SHORT COMMUNICATION

Distribution of hydroxyanthracene derivatives in *Cassia alata* and the factors affecting the quality of the raw material

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Abstract

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Analyses have been carried out on the content of hydroxyanthracene derivatives of the leaves, flowers and pods of *Cassia alata*, which had been collected at different harvesting times and different leaf-positions. It was found that when the leaves had been harvested in March, June or September, the hydroxyanthracene derivatives were accumulated more in the leaf-positions 1-3 (1.82, 1.25, 1.63 %w/w, respectively) and 4-6 (1.39, 1.58, 1.09 %w/w, respectively). In December (the flowering and fruiting season), hydroxyanthracene derivatives were accumulated more in the flowers (2.21%w/w) and the pods (1.82 %w/w), respectively. The method and temperature of drying markedly affected the hydroxyanthracene derivative content. Drying of the leaves in a hot air oven at 50ºC gave a higher hydroxyanthracene derivative content (1.43 %w/w) than drying in a hot air oven at 80ºC (0.44 %w/w) or drying in the sun (0.95 %w/w). Study on the stability of hydroxyanthracene derivatives in *C. alata* leaf powder, which was kept in tight container at room temperature, found that the hydroxyanthracene derivative content did not decrease within 9 months.

Key words : *Senna alata, Cassia alata*, hydroxyanthracene, harvesting, drying

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บทคัดย่อ

ภาคภูมิ พาณิชยูปการนันท์ และ นิวรรณ อินทรักษา
การกระจายของสารอนุพันธ์ไฮดรอกซี่แอนทราซีนในต้นชุมเห็ดเทศ และปัจจัยที่มีผลต่อคุณภาพวัตถุดิบ
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การวิเคราะห์ปริมาณสารอนุพันธ์ไฮดรอกซี่แอนทราซีนในใบ ดอก และฝักของชุมเห็ดเทศ (*Cassia alata*) ที่เก็บเกี่ยวในระเวลาและตำแหน่งของใบที่แตกต่างกัน พบว่าใบชุมเห็ดเทศที่เก็บเกี่ยวในเดือนมีนาคม มิถุนายน และกันยายน สารอนุพันธ์ไฮดรอกซี่แอนทราซีนถูกเก็บสะสมมากในใบที่เก็บจากตำแหน่งใบที่ 1 ถึง 3 (1.82, 1.25, 1.63 %w/w ตามลำดับ) และตำแหน่งใบที่ 4 ถึง 6 (1.39, 1.58, 1.09 %w/w ตามลำดับ) ในเดือนธันวาคม (เป็นฤดูที่ออกดอกออกผล) สารอนุพันธ์ไฮดรอกซี่แอนทราซีนถูกเก็บสะสมมากในส่วนของดอก (2.21 %w/w) และฝัก (1.82 %w/w) ตามลำดับ วิธีการและอุณหภูมิในการทำให้แห้งมีผลต่อปริมาณสารอนุพันธ์ไฮดรอกซี่แอนทราซีนอย่างเห็นได้ชัด การทำให้แห้งโดยใช้ตู้อบที่อุณหภูมิ 50°C มีผลทำให้ปริมาณสารอนุพันธ์ไฮดรอกซี่แอนทราซีน (1.43 %w/w) สูงกว่าการทำให้แห้งโดยใช้ตู้อบที่อุณหภูมิ 80°C (0.44 %w/w) หรือการตากแดด (0.95 %w/w) การศึกษาความคงตัวของสารอนุพันธ์ไฮดรอกซี่แอนทราซีนในผงใบชุมเห็ดเทศที่เก็บไว้ในภาชนะปิดสนิทที่อุณหภูมิห้อง พบว่าสารอนุพันธ์ไฮดรอกซี่แอนทราซีนในผงใบชุมเห็ดเทศไม่ลดลงเมื่อเก็บผงยาไว้ภายใน 9 เดือน

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Cassia alata L. (in Thai "Chumhetthet") is an herbal medicine that has been traditionally used for the treatment of constipation and skin disease (de Padua *et al*., 1999; Farnsworth and Bunyapraphatsara, 1992; Perry and Metzger, 1980). In Thailand, *C. alata* has been approved as a laxative drug in the Thai Herbal Pharmacopoeia 1998 and the Thai National List of Essential Drug 1999 (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998; National Drug Committee, 1999). Hydroxyanthracene derivatives were demonstrated as the active constituents in this plant (Elujoba *et al*., 1989). The efficiency of herbal medicines depends on the plant raw material quality, which is usually related to the content of the active compounds (Thaweephol *et al*., 1993). Recently, poor quality of *C. alata* leaves due to the content of hydroxyanthracene derivatives being lower than the standard value (that is not less than 1.0 %w/w of hydroxyanthracene derivatives, calculated as rhein-8-glucoside on a dried basis) in the monograph (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998) has been a major problem in the production of the herbal medicines from *C. alata*. As part of our interest in the effect of harvesting and post-harvesting factors on the quality of *C. alata* raw material, we have determined the distribution of hydroxyanthracene derivatives accumulated in the leaves at different leaf-positions, flowers and pods. The effects of the harvesting period and the drying method, and the stability of hydroxyanthracene derivatives in the leaf powder have also been studied. This work will contribute to our knowledge of the good harvesting and processing practices required for the production of herbal medicine from *C. alata*.

Materials and Methods

Plant Materials

The plant materials were harvested from five year-old *C. alata* plants grown in Songkhla province. The harvesting periods were March, June, September and December 2002.

Preparation of *C. alata* samples

The plant materials were collected and divided into the three groups according to plant parts: leaves, flowers and pods. The leaves were divided into six groups according to the positions of the leaves on the plant counted from the top, that is, leaf-positions 1-3, 4-6, 7-9, 10-12, 13-15, 16-19, respectively. The plant materials were dried at 50ºC for 24 hours, ground and passed through a No. 60 sieve. The amounts of total hydroxy-anthracene derivatives in the leaf powders were then determined.

The effect of drying methods

The *C. alata* leaves which had been collected from the leaf-positions 4-6, were divided into three parts and these were dried by three different methods, that is, in a hot air oven at 50ºC for over-night, in a hot air oven at 80ºC for overnight, and in the sun for three days. The amounts of total hydroxyanthracene derivatives in the three leaf samples were then determined.

Stability of hydroxyanthracene derivatives in *C. alata* leaf powder

Stability of hydroxyanthracene derivative in the leaf powder was studied by determining the total hydroxyanthracene derivative content in the leaf powder, which had been kept in the well-closed containers and stored in a dry place at room temperature, at intervals of three months for a period of one year.

Determination of hydroxyanthracene derivatives

Total content of hydroxyanthracene derivatives in the plant materials was measured by a spectrophotometric method according to the Thai Herbal Pharmacopoeia (1998), as follows; About 150 mg of the leaf powder was accurately weighed and placed in a 100-ml round-bottomed flask. A portion of water (30.0 ml) was added, mixed, weighed, and heated under a reflux condenser for 15 minutes. The cooled mixture was weighed and adjusted to the original weight with water. The mixture was centrifuged and the supernatant liquid (20.0 ml) was transferred to a 150-ml separator. 2 M hydrochloric acid (0.1 ml) was added and the mixture was shaken with three 15-ml portions of chloroform. The chloroform layer was discarded. Sodium hydrogen carbonate (100 mg) was added into the aqueous part and shaken for 3 minutes. After centrifugation, the supernatant liquid (10.0 ml) was transferred to a 100-ml round-bottomed flask. 10.5 %w/v solution of iron (III) chloride (20 ml) was added and the mixture was heated for 20 minutes under a reflux condenser. Hydrochloric acid (1 ml) was added and heated for a further 20 minutes with frequent shaking. After cooling, the mixture was transferred to a separator and shaken with three 25-ml portions of ether previously used to rinse the flask. The ether layers were combined and washed with two 15-ml portions of water. The ether layer was then transferred to a 100-ml volumetric flask and diluted with ether to the required volume. An aliquot of the solution (25.0 ml) was carefully evaporated to dryness at low temperature and the residue was dissolved in 10.0 ml of a 0.5 % w/v solution of magnesium acetate in methanol. The absorbance of the solution was measured by SPECTRO UV-VIS RS Spectro-photometer at 515 nm, using the magnesium acetate solution as the blank. The percentage of rhein-8-glucoside was calculated from the expression: A x 0.4283/w, where A is the absorbance measured finally at 515 nm, and w is the weight in g of the dried leaf powder used initially. The analyses in all experiments were in triplicate.

Satatistical analysis

Values are expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by the Scheffe's test (Snedecor, 1967). The level of statistical significance was taken at $P < .05$.

Results and Discussion

Distribution of hydroxyanthracene derivatives in *C. alata*

Determination of hydroxyanthracene derivatives content in the leaves of *C. alata*, which

Table 1. Hydroxyanthracene derivatives content in the leaves (collected at different leaf-positions), flowers and pods of *C. alata*, which had been harvested in different months.

Leaf-positions	% Hydroxyanthracene derivatives (Mean ± SEM)			
	March	June	September	December
1-3	1.82±0.02*	1.25±0.12	1.63±0.06*	1.09±0.10
4-6	1.39±0.02*	1.58±0.07	1.09±0.08	1.26±0.13
7-9	0.98±0.01	1.25±0.12	0.85±0.08	1.20±0.12
10-12	0.99±0.01	1.22±0.04	0.89±0.06	n.s.
13-15	n.s.	0.75±0.05*	n.s.	n.s.
Flower	n.s.	n.s.	n.s.	2.21±0.11*
Pod	n.s.	n.s.	n.s.	1.82±0.08*

The plant materials were dried by using a hot air oven at 50ºC for 24 hours. *n* = 3 individual experiments. * Significantly different from the others when compared within the column, *P* < .05. n.s. no sample.

were collected from different positions of the plant and at different period of times, has demonstrated that hydroxyanthracene derivatives markedly accumulated in the leaves that had been collected from the leaf-positions 1-3 and 4-6 (Table 1). The hydroxyanthracene derivative content was lower for the leaves collected from the lower positions (older leaves). This shows that hydroxyanthracene derivatives are concentrated more in the younger and the mature leaves rather than in the older leaves. Although the results agree with the harvesting method of *C. alata* leaves suggested by the Department of Medical Sciences, harvesting should be only of the mature leaves (Jaree Bansiddhi *et al*., 2002), they also clearly indicate the good quality of the young leaves. As regards the effect of harvesting period it was found that the young leaves harvested in March and September, and the mature leaves harvested in June, gave higher amounts of hydroxyanthracene derivatives (Table 1). In June *C. alata* contributed more leaves, which gave a higher hydroxyanthracene derivative content than the standard value of 1.0 % w/w. *C. alata* begins to blossom in November, and fruits in December. In December, the hydroxyanthracene derivatives were found to accumulate more in the flowers and pods than in the leaves. This indicates that the active compounds have been translocated from the leaves to accumulate in the flowers and pods, in the same manner as the anthraquinone glycosides in *Cassia senna* (Fairbairn and Shrestha, 1967). The result is harmonious with a previous report that the leaves harvested in the period of November to January gave poor quality materials (Thaweephol and Pranee, 1988). The results suggest that leaf havesting should be made before blossom, which agrees with the harvesting method suggested by the Department of Medical Sciences (Jaree *et al*., 2002).

Although the young and mature leaves (leaf-positions 1-6), which were collected in different periods, contain different amounts of hydroxyanthracene derivatives, the content is higher than the minimum value (1.0 % w/w) specified in the monograph for Chumhetthet in the Thai Herbal Pharmacopoeia 1998 monograph (Subcommittee on the Establishment of the Thai Herbal Pharmacopoeia, 1998). The results suggest that the *C. alata* leaves that are most suitable for the production of herbal medicines are the young and mature leaves, collected from leaf-positions 1-6.

The effect of drying methods

This study was designed to examine the general drying methods, including artificial heat and open-air drying, that are used for herbal pre-

Table 2. Effect of drying method and temperature on hydroxyanthracene derivatives content in *C. alata* leaves, which were harvested from the leaves positions 4-6 in June.

Drying method/Temperature	% Total hydorxyanthracene derivatives (Mean ± SEM)
Hot air oven	
- 50ºC	1.43±0.01
- 80ºC	0.44±0.01*
Dried in the sun (3 days)	0.95±0.01*

***n* = 3 individual experiments. * Significantly different from the others, *P* < .05.**

Table 3. Stability of hydroxyanthracene derivatives in *C. alata* leaf powder during storage. The leaves were harvested from the leaf-positions 4-6, in June.

Storage period (months)	% Total hydroxyanthracene derivatives (Mean ± SEM)
0	1.62±0.01
3	1.71±0.03
6	1.76±0.08
9	1.61±0.02
12	1.22±0.01*

***n* = 3 individual experiments. * Significantly different from the others, *P* < .05.**

paration. The effect of using artificial heat with a high temperature was also studied. It was found that the temperature and the method of drying both play an important role in the quality of *C. alata* raw material preparation (Table 2). The leaves that had been dried by using a hot air oven at 50ºC contained a higher hydroxyanthracene derivative content (1.43 %w/w) than either those by using the hot air oven at 80ºC (0.44 %w/w) or those that had been dried in the sun for three days (0.95 %w/w). This indicates that the hydroxyanthracene derivatives are not stable at the high temperatures. In the normal drying processes, leaves are generally dried at temperature between 20º and 40ºC. In tropical countries, however, use of lower temperatures in the herbal drying process often promotes the growth of microbes. Thus, in Thailand, herbs are usually dried at a temperature of about 50ºC in a hot air oven. In addition, the duration of the drying process also can have an effect on the stability of some active compounds. In general, drying by artificial heat is more rapid than open-air drying, such artificial drying is often necessary in tropical countries where the humidity may be very high (Evans, 1996). This study therefore suggests that rapid drying helps the *C. alata* leaves to retain a high content of the active compounds, but the temperature used should not be higher than 50ºC due to the lower stability of hydroxyanthracene derivatives under high temperature.

Stability of hydroxyanthracene derivatives in *C. alata* leaf powder

This study looked at the stability of the active compounds, hydroxyanthracene derivatives, which is related to the shelf-life of the herbal medicine. The results showed that when kept in a well-closed container and stored in a dry place at room temperature after nine months, the content of the hydroxyanthracene derivatives in the leaf powder did not decrease significantly, but it decreased by approximately 25% after one year. However, although the content of hydroxyanthracene derivatives decreased after being stored for one year, the final content was still higher than the standard value (1.0 %w/w). This suggests that so long as *C. alata* leaves that contain a high enough content of hydroxyanthracene derivatives (that is, higher than 1.4 %w/w) are used for herbal medicine production, the shelf-life of the leaf powder would be at least one year.

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