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Determination of degradation of barakol in extracts of *Senna siamea* and its herbal recipes

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Introduction

Senna siamea is widely cultivated in the Southeast Asia. *S. siamea* is normally found around Thailand. Barakol has been reported as a major active compound of *S. siamea* leaf and flower.[1] Barakol have hypnotic effect, unfortunately, herbal products containing *S. siamea* are withdrawn from the market due to their hepatotoxic effect.[2] According to Thai traditional medicines, herbal recipes are widely use than single herb. Purpose of the recipe using is offered synergistic effect of therapeutic efficacy while lessening toxicity according to Chinese traditional medicines.[3]

Quality control of herbal products by measuring chemical constituent stability is extremely challenging due to the complexity of extract of plant or herbal recipe. There are many factors affecting stability of herbal products such as pH, light, temperature, enzymatic degradation, water, metal ions, solvents, and concomitant compounds.[4] Barakol is used as active marker for control the quality of *S. siamea* and herbal recipes containing *S. siamea* in this study. Chantong et al. reported that barakol is extremely degraded under alkaline condition. Moreover, thermal stress and oxidative stress also enhanced degradation of barakol.[2] Our previous work determined barakol content in *S. siamea* and herbal recipes containing *S. siamea*.[5] However, stability of barakol in *S. siamea* and herbal recipes containing *S. siamea* in long term storage is not reported elsewhere. Thus, this study aimed to determine the degradation of barakol in extracts of leaf and flower of *S. siamea* after 10 months storage. Degradation of barakol in extracts of *S. siamea* contained herbal recipes was also investigate.

Methods

Sample extraction: Dried *S. siamea* leaf and flower were pulverized and passed through 40-mesh sieve. Two grams of each sample was extracted in 100 mL 95% ethanol using ultrasonication for 1 h, filtered, and evaporated by rotary evaporator (Buchi, Thailand) (n=3). In case of herbal recipes, each plant was ground and passed through 40-mesh sieve. After that, each herb was mixed with other plant to obtained Recipe 1-3. Recipe 1 was extracted using boiling water, water at ambient temperature, 60% ethanol, and 95% ethanol with ultrasonication for 1 h (n=3). Recipe 2 and 3 were extracted with the same method of Recipe 1, while, boiling water and 95% ethanol were used as solvent for Recipe 2 and 3. Extract solution was filtered and dried under freeze dryer and/or rotary evaporator.

Sample preparation: Extracts were dissolved in the extraction solvent to obtained 0.5 mg/mL, filtered through 0.22 µm membrane filter, and injected to HPLC instrument. Peak area of barakol was compared with calibration curve and calculated as barakol content in extracts. Barakol content at initial time and after 10 months storage (kept in desiccator at ambient temperature and protected from light) were compared.

Standard solution preparation: Barakol extracted from fresh young leaf of *S. siamea* from previous work[5] was used. Barakol with concentration of 0.25-40 µg/mL was prepared using absolute ethanol as solvent. The obtained solution was filtered through 0.22 µm membrane filter and injected to HPLC instrument. Calibration curve of barakol was constructed.

HPLC condition:
Analysis was performed on Agilent 1260 series equipped with photodiode array detector and autosampler (Agilent, USA). Luna C18(2) column (250×4.6 mm, i.d., 5 µm) was used. Column temperature was controlled at 25 ºC. Mobile phase consisted of ultrapure water and methanol with a flow rate of 1 mL/min. Injection volume was 10 µL. The gradient system of mobile phase was shown in Table 1.

Table 1. Mobile phase composition of gradient system

Time (min)	Water (%)	Methanol (%)
0	100	0
5	95	5
7	95	5
10	90	10
25	60	40
55	30	70
58	30	70
68	0	100
70	0	100
71	100	0
80	100	0

Results

Barakol content in extract of leaf of *S. siamea* at initial time varied from 0.40-5.01%. Degradation of barakol in *S. siamea* leaf of L2-L5 was 50.29-90.11% (Figure 1). The highest and lowest of degradation found in L2 and L5, respectively. While, degradation of barakol in L1 could not be calculated due to amount of barakol at 10 months storage was not found. Barakol in L1 might be almost degraded within less 10 months. Average data in Figure 1a calculated from difference of data at initial and after 10 months storage of L2-L5. After 10 months storage, barakol in leaf of *S. siamea* degraded approximately 64%.

In case of barakol content in flower of *S. siamea*, at initial time, barakol content in flower extracts ranged from 1.83-2.97%, which F3 and F5 had the highest and lowest barakol content, respectively. After 10 months storage, barakol decreased to 0.65-1.99%. Degradation of barakol in flower extract of *S. siamea* was approximately 49% (Figure 1b). From Figure 1, barakol in leaf extracts highly degraded compared to flower extracts.

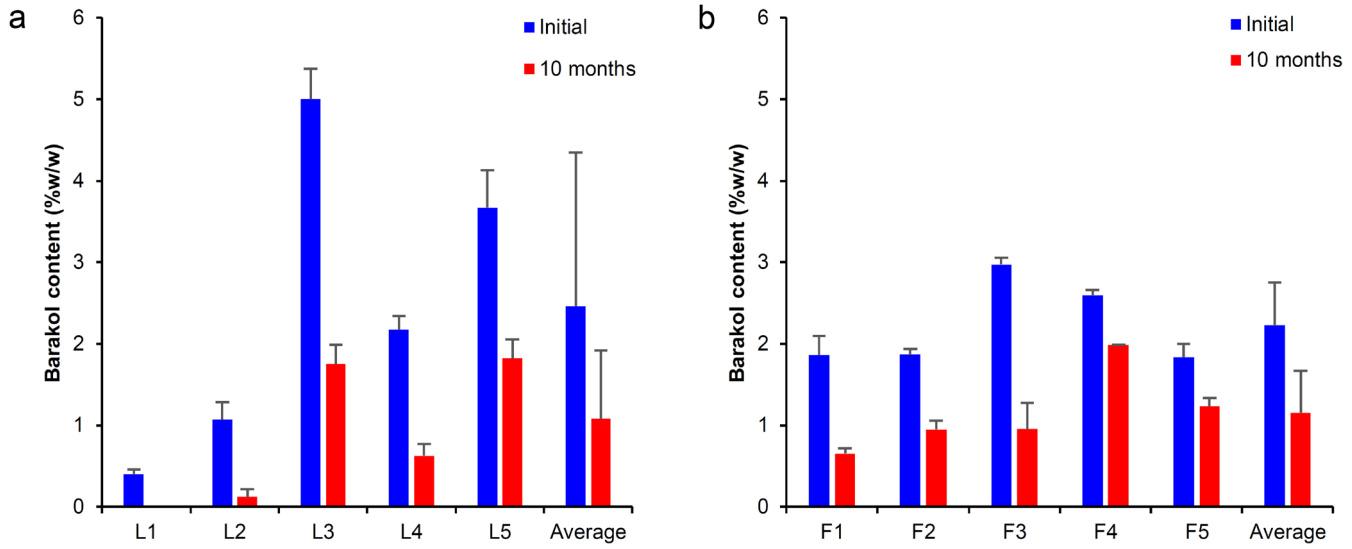

Figure 1. Barakol content in ethanolic extract of (a) leaf and (b) flower of *S. siamea* harvested from different places at initial time and after 10 months storage

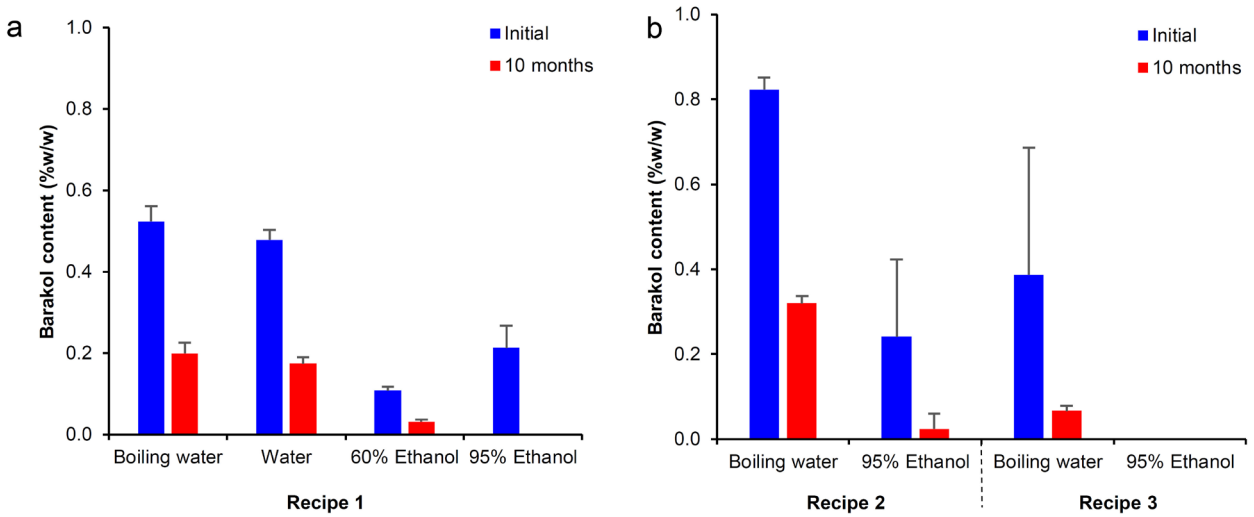

Figure 2. Barakol content in extract of (a) Recipe 1 and (b) Recipe 2 and 3 extracted using different solvents at initial time and after 10 months storage

Figure 2a showed that barakol in Recipe 1 extracted using boiling water was similar to water at ambiemt temperature. After 10 months storage, barakol degraded 61.98 and 63.65%, respectively. Ethanol based extracts showed higher degradation, 100% and 69.84% degradation were found in Recipe 1 extracted using 95% ethanol and 60% ethanol, respectively. The higher degradation of barakol in ethanol based extracts was observed in Recipe 2 (Figure 2b), 93.27% degradation was found in Recipe 2 extracted using 95% ethanol compared to 61.17% degradation of 60% ethanol extract. In case of Recipe 3, boiling water extract had 74.59% degradation, however, 95% ethanol could not be extracted barakol from Recipe 3.

Discussion

Barakol has been reported as a major active compound of *S. siamea* leaf and flower. Degradation of barakol means efficacy of plant or herbal recipes decreased. From Figure 1, barakol content in *S. siamea* leaf extracts was highly varied when compared to in flower. This result indicated that harvested place and part of plant affected the amount of barakol. Moreover, these two factors also affected degradation of barakol.

According to herbal recipes, degradation of barakol was low when extracted with boiling water. This result supported our previous study that boiling water is the most appropriate solvent for extraction of barakol from herbal recipe containing *S. siamea* due to it can extract the highest value of barakol. In addition, it can extract many types of chemical constituent from herbal recipe.[5] Herbal recipes extracted using boiling water and water at ambient temperature showed high stability of barakol. There were some reports of formation of artifact with organic solvents during extraction process or storage of herbal extracts.[4] Furthermore, degradation of barakol might be related to water content in extracts. Water is relate to redox reaction. Water allow redox reaction to produce hydroxyl radicals and hydrogen peroxide. These byproducts could cause degradation of active compound of herbal extract.[4] However, drying process of water extracts was freeze dried that had less water content compared to vacuum drying of 95% ethanol extract. Thus, water extract was highly stable than ethanol extract. In case of drying of herbal recipe water extract, spray dry could be use instead of freeze dry, extract with low moisture content obtained.[6] However, stability of chemical constituent in high temperature of spray drying process should be considered because barakol degraded under thermal stress.[2] Barakol in water extract of Recipe 3 had highly degradation compared to Recipe 1 and 2, indicated that interaction of chemical constituents in herbal recipe may affected stability of barakol in extracts. Alkaline condition and oxidative stress also affected barakol stability.[2] In addition, Cortés-Rojas et al. reported that storage condition and packing design also important for stability enhancement of herbal extract.[7]

Conclusion

This study determined the degradation of barakol in extract of *S. siamea* leaf and flower as well as in herbal recipe. Four factors affected on degradation of barakol in extract were plant harvested places, part used, composition of herbal recipe, and extraction solvent. In case of single herb, difference of barakol degradation in *S. siamea* harvested from different provinces was observed. Flower extracts had less degradation compared to leaf extracts. Furthermore, Recipe 3 showed high degradation compared to Recipe 1 and 2. In this work, boiling water extracts lessen barakol degradation compare to 95% ethanol extract.

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