# EXTRACTION METHOD FOR HIGH CONTENT OF ANTHRAQUINONES FROM *CASSIA FISTULA* PODS

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**ABSTRACT:** The ripe pod of *Cassia fistula* Linn. has long been used in traditional medicines as a laxative drug. The active principles are known to be anthraquinone glycosides of which rhein and aloe-emodin are major components. The pulp from ripe pods of *C. fistula* was extracted with 70% ethanol by maceration, percolation, and soxhlet extraction, and by decoction with water according to Thai traditional uses. The contents of total anthraquinone glycosides and total anthraquinones in the crude extracts prepared by each of extraction method were determined using a UV-vis spectrophotometer and the contents were calculated as rhein and aloe-emodin. The extract prepared by decoction method contained the highest content of total anthraquinone glycosides which are the active laxative form in the range of 0.2383 ± 0.0011 and 0.2194 ± 0.0077 %w/w calculated as rhein and aloe-emodin, respectively. Maceration exhibited the extract containing the highest content of total anthraquinones at 0.3139±0.0129 % w/w calculated as rhein and 0.2194±0.0088 % w/w calculated as aloe-emodin. Comparing all extraction methods, decoction is simple, convenient, carried low cost in terms of solvent and instrumentation and found to be the appropiate extraction method for the pulp of *C. fistula* pods for a laxative drug.

**Keywords:** *Cassia fistula*, anthraquinone, extraction method, maceration, decoction, rhein, aloe-emodin

**INTRODUCTION:** *Cassia fistula* Linn. (Caesalpiniaceae) known as Khun in Thai language[1], is a tropical plant which can be found in all parts of Thailand. The ripe pod contains several anthraquinones such as rhein, aloin, emodin, sennosides, and aloe-emodin, both in aglycone and glycoside forms[2,3]. In Thai traditional medicines, the ripe pods have been used as a laxative drug by boiling with water and the mixture is filtered through a muslin cloth. The filtrate is evaporated and the soft extract is made as small pills. *C. fistula* also exhibits antifungal, antibacterial[4], anti-inflammatory[5], and antioxidant[6] activities. The infusion of *C. fistula* pods possess a very low level of toxicity ($LD_{50}$ 6.6 g/kg) compared to a senokot tablet, and also shows no pathological effect on animal organs examined microscopically. Therefore, *C. fistula* pod is safe for using as a laxative drug[7]. The laxative effect is depended on degree of content of total anthraquinone glycosides[8-11]. Thus, it is interesting to investigate the contents of total anthraquinone glycosides and total anthraquinones in the pods of *C. fistula* to find a good source of laxative drug. In this study, we compared four extraction methods i.e. decoction with water according to Thai traditional preparation; maceration, percolation, and soxhlet extraction with 70% ethanol to find a suitable extraction method yielding high contents of total anthraquinones and total anthraquinone glycosides. The contents of anthraquinones determined by UV-vis spectrophotometric method were calculated as rhein and aloe-emodin.

## MATERIALS AND METHODS:

### Plant Materials

The ripe pods of *C. fistula* were collected from Ubonratchathani province, Thailand in May, 2007. The samples were identified by comparison with the plant specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (WCF0507) were deposited at Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

### Extraction Methods

**Decoction:** The fresh pulp (10.0 g) was separated from *C. fistula* pods and boiled with 100 ml distilled water for one hour at 95-98°C. The mixture was filtered through a muslin and the pulp was re-extracted with

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water for several times until the extraction was exhausted (tested by Borntrager's reaction). The decoction extracts were combined, filtered agian and the filtrate was evaporated to dryness on a boiling water bath to yield a decoction crude extract (4.8 g).

**Maceration:** The fresh pulp of *C. fistula* pod (10.0 g) was macerated with 100 ml of 70% ethanol. The extraction was repeated until exhausted. The maceration extracts were combined, filtered and evaporated to dryness on a boiling water bath to yield a maceration crude extract (4.9 g).

**Percolation:** The fresh pulp (10.0g) was moistened with 70% ethanol (30 ml) for 15 minutes. The moistened material was put in a percolator and 70% ethanol was added. The percolation was adjusted at a rate of 1-3 ml/min until the extraction was exhausted. The extracts were combined, filtered and evaporated to dryness on a boiling water bath to yield a percolation crude extract (5.2 g).

**Soxhlet Extraction:** The fresh pulp of *C. fistula* pod (10.0 g) was extracted with 300 ml of 70% ethanol in a soxhlet apparatus. The extraction was continued until the extraction was exhausted. The extracts were then combined, filtered and evaporated to dryness on a hot water bath to yield a soxhlet crude extract (4.3 g).

**Identification of Anthraquinones**

Borntrager's reaction was used to detect anthraquinone aglycones in the extract. Hydrochloric acid (2M) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution. The upper aqueous layer becomes pink- red.

**Quantitative Determination of Total Anthraquinones and Total Anthraquinone Glycosides by UV-Visible Spectrophotometric Method**

**Method validation**

**Linearity:** The calibration curves of rhein and aloe-emodin reference standards were made from 5 concentrations. The concentrations of rhein and aloe-emodin were ranged from 1.92-9.60 µg/ml and 1.56-25.00 µg/ml, respectively. These solutions were added with 0.5% w/v magnesium acetate in methanol and adjusted to 25 ml volume with methanol. Maximum absorbance of each mixture was measured at 515 nm by a UV-vis spectrophotometer. Each sample was done in triplicate. The relation between concentrations and absorbance was plotted. The linearity was evaluated by regression analysis and residual sum of squares.

**Precision**: The precision of the method was determined by repeatability and reproducibility. The repeatability was evaluated by assaying the sample for 6 replicates at the same concentration, twice a day. The reproducibility was evaluated by comparing the assays on three different days. The % R.S.D. was calculated.

**Quantitative Analysis of Total Anthraquinone Glycosides**[12)]

The extract (1.00 g) was accurately weighed and distilled water (30 ml) was added. The mixture was mixed, weighed and refluxed on a water bath for 15 minutes. The flask was allowed to cool, weighed, adjusted to the original weight with water and the mixture was centrifuged at 4000 rpm for 10 minutes. Twenty milliliters of the supernatant liquid was transfered to a separatory funnel and acidified with 2 M hydrochloric acid. Fifteen milliliters of chloroform was added, the mixture was extracted and the chloroform layer was discarded. The extraction was done triplicate. The aqueous layer was separated and 0.10 g of sodium bicarbonate was added. The mixture was then shaken for 3 minutes and centrifuged at 4000 rpm for another 10 minutes. Ten milliliters of the supernatant liquid was transferred to a 100 ml flask. The solution of 10.5% w/v ferric chloride hexahydrate (20 ml) was added and mixed. The mixture was refluxed on a boiling water bath for 20 minutes. Concentrated hydrochloric acid (1 ml) was added and the mixture was heated for 20 minutes, with frequently shaking to dissolve the precipitate. The mixture was cooled, transferred to a separatory funnel and shaken with 25

ml diethyl ether. The partition was repeated until anthraquinones were exhaustively extracted, tested by the Borntrager's reaction. The diethyl ether extracts were combined and washed with 15 ml distilled water twice. The combined diethyl ether was then transferred to a 100 ml volumetric flask and adjusted to volume. Twenty five milliliters of the solution was evaporated to dryness. The residue was dissolved with 10 ml of 0.5% w/v magnesium acetate in methanol yielding a red solution. The UV absorbance was measured at 515 nm.

**Selection of Appropriate Solvent for Extraction of Total Anthraquinones**

Distilled water, 50%, 70% and 95% ethanol were used for extraction total anthraquinones according to step I in scheme 1. Total anthraquinones content in each extract was according to step I in scheme 1. Total anthraquinones content in each extract was determined by UV-vis spectrophotometric method. A solvent promoted the extract with the highest yield of total anthquinones was choosen as the appropriate solvent. The results are shown in Table 1.

**Quantitative Analysis of Total Anthraquinones Content**[12)]

The extract (1.00 g) was accurately weighed and dissolved in 30 ml of distilled water which was a suitable solvent. The analysis procedure was followed the scheme 1.

**Table 1** The contents of total anthraquinones in the extracts prepared using different solvents

| Solvent | Total Anthraquinones (calculated as rhein, % w/w in wet pulp) |
|---|---|
| Distilled water | **0.0995** |
| 50% Ethanol | 0.0938 |
| 70% Ethanol | 0.0863 |
| 95% Ethanol | 0.0186 |

The contents of total anthraquinones and total anthraquinone glycosides in the extracts were calculated using the linear regression equations of reference standards rhein and aloe-emodin. The contents were expressed as mean ± standard deviation (SD).

## RESULTS AND DISCUSSION:

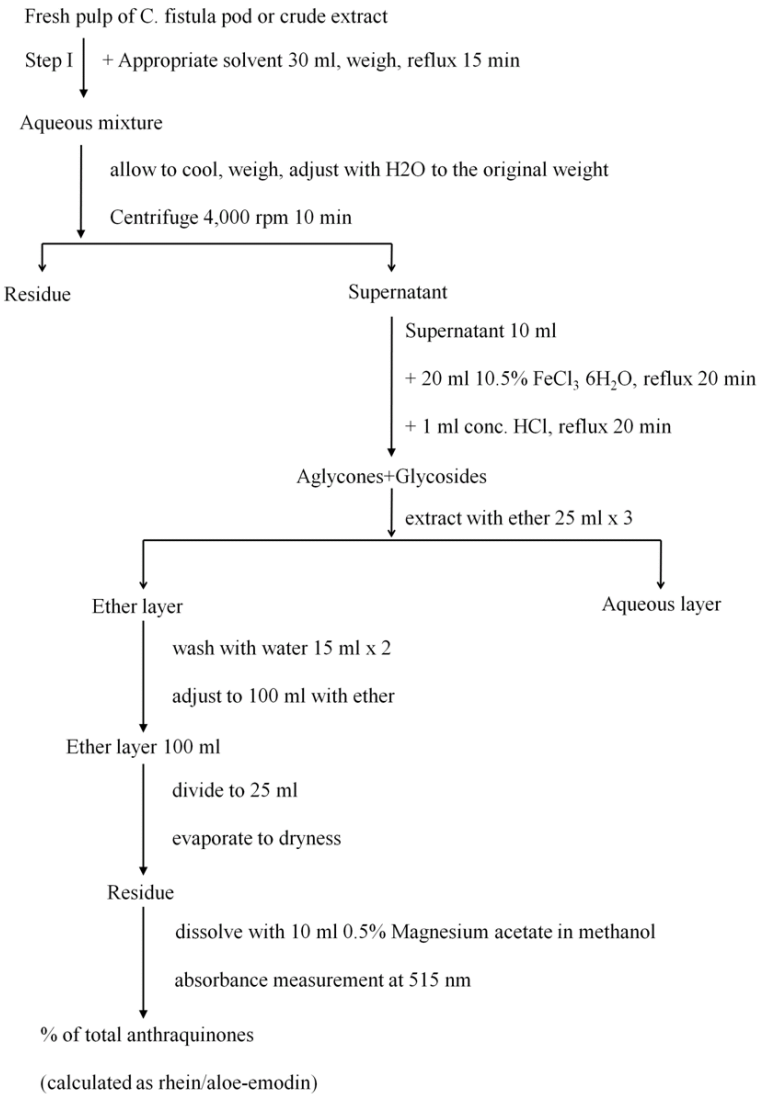
All extraction methods promoted the brownish-black extracts with characteristic odour. The extract ratio (crude drug/crude extract) was 2:1. The yields of crude extract, extraction time consuming, extraction temperature and solvent used for each extraction method are shown in table 2. For method validation, the linear equations of rhein and aloe-emodin reference standards are shown in table 3. The $r^2$ value was more than 0.999 while % R.S.D. was less than 5% for repeatability and reproducibility indicating good linearity, precission and accuracy.

**Table 2** Detail of each extraction method for extracting of the fresh pulp of *C. fistula* pod

| Method | Solvent | Volume of Solvent (ml) | Consumed time (hrs) | Temperature ($^{o}$C) | Weight of crude extract* (%w/w in wet pulp) |
|---|---|---|---|---|---|
| Decoction | Distilled water | 900 | 9 | 95 - 98 | 48.34 ± 0.01 |
| Maceration | 70% ethanol | 600 | 700 | Room temperature | 48.84 ± 0.02 |
| Soxhlet | 70% ethanol | 300 | 312 | 100 | 43.24 ± 0.23 |
| Percolation | 70% ethanol | 2,000 | 240 | Room temperature | 51.76 ± 0.15 |

**Table 3** Method validation

| Reference standards | Linear equation | $r^2$ | %RSD of repeatability | %RSD of reproducibility |
|---|---|---|---|---|
| Rhein | Y = 49209X – 0.0049 | 0.9992 | 1.21 | 1.21 |
| Aloe - emodin | Y = 72051X – 0.0170 | 0.9999 | 0.48 | 0.51 |




**Scheme 1** Quantitative analysis of total anthraquinones[12)]

Anthraquinone compounds in *C. fistula* comprise of both anthraquinone aglycones and anthraquinone glycosides, but only anthraquinone glucosides are active laxative property[8-10)]. The contents of total anthraquinones (total aglycones + total glycosides) and total anthraquinone glycosides are shown in table 4. The highest content of total anthraquinone glycosides, both calculated as rhien and aloe-emodin, was obtained in the extract prepared by decoction method while the highest content of total anthraquinones was found the maceration extract. Anyhow, the contents of total anthraquinones in the extracts prepared by percolation, soxhlet extraction and maceration were not much different, but significantly different from the content in the decoction extract. Anthraquinone glycosides are very soluble in water, while anthraquinone aglycones are freely soluble in ethanol and other organic solvents. The extract from decoction method using water as a solvent gave the equal amount of anthraquinone glycosides and total anthraquinones. This result indicates that water could extract only the glycosides, not aglycones while ethanol 70% (comprises 30 % of water) can extract most of anthraquinone aglycones and some of anthraquinone glycosides. So, maceration, percolation, soxhlet extraction using 70% ethanol as a solvent, yielded total anthraquinones more than total

**Table 4** Quantitative determination of total anthraquinones and total anthraquinone glycosides by UV-visible spectrophotometric method in the extracts prepared by different extraction methods and in the crude drugs, calculated as rhein and aloe-emodin

| Method | Total anthraquinones and total anthraquinone glycosides in crude extract* | | Total anthraquinones and total anthraquinone glycosides in crude drug* | |
|---|---|---|---|---|
| | %w/w calculated as rhein | %w/w calculated as aloe - emodin | %w/w calculated as rhein | %w/w calculated as aloe - emodin |
| Decoction | **0.2387 ± 0.0113** | **0.1803 ± 0.0288** | **0.1152 ± 0.0054** | **0.1032 ± 0.0155** |
| | **(0.2383 ± 0.0011)** | **(0.2194 ± 0.0077)** | **(0.1150 ± 0.0005)** | **(0.1075 ± 0.0053)** |
| Maceration | **0.3139 ± 0.0129** | **0.2194 ± 0.0088** | **0.1536 ± 0.0070** | **0.1536 ± 0.0063** |
| | **(0.2122 ± 0.0013)** | **(0.2122 ± 0.0088)** | **(0.0917 ± 0.0006)** | **(0.1071 ± 0.0065)** |
| Percolation | 0.2967 ± 0.0134 | 0.2122 ± 0.0072 | 0.1532 ± 0.0063 | 0.1532 ± 0.0045 |
| | (0.1503 ± 0.0006) | (0.2075 ± 0.0072) | (0.0762 ± 0.0003) | (0.0918 ± 0.0065) |
| Soxhlet | 0.3033 ± 0.0105 | 0.2075 ± 0.0092 | 0.1312 ± 0.0045 | 0.1312 ± 0.0070 |
| | (0.1259 ± 0.0005) | (0.1681 ± 0.0092) | (0.0544 ± 0.0002) | (0.0811 ± 0.0065) |

*Presented as mean ± SD (n=3), (…) content of anthraquinone glycosides

anthraquinone glycosides. Decoction method gave the highest yield of total anthraquinone glycosides which is the active form for laxative property. Compared all methods, decoction is simple, convenient, less time consuming and carried low cost in terms of solvent, electricity and instrumentation. Thus, decoction should be the recommended extraction method for the pulp of *C. fistula* pods for a laxative drug.

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## วิธีสกัดฝักคูนเพื่อให้ได้สารแอนทราควิโนนปริมาณมาก

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**บทคัดย่อ:** เนื้อในฝักแก่ของคูนใช้เป็นยาระบายในตำรับยาแผนโบราณมานานแล้วโดยมีสาระสำคัญเป็นสารในกลุ่มแอนทราควิโนน กลัยโคไซด์ ซึ่งสารสำคัญได้แก่ เรอินและอะโล-อีโมดิน เนื้อในจากฝักคูนถูกนำมาสกัดด้วยวิธีการสกัด 4 วิธี คือ การหมัก (Maceration) Percolation การสกัดแบบต่อเนื่อง (Soxhlet extraction) ด้วยตัวทำละลาย 70 % เอทานอล และโดยวิธีการต้ม (Decoction) ด้วยน้ำกลั่นโดยเลียนแบบการเตรียมยาระบายในตำรับยาแผนโบราณ จากการวิเคราะห์หาปริมาณแอนทราควิโนนทั้งหมด (Total anthraquinones) และปริมาณแอนทราควิโนนกลัยโคไซด์รวม (Total anthraquinone glycosides) ในสารสกัดเนื้อในฝักคูนที่เตรียมจากวิธีการสกัดทั้งสี่วิธีโดยวิธีวิเคราะห์ด้วย UV-vis spectrophotometer วัดการดูดกลืนแสงที่ความยาวคลื่น 515 nm พบว่า สารสกัดที่ได้จากวิธีการสกัดด้วยการต้มกับน้ำกลั่น มีปริมาณแอนทราควิโนน-กลัยโคไซด์รวม มากที่สุด ที่ร้อยละ 0.2383 ± 0.0011 และ 0.2194 ± 0.0077 โดยน้ำหนัก เมื่อคำนวณในรูปของสารมาตรฐานเรอินและอะโล-อีโมดิน ตามลำดับ ในขณะที่สารสกัดจากวิธีการหมักมีปริมาณแอนทราควิโนนทั้งหมดมากที่สุด ที่ร้อยละ 0.3139 ± 0.0129 และ 0.2194 ± 0.0088 โดยน้ำหนัก เมื่อคำนวณในรูปของสารมาตรฐานเรอินและอะโล-อีโมดิน ตามลำดับ เมื่อเปรียบเทียบวิธีการสกัดทั้งสี่วิธีพบว่า วิธีการสกัดด้วยวิธีการต้ม ให้ปริมาณแอนทราควิโนนกลัยโคไซด์รวมซึ่งเป็นสารออกฤทธิ์เป็นยาระบายในปริมาณมากที่สุด นอกจากนี้วิธีการสกัดแบบการต้มยังเป็นวิธีที่ง่าย สะดวก ใช้เวลาสกัดน้อย ประหยัดในด้านของตัวทำละลาย และค่าไฟฟ้า อีกทั้งเครื่องมือมีราคาถูก ดังนั้นวิธีการสกัดโดยการต้มนี้น่าจะเป็นวิธีที่เหมาะสมสำหรับการสกัดเนื้อในฝักคูน เพื่อใช้เป็นยาระบาย

**คำสำคัญ:** คูน แอนทราควิโนน การสกัด การต้ม การหมัก

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