BARAKOL CONTENTS IN FRESH AND COOKED *SENNA SIAMEA* LEAVES

Tanit Padumanonda and Wandee Gritsanapan

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

Abstract. *Senna siamea* (Lam.) Irwin & Barneby is a medicinal plant popularly used in Thailand. Young leaves and/or young flowers of this plant have been consumed by Thai people as a Khi Lek curry for a long time. The fresh young leaves and flowers are boiled with water 2-3 times to get rid of the bitterness and the boiled mush is used for curry cooking. Barakol, a major constituent of *Senna siamea* leaves was analyzed for its content in the fresh young leaves, the boiled leaves and the boiled filtrates by a high-performance thin-layer chromatographic method. Fresh young leaves of *S. siamea* contained 0.4035% w/w barakol. The amount of barakol in the first and second boiled filtrates were 0.2052 and 0.1079% fresh weight, while the first and second boiled leaves samples were 0.1408 and 0.0414% fresh weight, respectively. The results show the process of preparation of Khi Lek curry by boiling *S. siamea* young leaves twice with water reduced barakol content up to 90 % and the content of barakol in boiled leaves used for curry has much less tendency to cause liver toxicity. This may explain the reason why Thai Khi Lek curry has not caused hepatotoxicity, unlike *S. siamea* leaves consumed as a powdered capsule.

INTRODUCTION

Senna siamea (Lam.) Irwin & Barneby is found indigenously in Thailand and locally called "Khi Lek". It has a long history of use as a folk medicine and its therapeautic efficacy is well recognized. Different parts of *S. siamea* can be used for various medical purposes (Fiorino *et al*, 1998; Subhadhirasakul *et al*, 2000; Sukma *et al*, 2002). The fresh young flowers and/or young leaves have been used as vegetables in Thailand. They can be prepared as food 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 cooked by mixing with coconut milk and curry paste and cooked as a curry which is used as a mild laxative and sleeping aid. An aqueous extract of fresh or dried leaves of *S. siamea* has also been recommended for treatment of insomnia (Thongsaard *et al*, 1996). For the past few decades, barakol, a major biologically active constituent of *S. siamea*, especially in the young leaves, has been extensively studied for its anxiolytic activity (Thongsaard *et al*, 1997; Pooviboonsuk *et al*, 2000; Muangman *et al*, 2001).

Barakol(3,4-dihydroxy-2,5-dimethyl-1,4-dioxyphenalene,$C_{13}H_{12}O_4$, melting point 166-170ºC: decomposed) is unstable and converted to anhydrobarakol ($C_{13}H_{10}O_3$, melting point 163ºC) by losing a molecule of water (Fig 1). Barakol occurs as pale lemon-yellow needle crystals. It is soluble in methanol, ethanol and acetone, moderately soluble in chloroform and dichloromethane, and readily soluble in benzene, carbon tetrachloride, ethyl ether and water (Thongsaard, 1998).

S. siamea leaves were once marketed in Thailand as an herbal drug for sleep, available in capsule form containing 400 mg of leaf powder per capsule (10 mg of anhydrobarakol, a prodrug of barakol). The dosage of this herbal drug was 2-4 capsules before bed. Unfortunately, it was withdrawn from the market in 2003 due to hepatotoxicity (Hongsirinirachorn *et al*, 2003).

The cause of the hepatotoxicity of *S. siamea* capsules is still unexplained. In 2001, Chivapat *et al* studied the subchronic effect of barakol in rats and blood biochemistry parameters were investigated. The results revealed that *S. siamea* capsules caused degeneration and necrosis of

Correspondence: Wandee Gritsanapan, 447 Si 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

hepatocytes in Wistar rats and the severity of the lesion was dose dependent. Barakol probably acts as a hepatotoxin, but whether hepatitis developed as a result of the direct effect of barakol or due to the effect of one of its metabolites, needs further study. In contrast to *S. siamea* capsules, food from *S. siamea* leaves cooked as curry remains popular without any reports of hepatotoxicity for hundred years.

From the toxicity reports so far, it is still necessary to determine more accurately the barakol content in the leaves of *S. siamea.* Comparison of the barakol content in the food preparation and the medical preparation could give understanding of the toxicity, which could lead to the development of better pharmaceutical preparations of *S. siamea* and barakol in the future. Because barakol is an unstable compound, it is important to use an instrument that can determine barakol content within a short period of time.

There are a few methods to quantitate the barakol in the extracts. Siripunya (1997), reported an HPLC method to determine barakol in alcoholic solution and in syrup containing *S. siamea* extract. Thongsaard *et al* (2001) reported the application of an HPLC method with electrochemical detection to determine the purity and stability of the extracted barakol solution. We developed and validated a TLC-densitometric method, which is rapid, more convenient and requires less solvent than HPLC, to determine barakol content in different parts of *S. siamea* (Padumanonda *et al*, 2004). The aims of this study were to determine and compare barakol content in fresh young leaves of *S. siamea*, boiled fresh leaves prepared by cooking for Khi Lek curry, and the first and second discarded boiled filtrates using the TLC-densitometric method.

MATERIALS AND METHODS

Plant materials and reagents

S. siamea young leaves were collected from Bangkok, Thailand in June 2003 and identified by comparing with herbariums (BKF No. 65023, BKF No. 086142) at the Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok, Thailand. The voucher specimens (No.WSS0503) were kept at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. All reagents used for analysis were of analytical grade. Authentic barakol was kindly supplied by Dr C Chaichantipyuth, Chulalongkorn University, Thailand.

Extraction and purification of barakol for use as a standard

Fresh young leaves of *S. siamea* (6.0 kg) were sliced into small pieces and boiled with 12 l of 0.5 % sulfuric acid for 15 minutes; the mixture was then cooled and filtered. The mush was re-boiled with 0.5 % sulfuric acid using the same process as before. The filtrates were combined and basified with an adequate amount of sodium carbonate to pH 8. The basic solution was extracted with chloroform (3 x 4 liters) three times. The chloroform extracts were combined and shaken twice with deionized water. The chloroform layer was filtered and concentrated under reduced pressure until the volume was one-fourth the starting volume. An equal amount of deionized water was added and the mixture was cooled for 30 minutes in a refrigerator to get crude barakol, which was recrystalized with absolute ethanol to get green yellow crystals of barakol. Purified barakol was identified by comparing its UV, IR, H^1-NMR spectra, R_f value and melting point with those of the authentic sample.

Preparation of standard solutions

A stock solution of barakol was prepared by dissolving 10 mg of pure barakol in 10 ml of methanol in a volumetric flask. A standard solution of 100 ppm of barakol was prepared by diluting the stock solution with methanol.

TLC-densitometry instrumentation

A Camag TLC system (Switzerland) composed of an automatic TLC sampler (Linomat IV, Switzerland), TLC scanner and WINCATS 4 software was used for sample application and quantitative evaluation.

Chromatography was performed on a TLC pre-coated silica gel 60 F_{254} plate 20 x 10 cm using chloroform:methanol (85:15) as a mobile phase. The total volume of the solvent mixture was 30 ml. Sample bands were applied (6 mm

length) at 9.4 mm intervals under nitrogen stream. A constant application of 0.1 μl/sec was employed. The slit dimension was kept at 6 mm x 0.45 mm and the scanning speed was 10 mm/sec. The plates were developed in a Camag TLC chamber and equilibrated with the mobile phase for 40 minutes before inserting the plate. Chromatograms were evaluated using the peak area after scanning in absorbance mode at 366 nm for the barakol.

After development, the plate was air dried for 5 minutes, after which the sample and standard zones were quantified by linear scanning at 366 nm using a Camag TLC scanner III with a deuterium source and a tungsten source. The WINCATS software controlling the densitometer produced a calibration plot by linear regression analysis relating standard zone weights to their scan areas, and the content of the barakol in the samples was automatically interpolated from the calibration curve.

In addition to these components, a Camag video documentation system in conjunction with the reprostar 3 was used for imaging and archiving the thin-layer chromatogram. The HPTLC method was validated in terms of linearity, accuracy, precision, limit of detection and limit of quantitation (Padumanonda *et al*, 2004).

Preparation of samples for determination of total barakol content in *S. siamea* fresh leaves

Ethanol (15% v/v) was used for the extraction of barakol in *S. siamea* fresh leaves. The fresh leaves (20.0 g) were extracted with the solvent (100 ml) by sonication for 4 hours at room temperature and then filtered. The filtrate was collected and adjusted to the original volume in a volumetric flask with 15% ethanol. The solution was analyzed for barakol content by applying 3 μl of the solution on the TLC plate, followed by developing and scanning as described in the TLC-densitometry instrumentation section. The concentration of barakol content (mg/ml) in each sample was determined and the barakol content (% fresh weight) was then calculated.

Estimation and comparison of barakol content in the filtrates and boiled leaves

Preparation of the young *S. siamea* leaves mimicked the actual cooking process of Khi Lek curry. The young leaves (20 g) were accurately weighed and boiled with distilled water (60 ml) for 1 hour. The mixture was filtered to give the first boiled filtrate (filtrate I) and first boiled leaves (marc I).The weight and volume of filtrate I and marc I were recorded. Marc I was separated into two equal parts. The first part was for analysis while the second part was re-boiled for another 1 hour with distilled water (30 ml). After the second boiling, the mixture was filtered. The weights of the second boiled filtrate (filtrate II) and the second set of boiled leaves (marc II) were accurately recorded. The whole process was conducted in triplicate. Marc I and marc II were separately extracted with 15% (v/v) ethanol by sonicating at room temperature for 4 hours, then the mixtures were filtered. The ethanolic extracts of marc I, marc II, filtrate I and filtrate II were concentrated to exact volumes and spotted on a TLC plate which was further developed and analyzed for barakol content using the TLC-densitometer. The barakol content in marc I, marc II, filtrate I and filtrate II were then determined.

RESULTS

Identification of isolated pure barakol

The yield of barakol isolated from fresh young leaves of *S. siamea* was 0.086% w/w. The UV, IR and H^1-NHR spectral data and melting point of the purified barakol are shown in Table 1. The purity of the isolated barakol was established by TLC, which was shown as a single band chromatogram.

Estimation and comparison of barakol content in filtrates and boiled leaves

Barakol content in the fresh young leaves was 0.4035 ± 0.0055% w/w (Table 2). The amount of barakol in the first boiled filtrate, first boiled leaves, second boiled filtrate and second boiled leaves were 0.2052 ± 0.0029, 0.1408 ± 0.0096, 0.1079 ± 0.0040, and 0.0414 ± 0.0009% (w/w), respectively (Table 2). The total barakol content of the fresh leaves sample (0.4035 ± 0.0055 %) was the summation of the barakol contents of the first and second boiled filtrates and the second boiled leaves. The total of barakol content of the extracts from the cooked *S. siamea* fresh young leaves was

Table 1
Characteristics of barakol

Method of indentification	Characteristic
1. Melting point	167°C (decomposed)
2. UV	λmax (MeOH) at 247 and 375 nm
3. IR	- 3,453.7 cm^{-1} and 3,295.8 cm^{-1} (broad) [characteristic peak of hydroxyl group (-OH)]
	- 1,683.4 cm^{-1} (sharp) [characteristic peak of carboxyl group (C=O)]
	- 1,591.2 cm^{-1} and 1,564.9 cm^{-1} sharp (characteristic peak of aromatic rings)
	- 1,472.8 cm^{-1} (sharp) (characteristic peak of C-H stretching)
4. H^1 NMR	Chemical shift (ppm)
Position (as numbering in Fig 1)	
3	6.34(1H , *s*)
4	6.46 (1H , *d*, J_{meta})
6	6.57 (1H , *d*, J_{meta})
9	6.10 (1H , *s*)
2-Me	2.25 (3H , *s*)
8-Me	2.42 (1H , *s*)

Table 2
Barakol content in different samples of *S. siamea* determined by TLC-densitometry.

Sample	Content of barakol (% fresh weight)[a]	% [a,b] (compared to barakol content in fresh leaves)
Fresh young leaves	0.4035 ± 0.0055	100
First boiled filtrate (filtrate I)	0.2052 ± 0.0029	50.8550 ± 0.7148
First boiled leaves (marc I)	0.1408 ± 0.0096	34.8947 ± 2.3838
Second boiled filtrate (filtrate II)	0.1079 ± 0.0040	26.7410 ± 1.0116
Second boiled leaves (marc II)	0.0414 ± 0.0009	10.2602 ± 0.2225

[a]expressed as mean ± SD (n=3)
[b]when compared to barakol content in fresh leaves, which was considered as 100%
Total barakol content in cooked *S. siamea* leaves = 0.2052+0.1079+0.0414 = 0.3545% fresh weight

0.3545% (0.2052% + 0.1079% + 0.0414%) fresh weight or 87.86% of the total barakol in the fresh young leaves (Table 2).

DISCUSSION

The UV, IR and H^1-NMR spectral data and melting point of purified barakol (Table 1) were similar to the data of the authentic sample and to reported data (Hassanali *et al*, 1969; Bycroft *et al*, 1970; Teeyapant and Srikun, 1988; Siripunya, 1997). The present study analyzed and compared the barakol content of the discarded boiled filtrates (filtrate I and II) and boiled leaves (marc I and II) of *S. siamea* prepared similar to making Khi Lek curry. The TLC-densitometric method provided advantages in terms of high speed and less sample and solvents con-

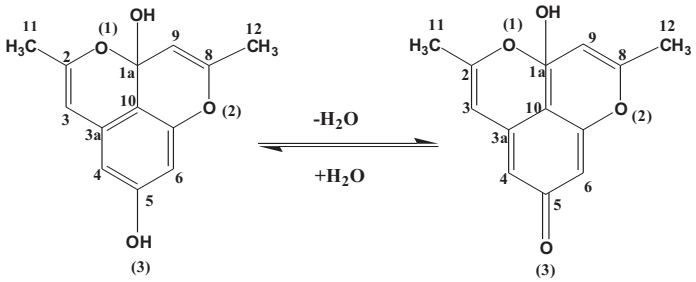

Fig 1–The conversion reaction of barakol and anhydrobarakol.

sumption than other techniques. The second boiled leaves, which were the actual part consumed as food by cooking with coconut milk, chili paste and meat, contained about one-third the barakol content of the first boiled leaves, the amount was about 10% of total barakol content in the fresh young leaves. It is safer to cook Khi Lek curry by boiling the *S. siamea* leaves twice. Boiling *S. siamea* not only reduces the bitterness but also reduces about 90 % of the barakol in the fresh leaves. One hundred grams of Khi Lek curry (1 bowl), which contains about 12 g of boiled leaves (cooked following the recipe of Kongpan (2005), gives about 5 mg of barakol. Thai people do not exclusively consumed Khi Lek curry, as a single dish. They consume it with other dishes. Normally, the consumption of Khi Lek curry per meal per person is approximately 1/4 bowl, which yields about 1.25 mg of barakol. Compared to the recommended dose of 2-4 Khi Lek capsules containing ≥10 mg of anhydrobarakol (prodrug of barakol) per capsule, barakol from the consumption Khi Lek curry is unlikely to cause hepatotoxicity. The consumption of unboiled *S. siamea* leaves without cooking gives a much higher barakol content. The cause of the hepatotoxicity found with *S. siamea* capsules is still unclear. The toxicity data of barakol in mice (intraperitoneal injection), showed that a lethal dose of barakol was high (LD_{50} = 324.09 mg/kg) (Jantarayota, 1988). Despite the toxicity reports for *S. siamea* capsules, Khi Lek curry is still a popular food in Thailand. Other ingredients in Khi Lek curry such as coconut milk, chilli paste and meat, may have an influence on the low level of toxicity found. The low content of barakol in Khi Lek curry may explain why consuming the curry causes no hepatotoxicity. People also do not consume Khi Lek curry everyday, unlike the drug. The findings from this study support the traditional uses of Thai medicinal plants, which should be considered along with the improvement of medicinal dosage forms.

In conclusion, by TLC-densitometric method, the barakol content in fresh young leaves of *S. siamea* was 0.4035% w/w. The boiled leaves used for cooking Khi Lek curry contained 0.0414% w/w of fresh leaves, this amount was about 10% of the barakol content in fresh young leaves. In one meal, if people consumed approximately $^1/_4$ bowl of Khi Lek curry, they may receive 1.25 mg of barakol. This amount is 16-32 times less than a normal dose of 2-4 Khi Lek capsules. The low content of barakol in the curry may explain why Thai people consuming Khi Lek curry have no reported hepatotoxicity. This is the first time this information regarding the tropical medicinal plant *S. siamea* has been reported.

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