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, semedin 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,
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 (r²) 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 hydroxyanthracone 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 linearity was obtained within the concentration range of 1.92-9.60 μg/ml of rhein with a correlation coefficient (r²) 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 the decoction extracts ranged from 0.6212 ± 0.0006 to 2.0077 ± 0.0016% w/w (average 1.7768 ± 0.1667% w/w) in the dried leaves while in the central samples 1.7768 ± 0.1667% w/w and 0.4627 ± 0.0586% w/w, respectively were found in the southern samples (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 ± 0.0684% 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.
Anthraquinone glycosides in *Cassia fistula* leaves. Sakulpanich and Gritsanapan

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* 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*...
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 affecting 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 (Sukulpanich 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.

ACKNOWLEDGEMENT

This project was granted by The Thailand Research Fund (TRF) with Office of Small and Medium Enterprises Promotion (OSMEP).

REFERENCES


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.

<table>
<thead>
<tr>
<th>Part of Thailand</th>
<th>Provinces</th>
<th>% w/w in decoction extract*</th>
<th>Total anthraquinone glycosides calculated as rhein</th>
<th>% w/w in dried leaves*</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>Sukhothai (1)</td>
<td>1.8960 ± 0.0014</td>
<td>1.6206 ± 0.3894</td>
<td>0.2868 ± 0.0014</td>
<td>0.3293 ± 0.0601</td>
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<tr>
<td></td>
<td>Nan (2)</td>
<td>1.435 ± 0.0024</td>
<td></td>
<td>0.3717 ± 0.0024</td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>Udon Thani (3)</td>
<td>1.853 ± 0.0005</td>
<td>1.6731 ± 0.4035</td>
<td>0.4496 ± 0.0005</td>
<td>0.4649 ± 0.1490</td>
</tr>
<tr>
<td></td>
<td>Roi Et (4)</td>
<td>1.0906 ± 0.0029</td>
<td></td>
<td>0.2698 ± 0.0029</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kalasin (5)</td>
<td>2.0077 ± 0.0016</td>
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<td>0.634 ± 0.0016</td>
<td></td>
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<td></td>
<td>Ubon Rajathani (10)</td>
<td>1.7409 ± 0.0015</td>
<td></td>
<td>0.432 ± 0.0015</td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>Bangkok (8)</td>
<td>1.8946 ± 0.0013</td>
<td>1.7768 ± 0.1667</td>
<td>0.5041 ± 0.0013</td>
<td>0.4627 ± 0.0586</td>
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<td>Saraburi (9)</td>
<td>1.6589 ± 0.0018</td>
<td></td>
<td>0.4212 ± 0.0018</td>
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</tr>
<tr>
<td>South</td>
<td>Phuket (6)</td>
<td>1.0536 ± 0.0008</td>
<td>0.8374 ± 0.3058</td>
<td>0.1732 ± 0.0008</td>
<td>0.1304 ± 0.0604</td>
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<tr>
<td></td>
<td>Nakhon Si Thammarat (7)</td>
<td>0.6212 ± 0.0006</td>
<td></td>
<td>0.0877 ± 0.0006</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>1.5162 ± 0.4642</td>
<td></td>
<td>0.3632 ± 0.1618</td>
<td></td>
</tr>
</tbody>
</table>

* expressed as mean ± SD (n = 3)


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