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COMPARISON OF HPLC AND TLC DENSITOMETRIC METHODS FOR THE QUANTIFICATION OF RHEIN IN *CASSIA FISTULA* POD PULP EXTRACT

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KEYWORDS: *Cassia fistula*, HPLC, rhein, TLC densitometric method

INTRODUCTION

Cassia fistlula Linn. (Fabaceae) is commonly known as "Khun" and "Ratchapruek" in Thai. *C. fistula* pod pulp is used in traditional medicine as a purgative/laxative drug and also used against various disorders such as skin diseases, diabetes and other ailments [1,2]. The major anthraquinone derivative in the pod pulp is rhein[3,4]. Although high performance liquid chromatography (HPLC) is the most widely used method for quality assessment of herbal preparations, it requires high operational cost and skilled operator. Therefore, the purpose of this study is to compare the quantitative results obtained by validated HPLC and TLC densitometric methods in the analysis of rhein content in the extracts of *C. fistula* pod pulp. From our data, there is no significant difference ($P > 0.05$) between the mean content of rhein measured by HPLC and TLC densitometric methods. Thus, TLC densitometric method could be alternatively used as a routine analysis of rhein in *C. fistula* pod pulp. Moreover, TLC densitometric method is simple, less expensive and can provide analytical data faster than HPLC.

MATERIALS AND METHODS

Plant Materials Six samples of ripe pods of *C. fistula* were collected from Nakhon Si Thammarat province, Thailand, in March 2010. They were identified by comparing with herbarium specimens at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimens (WCF0401-6) were 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 Each sample of fresh pulp of *C. fistula* ripe pod (20 g) was boiled with distilled water (200 mL) for one hour at 95-98°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 Decoction extracts of samples of *C. fistula* pod pulp (each 0.1 g) were accurately weighed, separately dissolved in 60% (v/v) methanol, then adjusted to 10 mL in volumetric flasks for both methods. Each solution was filtered through a 0.45 µm nylon membrane filter and analyzed in triplicate.

Preparation of Standard Solution Rhein reference standard was accurately weighed and dissolved in methanol in a volumetric flask as a stock solution (1 mg/mL). Standard working solutions of rhein were prepared by diluting the stock solution with 60% (v/v) methanol in the range of 1.25-20 µg/mL and 25-250 ng/spot for HPLC and TLC densitometric analysis, respectively.

HPLC Method A validated HPLC analysis[5] was performed on a Shimadzu Technologies modular model Class *VP* system consisting of a SCL-10A system, a UV-vis SPD-10A detector, LC-10 AD and auto injector SIL-10A (Shimadzu, Japan). The analysis was carried out using a BDS Hypersil C18 column (250×4.6 mm, i.d. 5 µm) (Thermo Fisher Scientific Inc., USA) with a BDS Hypersil C18 guard column (10×4 mm, i.d. 5 µm) (Thermo Hypersil-Keystone, USA). The isocratic mobile phase was 0.5% aqueous acetic acid solution and methanol (40:60). The total running time was 30 min and the flow rate was 1.0 mL/min. The UV detector monitored at 435 nm while the injection volume was 20 µL.

TLC Densitometric Method Thin layer chromatography was performed on an aluminum sheet of silica gel60 F_{254} (20 cm x 10 cm). 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 consisted of ethyl acetate-methanol-water (100:17:10, *v/v/v*). The plate was developed to a distance of 8 cm in a Camag twin trough chamber. The densitometric scanning was performed by using a TLC Scanner 3 (Camag, Switzerland) with winCATS software. The

wavelength of the detector was set at 435 nm. The slit dimension was 6.00 x 0.45 mm and the scanning speed was 20 mm s^{-1}. The sample was applied at 10 µL/spot. The used method was validated as reported in our previous work[6)]

Statistical Analysis The mean values of rhein content in *C. fistula* pod pulp extracts determined by HPLC and TLC densitometric methods were tested by paired *t*-test at 95% confident level.

RESULTS AND DISCUSSION

The contents of rhein in *C. fistula* pod pulp extracts were in the range of 0.0851-0.1147% w/w. HPLC chromatograms of the extract showed similar pattern with a major peak of rhein at a retention time of 15 minute. Regarding the quantitative analysis of rhein content by the validated TLC densitometric method, rhein was found in the TLC chromatogram at Rf = 0.49 separated from other components (**Figure 1**). The contents of rhein in the six samples of *C. fistula* pod pulp extracts analyzed by both methods are shown in **Table 1**. The paired *t*-test showed no statistically significant difference ($P > 0.05$) between the mean contents of rhein performed by HPLC method and TLC densitometric method. In conclusion, the proposed TLC densitometric method could be used as an alternative method for the quantitative analysis of rhein content in *C. fistula* pod pulp extract. This method showed several advantages such as simplicity, fast data acquisition, and high efficacy.

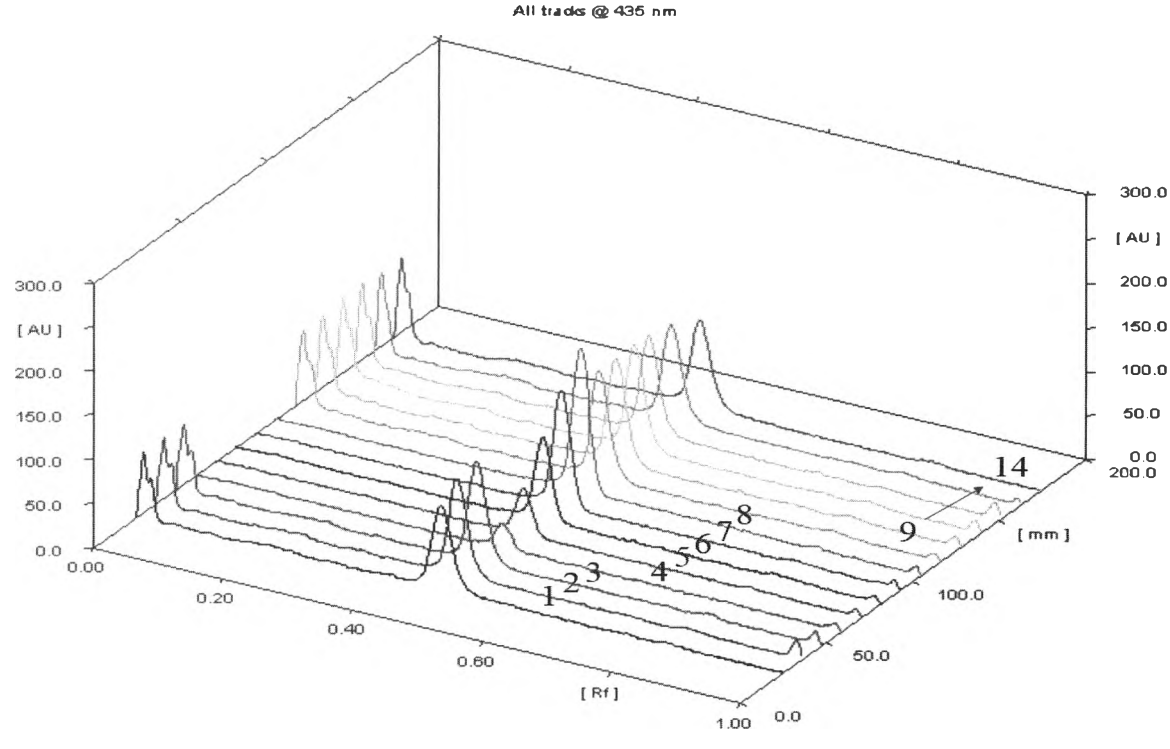

Figure 1 3D TLC densitogram of rhein standard (track no. 4-8) and *C. fistula* pod pulp extract (sample#1 lot 1= track no. 1-3, lot2 = track no.9-11, lot 3= track no. 12-14)

Table 1 The contents of rhein in 6 samples of *C. fistula* pod pulp extracts analyzed by HPLC and TLC densitometric methods

Sample no.	Content of rhein (% w/w)*	
	HPLC	TLC densitometry
1	0.1147± 0.004	0.1041 ± 0.006
2	0.1069 ± 0.002	0.0945 ± 0.003
3	0.0965 ± 0.001	0.0973 ± 0.004
4	0.0998 ± 0.002	0.0986 ± 0.006
5	0.0929 ± 0.003	0.1041 ± 0.004
6	0.0851 ± 0.001	0.0846 ± 0.001
average	**0.0993 ± 0.002**	**0.0972 ± 0.004**

*Expressed as mean ± SD (n = 3)

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