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Full Length Research Paper

Thin layer chromatography (TLC)-densitometric method for chemical stability evaluation of *Cassia fistula* pod pulp extract: An alternative laxative drug

Savita Chewchinda, Pongtip Sithisarn and Wandee Gritsanapan*

Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand.

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This study aimed to evaluate the chemical stability of *Cassia fistula* Linn. pod pulp extracts which were freshly prepared and were stored under accelerated and real time storage conditions for 6 months according to ASEAN guideline on drug stability study. A thin layer chromatography (TLC)-densitometric method was developed and validated for quantitative analysis of the content of rhein, a major anthraquinone constituent, in the extracts. The TLC was carried out on aluminium sheet of silica gel 60 F_{254} using ethyl acetate:methanol:water (100:17:10, v/v/v) as a mobile phase. The wavelength of the ultraviolet (UV) detector was set at 435 nm. The proposed TLC-densitometric method showed acceptable validation parameters. The content of rhein in the decoction extracts remained more than 95% (96.40 to 99.65%) of the initial amount (0.1446% w/w) for all storage conditions. There was no significant change of the extracts kept in glass vials and in aluminium foil bags. TLC-densitometric method is simple, rapid, sensitive, and economical for routine analysis of rhein content in *C. fistula* pod pulp extracts. The good chemical stability of the extracts indicates the suitability of *C. fistula* pod pulp extract as a raw material for the development of modern herbal laxative formulations.

Key words: *Cassia fistula*, chemical stability, laxative, thin layer chromatography (TLC)-densitometric method.

INTRODUCTION

Cassia fistula Linn. (Fabaceae) is commonly known as golden shower, Indian larburnum, and pudding pine tree. It is a medium-sized, deciduous tree widely grown in tropical and subtropical areas as a popular ornamental plant due to its beautiful, bright yellow flowers. *C. fistula* is widely used in traditional medicines for various medicinal properties. The pulp of the ripe pods possesses a mild, pleasant purgative action (Bahorun et al., 2005). Various biological activities of the pod pulp such as antibacterial, antifungal, antioxidant, antileishmanial, and hypolipidemic activity were reported (Duraipandiyan and Ignacimuthu, 2007; Siddhuraju et al., 2002; Satorelli et al., 2007; Gupta and Jain, 2009). In Ayurvedic medicinal system, *C. fistula* was used against various disorders such as haematemesis, pruritus, diabetes, and other ailments (Alam et al., 1990; Asolkar et al., 1992).

The major anthraquinone constituent in the pod pulp of *C. fistula* is rhein, while minor constituent is sennoside. Anthraquinone compounds are famous for their laxative property which is caused by changing in colonic motility and alteration in colonic absorption, resulting in fluid accumulation that cause diarrhea (Van Gorkom et al., 1998). High concentration of soluble sugars, volatile oils, waxy, and resinous substances were also found in the pod pulp (Bahorun et al., 2005; Rizvi et al., 2009). Gritsanapan and Nualkeaw (2008) reported the content of total anthraquinone glycosides in the ripe pods of *C. fistula* in Thailand in a range of 0.21 to 0.67% (average 0.44) dry weight, and the rhein content analysed by thin layer chromatography (TLC)-densitometric method was 0.05 to 0.14% (average 0.09) dry weight.

C. fistula is a national tree of Thailand and can be

*Corresponding author. E-mail: pywgs@mahidol.ac.th. Tel: +662-644-8678/5530. Fax: +662-644-8701.

found in every region of the country. In Thai traditional medicines, laxative pills obtained by boiling the ripe pod pulp of *C. fistula* with water and the decoction mixture is filtered through muslin cloth, then the filtrate is evaporated to yield a soft extract for making pills (Pongboonrod, 1979). However, it is inconvenient to make these laxative pills in addition to their unpleasant taste and odor.

The stability of herbal extract is necessary to ensure the quality, safety, and efficacy of the product. Physical stability concerns physical appearances such as color, odor, hardness, and degradation of the product. Chemical stability of the extracts depends mainly on the contents of active compounds. Therefore, the aims of this study were to develop and validate a TLC-densitometric method to determine rhein content for chemical stability of the *C. fistula* pod pulp extract and to evaluate its suitability as a raw material for further development of modern alternative herbal laxative formulations. Some characteristics, such as color, odor, and taste of the extracts of various storage conditions were also investigated and compared.

MATERIALS AND METHODS

Plant

The ripe pods of *C. fistula* were collected from Maha Sarakham province, Thailand, in April 2010. They were identified by comparing to herbariums at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimen (WCF0410) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. The ripe pods were cleaned with tap water and the pod pulp (without seed) was separated and kept in a tight container at 4°C until used.

Preparation of *C. fistula* pod pulp extract

Fresh pulp of *C. fistula* ripe pod (20 g) was boiled with distilled water (200 ml) for 1 h at 95 to 98°C and the mixture was filtered. The extraction process was repeated until anthraquinones in the pulp were exhaustively extracted (tested by Borntrager's reaction). The filtrates were combined and evaporated to dryness on a boiling water bath to yield a decoction crude extract. The yield of crude extract was recorded and the extract ratio (weight of pod pulp: 1 g extract) was calculated.

Preparation of sample solution

The decoction extract of *C. fistula* pod pulp (each 0.2 g) was accurately weighed, dissolved in methanol and adjusted to 10 ml in a volumetric flask. All solutions were filtered through a 0.45 µm nylon membrane filter. Sample solution (5 µl/spot) was applied (n = 3).

Preparation of standard solution

Rhein reference standard (Sigma, USA) was accurately weighed for the preparation of stock solution (0.96 mg/ml). Standard working solution of rhein was prepared by diluting 0.3 ml of the stock solution with methanol in a 10 ml volumetric flask to obtain working standard solution at the concentration of 28.8 µg/ml.

Instrumentation and chromatographic condition

TLC was carried out on an aluminium sheet of silica gel 60 F_{254} (20 × 10 cm with 0.2 mm thickness; E. Merck, Darmstadt, Germany). Sample and standard solutions were applied on the plate as 7 mm band with a Linomat V automatic sample spotter (Camag, Switzerland) under nitrogen flow, positioned at 10 mm from the bottom of the plate. The mobile phase ratio of ethyl acetate: methanol:water is 10:17:10 v/v/v. The plate was developed to a distance of 8 cm in a Camag twin trough chamber. Denstiometric scanning was performed by using a TLC Scanner 3 (Camag, Switzerland) with winCATS software. The wavelength of the detector was set at 435 nm.

Method validation

The method was validated by the evaluation of linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ) according to the International Conference on Harmonization guideline (2005).

Linearity

From a working standard solution of rhein (28.8 µg/ml), 1 to 8 µl of the solution was applied on the plate, corresponding to concentrations of 28.8 to 230.4 ng/spot. The calibration curves were obtained by plotting the peak areas versus the concentrations of the standard solutions.

Precision

The precision was determined by analyzing 28.8, 115.2, and 230.4 ng/spot of the rhein standard solution after applying on a TLC plate (n = 3) on the same day for intraday precision and on 3 different days for interday precision. The precision was expressed as percent relative standard deviation (%RSD).

Accuracy

Accuracy of the method was confirmed by determination of recovery. The recovery of rhein from the extract was performed on sample spiked with three concentration levels of standard rhein (approximately 50, 100, and 150% of the determined content of the *C. fistula* extract solution) (n = 3).

LOD and LOQ

LOD and LOQ were determined by scanning the blank (methanol) spot and noise. Signal-to-noise ratios of 3:1 and 10:1 were considered as LOD and LOQ, respectively.

Stability study of *C. fistula* pod pulp extract

Stability study condition

Three batches of *C. fistula* pod pulp extract were kept in glass vials and aluminium foil bags at the accelerated (40 ± 2°C/75 ± 5%

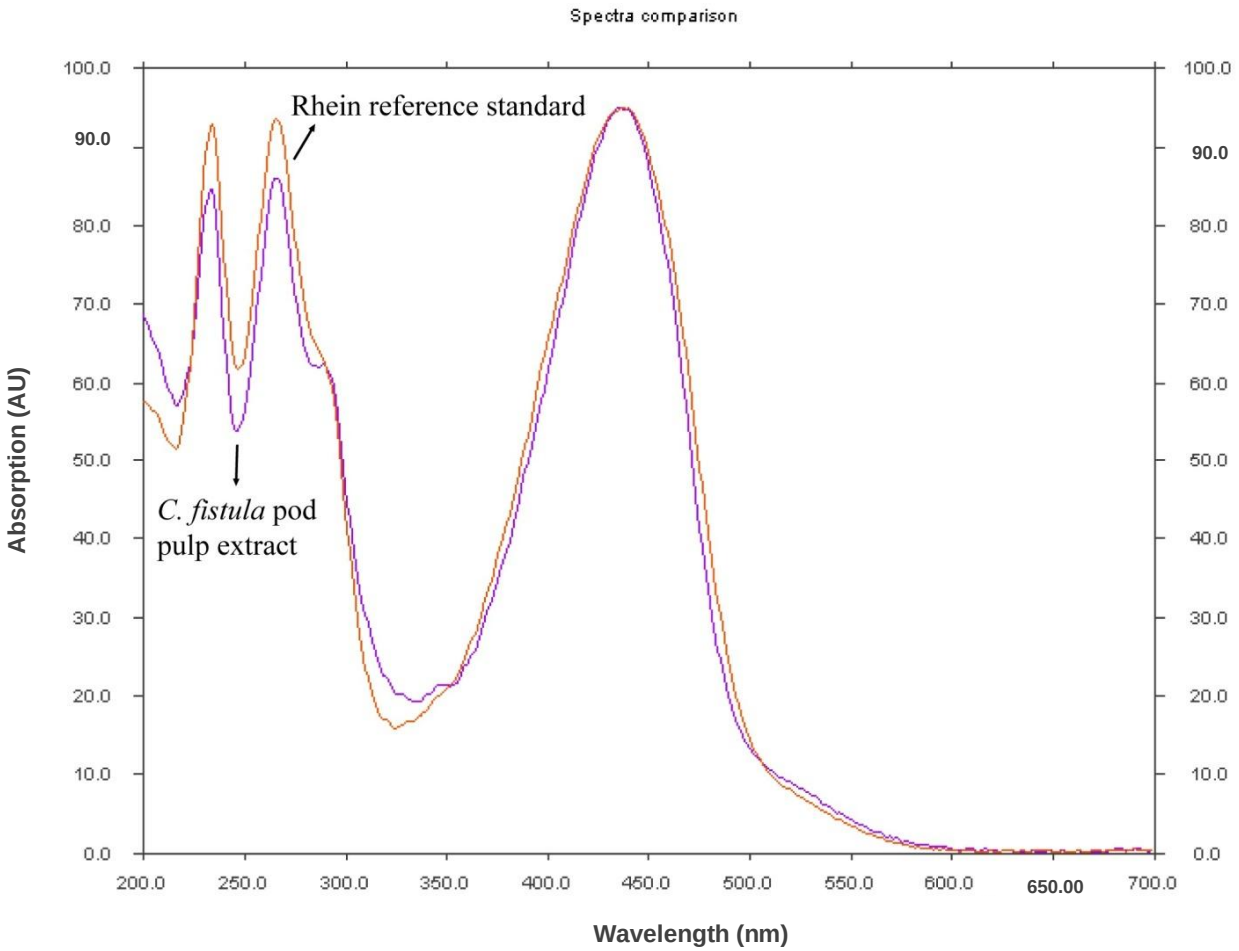

Figure 1. Overlay UV absorption spectra of rhein reference standard and *C. fistula* pod pulp extract.

relative humidity (RH)) and real time storage conditions (30 ± 2°C/75 ± 5% RH) as described in ASEAN guideline on stability study of drug product (2005).

Chemical stability evaluation

The extract of *C. fistula* pod pulp of each storage condition was analyzed for rhein content by the proposed TLC-densitometric method. The peak areas of standard and sample were compared to obtain the concentration of samples.

RESULTS AND DISCUSSION

TLC was developed using the mobile phase of ethyl acetate:methanol:water (100:17:10, v/v/v), and it yielded acceptable resolution and separation of the components in the extracts. The specificity of the bands of rhein (Rf = 0.49) in the *C. fistula* pod pulp extracts was confirmed by overlaying the absorption spectra of the extract with rhein reference standard solution (Figure 1). The maximum absorption of rhein was at 435 nm; and this wavelength was chosen for the analysis. Densitograms of standard rhein and other constituents in the decoction extract of *C. fistula* pod pulp are as shown in Figure 2. The proposed TLC-densitometric method showed acceptable validation parameters (Table 1). The calibration curve of rhein was linear over the range of 28.8 to 230.4 ng/spot. The correlation coefficient value was ≥ 0.999, confirming the linearity of the method (Figure 3). The percentage of relative standard deviation value of intraday and interday precisions was lower than 2% and the average recovery of rhein was 101.21 ± 2.58%, indicating the high precision and accuracy of the method. LOD and LOQ were found to be 3.16 and 10.52 ng/spot, respectively.

According to the evaluation criteria of ASEAN guideline on stability study of drug product (ASEAN countries, 2005), significant change is defined at more than 5% difference from its initial value and any degradation product exceeding the acceptance criteria or failure to meet the acceptance criteria of appearance, physical attributes, and functionality tests (e.g. color, phase separation, resuspendability, caking, hardness).

The contents of rhein in *C. fistula* pod pulp extracts of various storage conditions remained more than 95%

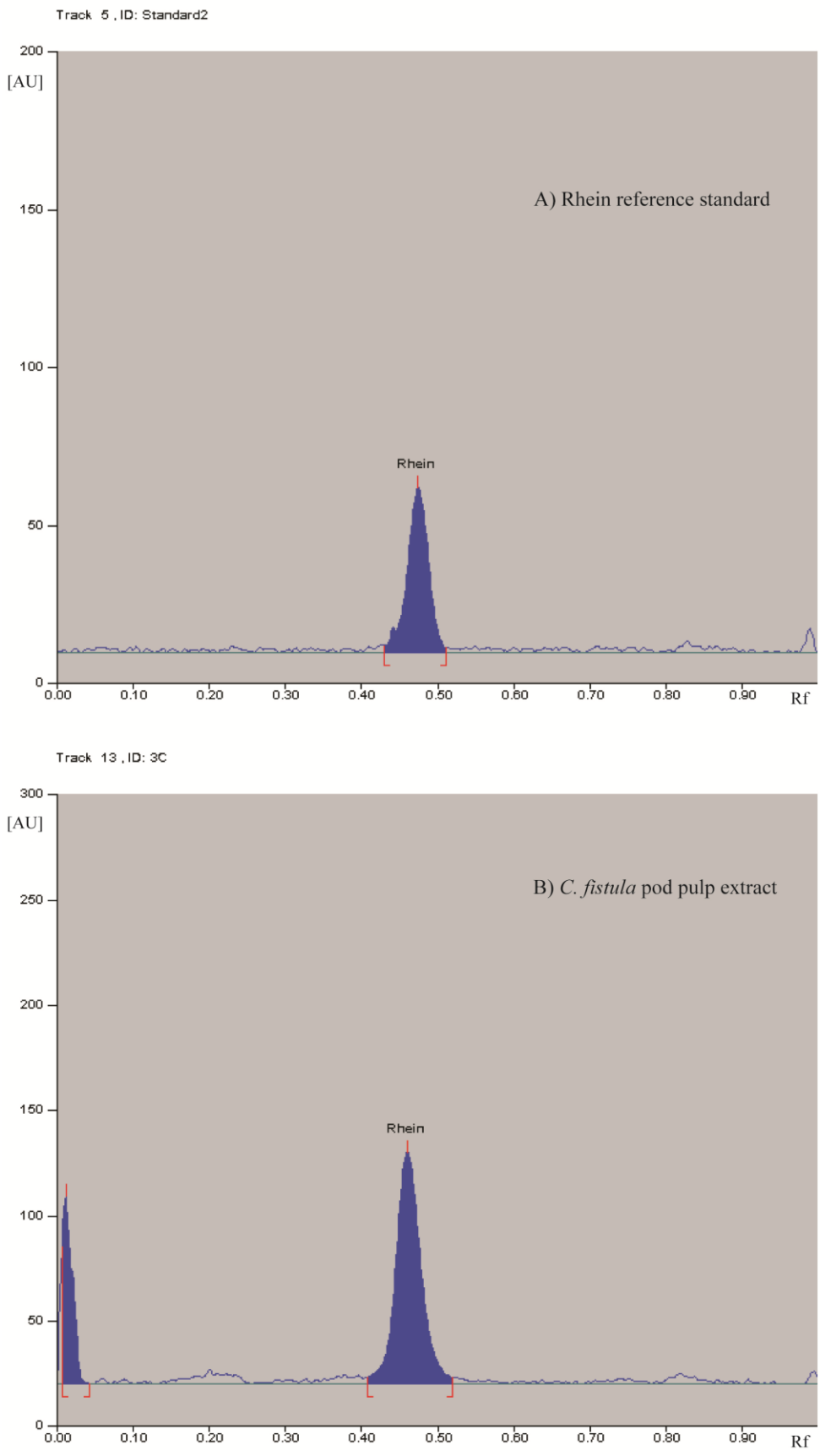

Figure 2. Densitograms of (A) Rhein reference standard and (B) *C. fistula* pod pulp extract.

Table 1. Method validation parameters by the proposed TLC-densitometric method.

Parameter	Result
Range of linearity	28.8-230.4 ng/spot
Regression equation	Y = 244.541 + 19.049X
Correlation coefficient (r^2)	0.99976
% RSD intraday precision (n = 6)	0.59%
% RSD interday precision (n = 3)	1.87%
% recovery	101.21 ± 2.58%
Limit of detection	3.16 ng/spot
Limit of quantitation	10.52 ng/spot

X = Concentration of rhein in ng/ml, Y = peak area.

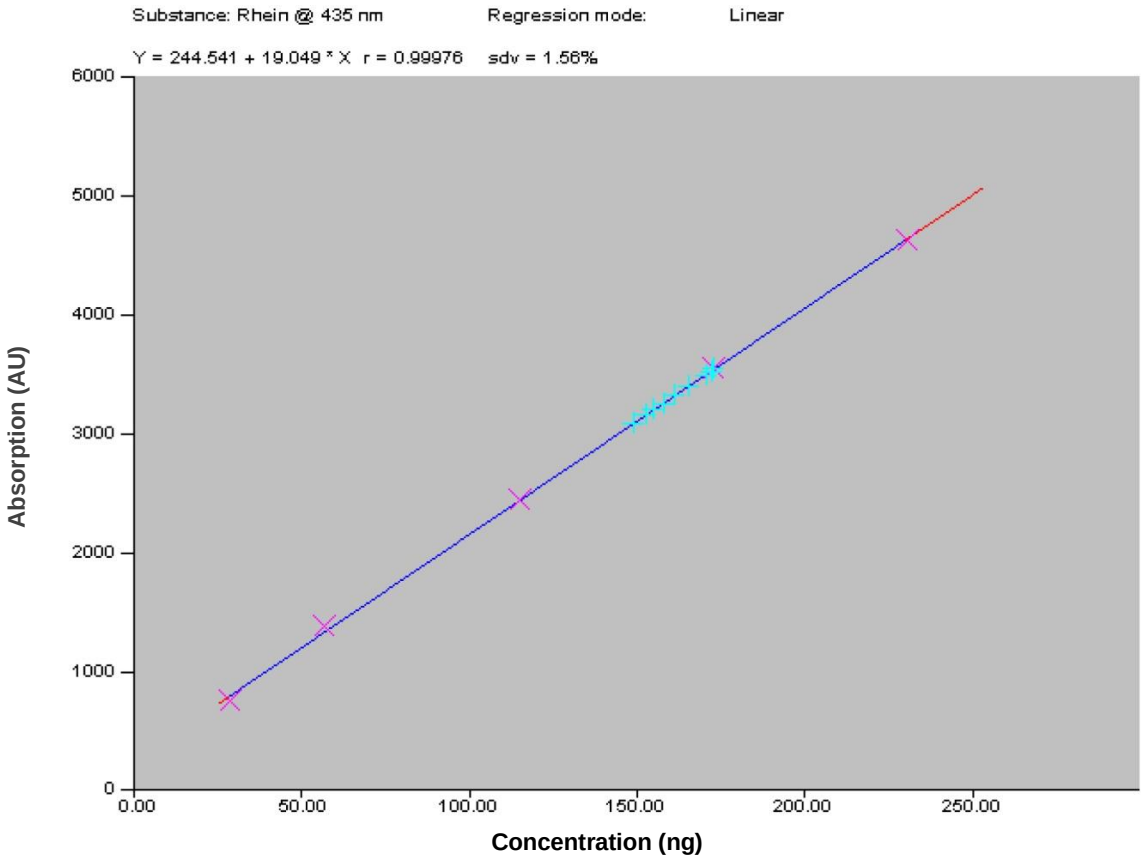

Figure 3. Calibration curve of rhein standard (λ_{max} = 435 nm).

(96.40 to 99.65%) of the initial content (0.1446 ± 0.0099%, w/w) (Table 2). From the results, there was no significant change of the extracts kept in glass vials and in aluminium foil bags and the acceptance criteria were met. Regarding physical attributes, all of the pod pulp extracts had brownish-black, semi-solid with characteristic odor and mild sweet taste. After 6 months of storage under previously mentioned conditions, no significant change was found, indicating good physical and chemical stabilities of the *C. fistula* pod pulp extract. Storing the extract in a glass vial or in an aluminium foil bag promoted no difference on the stabilities of the extract.

Conclusion

TLC-densitometric method is a simple, rapid, sensitive,

Table 2. The content of rhein in *C. fistula* pod pulp extracts of both storage conditions for 6 months determined by the validated TLC-densitometric method.

Time (month)	Packaging	Storage condition	Rhein content (%, w/w)[a]	Color/Characteristics
0	Glass vial	-	0.1446 ± 0.0099 (100%)	Brownish-black/semi-solid
	Aluminum foil	-		
3	Glass vial	Real time	0.1426 ± 0.0108 (98.62%)	brownish-black/semi-solid
	Aluminum foil	Real time	0.1441 ± 0.0096 (99.65%)	
	Glass vial	Accelerated	0.1408 ± 0.0100 (97.03%)	
	Aluminum foil	Accelerated	0.1432 ± 0.0095 (97.93%)	
6	Glass vial	Real time	0.1403 ± 0.0075 (97.37%)	brownish-black/semi-solid
	Aluminum foil	Real time	0.1416 ± 0.0082 (99.03%)	
	Glass vial	Accelerated	0.1394 ± 0.0086 (96.40%)	
	Aluminum foil	Accelerated	0.1397 ± 0.0091 (96.61%)	

(…) Content of rhein in *C. fistula* extract when compared to 100% at day 0, [a]Expressed as mean ± SD (n = 3).

and economical alternative method for routine analysis of rhein content in *C. fistula* pod pulp extracts and the products containing them. The extract was physically and chemically stable after 6 months of storage at the accelerated and real time storage conditions, which could be implied that *C. fistula* pod pulp extract shall enable a tentative shelf-life of 24 months (World Health Organization, 1996). Glass vials and aluminium foil bags promoted no difference on the stability of the pod pulp extracts. This indicated that the decoction extract, prepared in the same way as the traditional laxative drug from *C. fistula* pod pulp, has good physical and chemical stabilities and is suitable for further development as modern pleasant alternative laxative products. TLC-densitometry is convenient for the analysis of rhein content in the pod pulp extract during the stability studies.

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REFERENCES

Alam M, Siddiqui B, Hussian W (1990). Treatment of diabetes through herbal drugs in rural India. Fitoterapia 61:240-242.

ASEAN Countries (2005). ASEAN Guideline on stability study of drug product. 9th ACCSQ-PPWG Meeting, the Philippines.

Asolkar V, Kakkar K, Chakre J (1992). Second supplement to glossary of Indian medicinal plant with active principles. In Publication and Information Directorate, CSIR, New Delhi, India p.177.

Bahorun T, Neergheen VS, Aruoma OI (2005). Phytochemical constituents of *Cassia fistula*. Afr. J. Biotechnol. 4:1530-1540.

Duraipandiyan V, Ignacimuthu S (2007). Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant. J. Ethnopharmacol. 112:590-594.

Gritsanapan W, Nualkaew S (2008). Variation of anthraquinone content in the leaves and pods of *Cassia fistula*. Planta Med. 74:1085.

Gupta UC, Jain GC (2009). Study on hypolipidemic activity of *Cassia fistula* legume in rats. Asian J. Exp. Sci. 23(1):241-248.

ICH steering Committee (2005). ICH Q2(R1). Validation of analytical procedures: text and methodology. International Conference on Harmonization, Geneva.

Pongboonrod S (1979). Mai Tet Meuang Thai. Kasembannakit Press, Bangkok, Thailand pp.137-139.

Rizvi A, Irshad M, Hassadi E, Younis B (2009). Bioefficacies of *Cassia fistula*: an Indian labrum. Afr. J. Pharm. Pharmacol. 3:287-292.

Satorelli P, Samanta PA, Marcia SC, Frederico OP, Andre GT (2007). Isolation of antileishmanial sterol from the fruits of *Cassia fistula* using bio-guided fractionation. Phytother. Res. 21(7):644-647.

Siddhuraju P, Mohan PS, Becker K (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem, bark, leaves, flowers and fruit pulp. Food Chem. 79(1):61-67.

Van Gorkom BAP, De Vries EGE, Karrenbeld A, Kleibeuker JH (1998). Anthranoid laxatives and their potential carcinogenic effects. Aliment Pharmacol. Ther. 13(4):443-452.

World Health Organization (WHO) (1996). Expert Committee on Specifications for Pharmaceutical Preparations. WHO Technical Report Series. Thirty-fourth report, Geneva p.863.