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## Establishing the Specifications of Cassia tom Linn. Seeds(การจัดทำข้อกำหนดเฉพาะของเมล็ดชุมเห็ดไทย)

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## ปริญญานิพนธ์

# การจัดทำข้อกำหนดเฉพาะของเมล็ดชุมเห็ดไทย

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## บทคัดย่อ

ได้ศึกษาคุณภาพเมล็ดชุมเห็ดไทยเพื่อจัดทำข้อกำหนดเฉพาะเมื่อกำหนดจำนวนตัวอย่างที่ระดับนัยสำคัญ 0.05 และให้ความผิดพลาดสูงสุดที่ยอมรับให้เกิดเป็นร้อยละ 10 และ 20 ปรากฏว่าในความชื้นร้อยละ 95 ช่วงค่าเฉลี่ยของปริมาณดังต่อไปนี้คือการสูญเสียน้ำหนักเมื่ออบแห้ง, สารสกัดที่ละลายในน้ำ, สารสกัดที่ละลายในเอทานอล, เถ้าทั้งหมด, เถ้าที่ไม่ละลายในกรด, และสิ่งแปลกปลอมเท่ากับ 10.5 - 11.8 %, 20.5 - 22.5 %, 8.6 - 10.8 %, 5.5 - 6.4 %, 0.13 - 0.19 %, และ 1.4 - 2.8 % โดยน้ำหนักตามลำดับ พิสัยของปริมาณโลหะหนักอยู่ในระหว่าง 10 และ 20 ppm นอกจากนี้ สาร emodin จากสารสกัดคลอโรฟอร์มมีการแยกตัวได้ดีโดยวิธีแรงเฉื่อยแบบมิติเดียว และแบบสองมิติ ในโมบิลเฟสต่างกัน 2 ชนิด

## กุญแจคำ

เมล็ดชุมเห็ดไทย, ข้อกำหนดเฉพาะ

*Original Article***Establishing the Specifications of *Cassia tora* Linn. Seeds**

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*\*Division of Drug Analysis, Department of Medical Sciences, Ministry of Public Health, Tiwanond Road, Nonthaburi 11000.***Abstract**

The quality of *Cassia tora* Linn. seeds was investigated in order to set up specifications. The sample estimates at the significance level ( $\alpha$ ) of 0.05 with desired limits of error of 10 % and 20 % were determined. The 95 % confidence intervals for the sample means of the amount of loss on drying, water-soluble extractive, ethanol-soluble extractive, total ash, acid-insoluble ash, and foreign matter were found to be 10.5 to 11.8 %, 20.5 to 22.5 %, 8.6 to 10.8 %, 5.5 to 6.4 %, 0.13 to 0.19 %, and 1.4 to 2.8 % w/w, respectively. The range of the heavy metal values was from 10 to 20 ppm. By means of one-dimensional and two-dimensional thin-layer chromatography, the resolution of emodin in the chloroform extract of the seeds was accomplished with two different mobile phases.

**Key words***Cassia tora* Linn. seeds, Specifications**Introduction**

*Cassia tora* Linn. (Leguminosae), an herb or undershrub up to 1 m high, is a common weed throughout the country. The pods occur in terete, linear shape, 10 to 15 cm long, 0.5 cm wide and the seeds are glossy, rhomboidal, about 5 mm in diameter. Its vernacular names are Chumhet Thai (central) or Lap Muen (northern) or Ya Luek Luen (south-eastern) (1). It is reputed for its medicinal value as laxative, anthelmintic and diuretic (2). The major compounds in the seeds were reported as anthraquinone glycosides, aglycones, naphthapyrone glycosides, fatty acids and sterols (3-6). Poethke et al. (7) revealed the presence of the aglycone chrysophanol, physcion, emodin, aloemodin, aloe-emodin, and rhein in the chloroform and benzene extracts of the seeds.

Seeds of *Cassia tora* Linn. are included in the selection list of the Thai Herbal Pharmacopoeia. In an attempt to establish its specifications, the preliminary study (8, 9) of the amount of loss on drying, water-soluble extractive, ethanol-soluble extractive, total ash, acid-insoluble ash, and foreign matter in a small group of samples has been performed. However, the results were not substantive enough to construct the specifications

due to the limited number of samples. It is thus an aim of this study to use more appropriate sample sizes compared with previous data and to further investigate on the same parameters for more precise and conclusive results. In addition, the range of heavy metals is determined and the seeds identified employing the thin-layer chromatography (TLC) technique with emodin as a marker.

**Materials and Methods*****Materials and apparatus***

Dry mature seeds of *Cassia tora* Linn. were purchased from traditional retail stores both in Bangkok and in other provinces, i.e., Songkhla, Khon Kaen, Udon Thani, Chonburi, and Nonthaburi. The purchase was made from 1998 until 2000. Before subjected to the experiment, they were authenticated by Pharmacognosy Section, Medicinal Plant Research Institute, Department of Medical Sciences. Emodin, Lot 069H3406, was from Sigma Co., USA. Silica gel 60F254 pre-coated plastic sheets were from E. Merck Co., Germany. All chemical reagents were of analytical grade and were used as purchased.

Muffle furnace was from Heraeus and Video Store 2 Version 3.00 was from Camag, Germany.

### ***Determination of loss on drying***

The seeds were ground and sieved (12 mesh or 1.70 mm) before the determination. The procedure described in the Thai Herbal Pharmacopoeia (10) was followed using the 2-g ground seeds. The heating condition of 105°C for 5 hours was employed.

### ***Determination of foreign matter, total ash, acid - insoluble ash, water - soluble extractive, and ethanol - soluble extractive***

The procedures described in the Thai Herbal Pharmacopoeia (10) were followed using the 12-mesh ground seeds.

### ***Identification by TLC***

***Sample preparation*** The 10-g, ground seeds were defatted by either extracting in a Soxhlet apparatus for 2 hours with petroleum ether (35-60 °C) or standing overnight with petroleum ether without heating. The defatted seed powder was refluxed with 100 ml of chloroform for 1 hour. The chloroform extract was filtered and evaporated to dryness on a water-bath. The residue was taken in 1 mL of methanol.

***Standard preparation*** Five mg of emodin was dissolved in 5 mL of methanol.

***One-dimensional development*** A 10- $\mu$ L portion each of sample preparation and standard preparation was chromatographed on a 20-cm  $\times$  20-cm silica gel 60F254 precoated plastic sheet, using a mixture of toluene:ethyl acetate (75:25) as the mobile phase and allowing the solvent front to ascend 15 cm above the line of application. After being developed, the sheet was examined under ultraviolet light (254 nm) and subsequently exposed to ammonia vapour.

The same procedure was repeated on another sheet with a change of the mobile phase to a mixture of hexane:ethyl acetate:glacial acetic acid (75:25:1).

***Two-dimensional development*** The two-dimensional TLC was carried out employing the same chromatographic conditions as those in the one-dimensional TLC. The development in the first direction was performed in a mixture of toluene:ethyl acetate (75:25); whereas in the second

direction, a mixture of hexane:ethyl acetate:glacial acetic acid (75:25:1) was used.

### ***Determination of heavy metals***

The procedure described under Method II of Limit Test for Heavy Metals in the Thai Pharmacopoeia (11) was followed, using the 12-mesh ground seeds. The 5.0, 10.0 and 15.0-mL portions of lead standard solution (1 ppm Pb) were used as the standard preparations.

### ***Statistical analysis of data***

The analysis of variance (ANOVA) and the test for normal distribution of data (Kolmogorov-Smirnov One-sample Test) were performed on a digital computer using the SPSS program (12). The significance level ( $\alpha$ ) was set at 0.05.

### ***Estimation of sample size***

The size of the sample ( $n$ ) was estimated by the method of Cochran (13) using the formula

$$n = t_{\infty}^2 S^2 / d^2$$

where  $d$  is the chosen margin of error in estimating the sample mean,  $S^2$  is the variance from previous sampling of a similar or related population,  $t_{\infty}$  is 1.96 (two-tailed,  $\alpha = 0.05$ ).

## **Results and Discussion**

### ***Physicochemical studies***

Based on the previous data (8, 9), the estimation of sample sizes was calculated by the method of Cochran (13) with the significance level ( $\alpha$ ) of 0.05. The desired limits of error in the sample estimates were 10 percent for the determination of loss on drying, water-soluble extractive, ethanol-soluble extractive, total ash, and acid-insoluble ash and 20 percent for the foreign matter (Table 1). It was desirable to tolerate more for the determination of foreign matter because the variation observed for foreign matter values in the previous data was relatively high (maximum 3.59 % w/w, minimum 0.65 % w/w). In addition, such determination was less precise in comparison to others.

The initial sample sizes estimated for the determination of acid-insoluble ash and foreign matter were relatively large due to the large variations observed in the preliminary test. The source of variation

**Table 1.** Estimation of sample size.

Test	Initial Sample Size	Standard Deviation	Desired Limit of Error	Estimated Sample Size
Loss on drying	6	1.35	10%	5
Water-soluble extractive	7	2.59	10%	6
Ethanol-soluble extractive	7	1.36	10%	16
Total ash	6	0.37	10%	2
Acid-insoluble ash	15	0.04	10%	19
Foreign matter	15	0.88	20%	23

**Table 2.** Results of physicochemical studies.

Test	Number of Samples	Max./Min.	Mean $\pm$ SE	95% Confidence Interval
Loss on drying (% w/w)	23	13.7/8.3	11.2 $\pm$ 0.31	10.5-11.8
Water-soluble extractive (% w/w)	26	27.2/16.2	21.5 $\pm$ 0.47	20.5-22.5
Ethanol-soluble extractive (% w/w)	26	15.4/4.7	9.7 $\pm$ 0.55	8.6-10.8
Total ash (% w/w)	25	9.3/4.4	6.0 $\pm$ 0.22	5.5-6.4
Acid-insoluble ash (% w/w)	25	0.35/0.06	0.16 $\pm$ 0.01	0.13-0.19
Foreign matter (% w/w)	26	8.7/0.4	2.1 $\pm$ 0.34	1.4-2.8

in the foreign matter determination was likely the differences in sample sources, particularly the deliberate adulteration by the retailers. As for the determination of acid insoluble ash, the source of variation was possibly related to the limited sensitivity in measuring the minute amount of ash, which was a common problem in the process. To gain the most practical sample sizes for those particular determinations, additional work employing the same methodology and sampling technique was performed. The results were subsequently combined with the original data. In this manner, the calculation of sample sizes for the determinations of acid-insoluble ash and foreign matter included not only the data from the preliminary study (8, 9) but also those from the additional work.

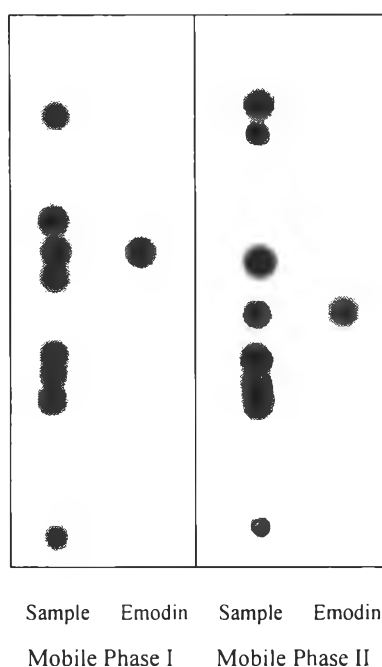
Based on the estimated sample sizes, the amount of loss on drying, water-soluble extractive, ethanol-soluble extractive, total ash, acid-insoluble ash, and foreign matter were obtained as shown in Table 2. The data in Table 2 showed that the distribution of all values was normal ( $p > 0.05$ ). The 95 % confidence intervals for the sample

means of the amount of loss on drying, water-soluble extractive, ethanol-soluble extractive, total ash, acid-insoluble ash, and foreign matter were described in the table.

The range of the heavy metals values found in ten batches of the seeds were from 10 to 20 ppm. The mode of these values was found to be 10 ppm. However, this value only gave information about the probable content of the heavy metals in the seeds of *Cassia tora* Linn. Further work should be done in order to set its limit in the monograph.

#### **Identification by TLC**

The chemical tests and the TLC technique utilizing chrysophanol as a marker have been reported in the previous study (8, 9). However, the claimed efficacy of the seeds as laxative was of more interest. In this study, emodin, one of the anthraquinone aglycones responsible for that effect, was therefore employed as a marker. The isolation of emodin from the defatted seed was readily achieved with chloroform as the extracting solvent. To reach the optimum chromatographic conditions, several mixtures of solvents were experimented to



**Figure 1.** One-dimensional thin-layer chromatograms of the chloroform extract of *Cassia tora* Linn. seeds. The mobile phase I was toluene:ethyl acetate (75:25) and the mobile phase II was hexane: ethyl acetate: glacial acetic acid (75:25:1). The photograph was obtained using Videostore 2 Version 3.00.

**Table 3.**  $hR_f$  Values of components in the chloroform extract of *Cassia tora* Linn. seed.

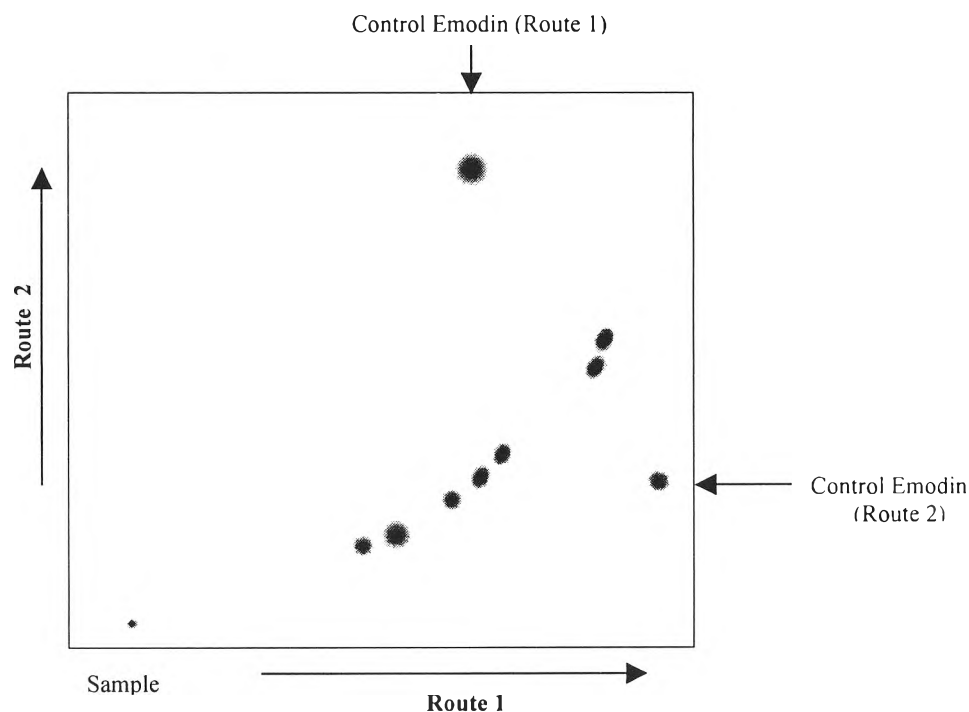
Mobile Phase	Spot	$hR_f$ Value	Detection with	
			UV 254	ammonia vapour
Toluene : ethyl acetate (75:25)	1	26-28	quenching	reddish purple
	2	31-34	quenching	reddish purple
	3	34-37	quenching	yellowish brown
	4	47-54	quenching	dark orange
	5*	59-60	quenching	reddish purple
	6	66-68	quenching	dark orange
	7	89-92	quenching	reddish purple
Hexane : ethyl acetate: glacial acetic acid (75:25:1)	1	19-25	quenching	violet
	2	25-29	quenching	yellowish brown
	3	32-38	quenching	reddish purple
	4*	41-47	quenching	reddish purple
	5	50-55	quenching	reddish purple
	6	75-80	quenching	reddish purple
	7	81-85	quenching	reddish purple

\*corresponding to emodin

permit the migration of emodin to approximately the middle of the TLC plate. It was found that the nonpolar solvent mixtures rendered better separation than the polar ones. The solvent mixture comprising toluene and ethyl acetate (75:25) and the one comprising hexane, ethyl acetate and glacial acetic acid (75:25:1) were the mobile phases

of choice. These two mobile phases offered equally good resolution of emodin as illustrated in Figure 1.

The quenching spot of emodin, when observed under ultraviolet light (254 nm), was revealed as a reddish purple spot after exposing to ammonia vapour. A good correlation of emodin spot in the extract with that of the standard spot was obtained.



**Figure 2.** Two-dimensional thin-layer chromatogram of the chloroform Extract of *Cassia tora* Linn. Seeds. Route 1 was the direction of chromatogram development in the solvent system of toluene: ethyl acetate (75:25); while Route 2 was the direction of chromatogram development in the solvent system of hexane: ethyl acetate: glacial acetic acid (75:25:1). The photograph was obtained using Videostore 2 Version 3.00.

The  $R_f$  values of each spot in the chromatogram were described in Table 3. These values were achieved from running the chromatograms of six batches of the seeds. By means of two-dimensional TLC, the presence of emodin in the seed extract was confirmed. The two solvent systems of different compositions are those used in one-dimensional TLC as mentioned earlier. The correspondence in position and colour of the sample and the standard emodin spot was conceivably a criterion for identification of emodin in the seed extract (Figure 2).

## Conclusion

From the present study, the following specifications of the seed of *Cassia tora* Linn. can be set up,

loss on drying: not more than 11.8% w/w;  
 water-soluble extractive: not less than 20.5% w/w;  
 ethanol-soluble extractive: not less than 8.6% w/w;

total ash: not more than 6.4 % w/w;  
 acid-insoluble ash: not more than 0.2% w/w;  
 foreign matter: not more than 2.8% w/w.

The mode of the heavy metal values was 10 ppm. The one-dimensional TLC using either studied solvent systems could reveal the presence of emodin in the chloroform extract of the seeds. Both systems were proved to be efficient for separation of components in the seed extract.

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