RESEARCH NOTE

LAXATIVE ANTHRAQUINONE CONTENTS IN FRESH AND COOKED *SENNA SIAMEA* LEAVES

Aurapa Sakulpanich and Wandee Gritsanapan

Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

Abstract. This study determined the contents of total anthraquinone glycosides in *Senna siamea*, which are active laxative form, and total anthraquinones in the fresh young leaves, the boiled leaves, and the boiled filtrates by a UV-vis spectrophotometric method. Total anthraquinone glycosides and total anthraquinones, calculated as rhein, in the fresh young leaves were 0.0523 and 0.0910% w/w, respectively. The first and second boiled filtrates contained total anthraquinone glycosides 0.0334 and 0.0031% fresh weight, respectively. The first boiled leaves contained 0.0161% fresh weight and the second boiled leaves contained non-detected amount. Total anthraquinones contents in the first and second filtrates and the first and second boiled leaves were found to be 0.0721, 0.0069, 0.0167% fresh weight and non-detected amount, respectively. The results showed that the process of preparation of *khi lek* curry by boiling *S. siamea* young leaves twice with water reduced total anthraquinone glycosides content more than 75%. This confirms the traditional use of *khi lek* curry as a very mild laxative drug.

INTRODUCTION

Senna siamea (Lam.) Irwin and Barneby is a well-known medicinal plant in Thailand and other Asian countries. It is used in a popular curry called *khi lek*. It has a long history of use as a traditional medicine, and its therapeutic efficacy is well recognized. The leaves have been used as a mild laxative, somnolent, antipyretic, and anti-hypertensive. It is recommended in primary health care as an antipyretic in Indonesia (Singhabutra, 1992). The aqueous extract of fresh or dried leaves of *S. siamea* has been recommended for treatment of insomnia (Thongsaard *et al*, 1996; Pooviboonsuk *et al*, 2000). The fresh young flowers buds and/or young leaves have been used as vegetables in Thailand. They are popularly prepared as a *khi lek* curry by boiling with water at a ratio of 1:3, for 1 hour, 2-3 times to reduce the bitterness. The water is then discarded and the boiled leaves are mixed with coconut milk and curry paste and cooked as a curry that is consumed as a food promoting very mild laxative activity and sleeping-aid. The laxative effect comes from anthraquinone glycosides while the somnolent effect comes from barakol, a major chemical constituent (Chaichantipyuth, 1979; Gritsanapan, 1983). The preparation of *khi lek* curry by boiling *S. siamea* leaves twice with water can reduce the barakol content by

Correspondence: Wandee Gritsanapan, Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri Ayutthaya Road, Ratchathewi, Bangkok 10400, Thailand.
Tel: +66 (0) 2644 8677 ext 1500, 5530; Fax: +66 (0) 2644 8701
E-mail: pywgs@mahidol.ac.th

up to 90% (Padumanonda and Gritsanapan, 2006). Our previous study found that the barakol content was, in descending order, in the fresh young leaves of *S. siamea* (0.4035% w/w) > the first boiled filtrate (0.2052% w/w) > the first boiled leaves (0.1408% w/w) > the second boiled filtrates (0.1079% w/w) > the second boiled leaves (0.0414% w/w) (Padumanonda and Gritsanapan, 2006).

Anthraquinone compounds are famous for their laxative and antifungal properties. Glycosides of anthraquinones, which are hydrolyzed by β-glucosidase of the intestinal flora to free anthraquinones and are further reduced to anthrones, are active forms for the laxative effect (Bruneton, 1995). Anthraquinone aglycones are active for the antifungal property (Kupitayanant *et al*, 2001). Thus, the quantity of total anthraquinone glycosides in the plants indicates the strength of laxative or purgative activities. One of the important sources of anthraquinones is the *Cassia*/*Senna* plant. The leaves of *S. siamea* were reported to contain anthraquinones, both aglycones and glycosides. Anthraquinones found in the leaves of *S. siamea* are rhein, cassiamin, physcion, chrysophanic acid, and sennosides (Gritsanapan, 1983; Nualkaew, 1999). Anthraquinones stimulate Cl^- secretion and/or inhibit Na^+ absorption, resulting in an accumulation of fluid and subsequent increased colonic motility. The increased Cl^- secretion by anthranoid laxatives is due to disruption of epithelial tight junctions, leading to increased permeability of the epithelium and laxative effect (Ewe, 1980; Wanitschke, 1980; van Gorkom *et al*, 1999). The content of barakol in the fresh and cooked leaves has been reported (Padumanonda and Gritsanapan, 2006). The aim of this study was to determine, using a UV-vis spectrophotometric method, the contents of total anthraquinone glycosides and total anthraquinones in the fresh young leaves, the boiled leaves, and the filtrates of *S. siamea* when following the normal process of cooking *khi lek* curry. The results could confirm the mild laxative effect of *khi lek* curry, which has been a popular dish of Thai people since ancient times.

MATERIALS AND METHODS

Plant materials and reagents

The young leaves of *S. siamea* were collected in March 2008 from Siri Ruckhachati Medical Plant Garden, Nakhon Pathom, Thailand. The plant samples were identified by comparison with the herbariums (BKF No. 65023, BKF No. 086142) at The Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimen (WCS001YL) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. All reagents used for analysis were analytical grade.

Preparation of young *S. siamea* leaves

The cleaned fresh young leaves were accurately weighed (100 g) and boiled with distilled water (1:3) for one hour. The mixture was filtered to give the first filtrate (Filtrate I) and first boiled leaves (Marc I). The weight and volume of Filtrate I and Marc I were recorded. Filtrate I was centrifuged at 10,000 rpm for 15 minutes, and the supernatant was dried on a boiling water bath to give the dried extract of Filtrate I. Marc I was divided into two parts. The first part was analyzed for the contents of total anthraquinone glycosides and total anthraquinones. The second part was re-boiled with distilled water (1:3) for another one hour. After the second boiling, the mixture was filtered. The weights of the second filtrate (Filtrate II) and the second boiled leaves (Marc II) were recorded. The whole process was repeated. Filtrate II was centrifuged at

10,000 rpm for 15 minutes, and the supernatant was dried on a boiling water bath to give dried extract of Filtrate II. Marc I, Marc II, and Filtrate I and Filtrate II dried extracts were further separately extracted for total anthraquinone glycosides and total anthraquinones (Fig 1).

Extraction of total anthraquinone glycosides

The method of extraction of total anthraquinone glycosides (Fig 1) was modified from that described for hydroxyanthracene derivatives of *Senna alata* (L.) Roxb. in ASEAN Herbal Medicine (ASEAN, 1993). The fresh young leaves, dried extracts of Marc I, Marc II, and Filtrates I and II (each 1.5 g) were used for extraction and analysis. The calibration curve of rhein standard was determined by UV-vis spectrophotometric method (Fig 2).

Extraction of total anthraquinones

Sample extraction of total anthraquinones was performed by the same procedure as total anthraquinone glycosides (Fig 1) except for omitting the extraction of free anthraquinone aglycones from the sample (Steps I and II).

Analysis of anthraquinones

A validated UV-vis spectrophotometric method (Sakulpanich and Gritsanapan, 2008) was used for the analysis of total anthraquinone glycosides and total anthraquinones using linear regression equation of standard curve of rhein, an anthraquinone component in *S. siamea* leaves. The maximum absorbance was measured at 515 nm.

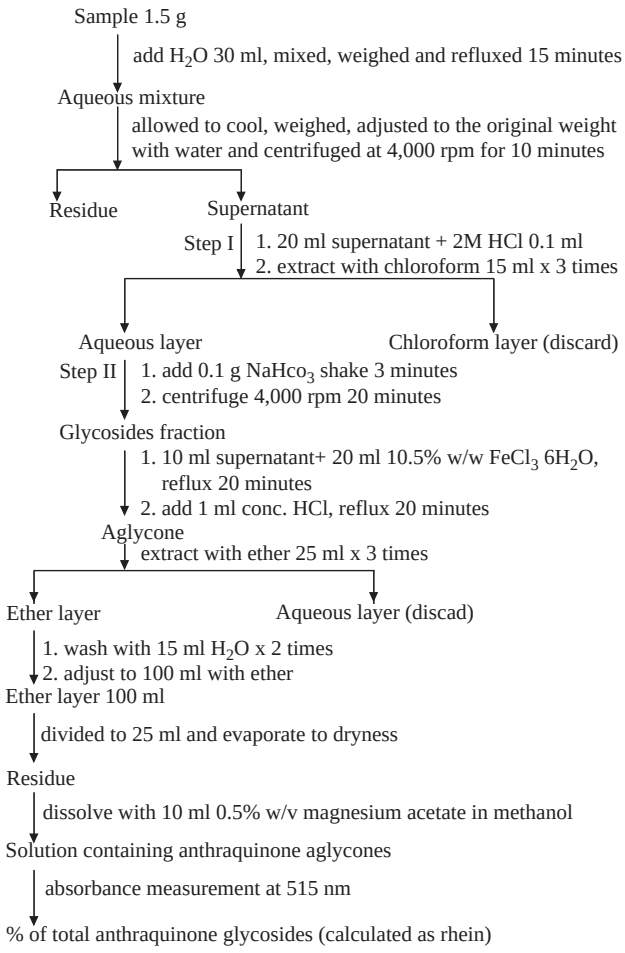

Fig 1–Extraction of total anthraquinone glycosides.

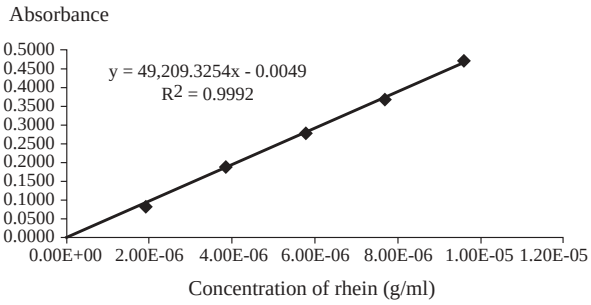

Fig 2–Calibration curve of rhein in 0.5% magnesium acetate solution in methanol determined by UV-vis spectrophotometric method.

RESULTS

Fresh young leaves contained total anthraquinones 0.0910 % w/w and total anthraquinone glycosides 0.0523% w/w calculated as rhein (Table 1). In the preparation simulating *khi lek* curry, the highest content

Table 1
Total anthraquinones and total anthraquinone glycosides in the filtrates and the boiled young leaves (marc) prepared as *khi lek* curry.

Sections	Fresh young-leaves[a]		Comparison with fresh young leaves[b]	
	Total anthraquinones (%w/w)	Total anthraquinone glycosides (%w/w)	Total anthraquinones (%)	Total anthraquinone glycosides (%)
Young leaves	0.0910±0.0015	0.0523±0.0010	100	57.47 (100)
Filtrate I	0.0721±0.0039	0.0334±0.0017	79.23	36.70 (63.86)
Marc I	0.0167±0.0002	0.0161±0.0900	18.35	17.69 (30.78)
Filtrate II	0.0069±0.0037	0.0031±0.0004	7.58	3.41 (5.93)
Marc II	ND	ND	ND	ND

ND, could not be detected; [a] expressed as mean ± SD (n = 3); [b] total anthraquinones content in fresh young leaves considered as 100%.

of total anthraquinones and total anthraquinone glycosides were found in the Filtrate I (0.0721 and 0.0334% fresh weight, respectively), while Marc II contained a very low amount that could not be detected. The contents of total anthraquinones and total anthraquinone glycosides decreased, in the descending order of Filtrate I > Marc I > Filtrate II > Marc II.

If the total anthraquinone content in the fresh young leaves was considered as 100%, the total anthraquinone glycosides in the fresh leaves would be about 57% of the total anthraquinones. The combination of total anthraquinone glycosides in the Filtrate I (0.0334% fresh weight) and Filtrate II (0.0031% fresh weight) was about 40% of total amount of anthraquinones. The first boiled leaves contained total anthraquinone glycosides (0.0161% fresh weight) estimated as about 18% of the total anthraquinones of fresh leaves.

DISCUSSION

Anthraquinone glycosides in the fresh young leaves (0.0523% w/w) were estimated to be about 57% of total anthraquinones. According to Thai preparation of *khi lek* curry, the young leaves are boiled with water 2-3 times to eliminate the bitterness. After the first boiling, 79% of all anthraquinones in the leaves was extracted into the Filtrate I, while about 18% of anthraquinones was still in the boiled leaves (Marc I). After the second boiling, 7.5% of total anthraquinones was extracted into Filtrate II. A very low amount of anthraquinones was left in the second boiled leaves, which were the actual part consumed as food by cooking with coconut milk, chili paste, and meat as *khi lek* curry.

Anthraquinone glycosides in the second boiled leaves should be less than 0.013% fresh weight, which is the difference between the amount of anthraquinone glycosides in the first boiled leaves and the amount in the second boiled filtrate. One hundred grams of *khi lek* curry (one bowl), which contains about 12 g of the second boiled leaves (cooked according to the recipe of Kongpan, 2005), gives less than 1.56 mg of total anthraquinone glycosides. Thai people do not consume *khi lek* curry, as a single dish but

they consume it with other dishes. Normally, the consumption of *khi lek* curry per meal per person is approximately $^1/_4$ bowl (3 g of second-boiled leaves), which yields less than 0.40 mg of total anthraquinone glycosides. When compared to the recommended daily dose of Senna leaves (0.6-2 g), which contains anthraquinone glycosides 30 mg (European Medicines Agency, 2006), this amount is 75 times greater than the amount of anthraquinone glycosides consumed from *khi lek* curry in each meal.

In conclusion, the content of total anthraquinone glycosides in the boiled young leaves of *S. siamea* was very low. Therefore, anthraquinone glycosides in the *khi lek* curry consumed in each meal produced a very mild laxative activity, which confirmed the traditional use of *khi lek* curry as a mild laxative drug.

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