in µg/ml, y is the absorbance at 515 nm

which indicates the degree of laxative potency of plant material/extract. The Standard of ASEAN Herbal Medicine recommends that the percentage of hydroxyanthracene glycosides in dried leaves of *S. alata* should not less than 0.5% w/w. In the European Pharmacopoeia, the percentage of hydroxyanthracene glycosides in *C. angustifolia* dried leaves recommended should not less than 2.5. Since the content of total anthraquinone glycosides in the dried leaves of *C. fistula* was 0.09-0.63% w/w (average 0.36% w/w), when compare the contents of hydroxyanthracene glycosides in dried leaves of *S. alata*, *C. angustifolia*, and *C. fistula*, the content of anthraquinone glycosides in *C. fistula* leaves is about 72 and 15% of the anthraquinone glycosides contents in *S. alata* and *C. angustifolia* leaves, respectively.

For the aqueous extract, the content of total anthraquinone glycosides in the decoction leaf extract of *C. fistula*

Table 3 The contents of total anthraquinone glycosides in the decoction leaf extracts and dried leaves of *C. fistula* from various locations calculated as rhein.

Part of Thailand	Provinces	Total anthraquinone glycosides calculated as rhein			
		% w/w in decoction extract*	Average	% w/w in dried leaves*	Average
North	Sukhothai (1)	1.8960 ± 0.0014	1.6206 ± 0.3894	0.2868 ± 0.0014	0.3293±0.0601
	Nan (2)	1.3453 ± 0.0024		0.3717 ± 0.0024	
Northeast	Udon Thani (3)	1.8533 ± 0.0005	**1.6731 ± 0.4035**	0.4496 ± 0.0005	**0.4469 ± 0.1490**
	Roi Et (4)	1.0906 ± 0.0029		0.2698 ± 0.0029	
	Kalasin (5)	**2.0077 ± 0.0016**		**0.6340 ± 0.0016**	
	Ubon Rajchathani (10)	1.7409 ± 0.0015		0.4342 ± 0.0015	
Central	Bangkok (8)	1.8946 ± 0.0013	**1.7768 ± 0.1667**	0.5041 ± 0.0013	**0.4627 ± 0.0586**
	Saraburi (9)	1.6589 ± 0.0018		0.4212 ± 0.0018	
South	Phuket (6)	1.0536 ± 0.0008	**0.8374 ± 0.3058**	0.1732 ± 0.0008	**0.1304 ± 0.0604**
	Nakhon Si Thammarat (7)	**0.6212 ± 0.0006**		**0.0877 ± 0.0006**	
Average		1.5162 ± 0.4642			0.3632 ± 0.1618

* expressed as mean ± SD (n = 3)

was 0.62-2.01% w/w (average 1.52% w/w) which is about 3.5-5.0 times less than the hydroxyanthracene glycosides content in senna leaf extract (5.5-8.0% w/w) (Council of Europe 2000).

The recommended dose of hydroxyanthracene glycosides in the senna leaf extract is 15-30 mg (Special expert committee of the German federal institute for drugs and medical devices 1998; European Medicines Agency 2006). Thus, the dose of *C. fistula* decoction leaf extract equivalents to the dose of senna leaf extract should be 1-2 g (0.99-1.97 g) while the dose of the dried leaves should be 4-9 g (4.17-8.33 g).

Region and weather of cultivation are important factors effecting the amount of total anthraquinone glycosides in the leaves of *C. fistula*. The Central and the North-East area of Thailand, where the weather is warm in summer and not too cool in winter, the leaves contained a higher amount (average 0.46 and 0.45% dry weight, respectively) of total anthraquinones glycosides than the leaf samples collected from the South, where the weather is cool and raining all year round. All decoction extracts showed the same pattern of TLC fingerprints, while rhein was a major component just as the pod extract (Sakulpanich and Gritsanapan 2008). Thus, the central and north-eastern leaf samples of *C. fistula*, which contained about 0.5% of total anthraquinone glycosides, might be used as an alternative source for laxative drugs. Also, the leaves of *C. fistula*, which are available throughout the year, might be used instead of the pods which are available only once a year, for laxative purposes.

CONCLUSION

The contents of anthraquinone glycosides in the leaves and decoction leaf extracts of *C. fistula* will be useful for finding a good source of alternative herbal laxative drugs and promoting standardization of plant raw material and its extract. Anthraquinone glycoside content indicates the laxative potency of *C. fistula* leaves and their extracts. The Central and North-East dried leaf samples contained about 0.5% w/w of total anthraquinone glycosides. Thus, they had ability to be developed as an alternative herbal laxative drug and used instead of *C. fistula* pods which are available once a year.

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