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Development and Validation of a HPTLC method for determination of Karanjin in *Pongamia pinnata*: A novel Indian medicinal plant

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ABSTRACT

Pongamia pinnata is a popular Indian medicinal plant used for treatment of bronchitis, whooping cough, rheumatic arthritis, and diabetes diseases. Karanjin in one of the prevalent phytoconstituent present in this plant. A sensitive, selective and precise thin-layer chromatographic method has been developed and validated for the analysis of Karanjin in Pongamia pinnata. Separation and quantification was achieved by TLC using mobile phase of ethanol-ethyl acetatate (7:3, v/v), (R_F 0.69) on precoated silica gel 60F_{254} aluminium plates and densitometric determination was carried out in reflection/absorption mode at 260 nm. The calibration curve was linear in the concentration range of 0.1µg-1.2µg spot[1]. The method was validated for precision, repeatability and accuracy. The proposed method was found to be simple, precise, specific, sensitive and accurate for the quantification of Karanjin.

Key words: Karanjin; *Pongamia pinnata;* Standardization; HPTLC.

INTRODUCTION

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine [1]. *Pongamia pinnata*, locally known as karanja, is a mangrove plant belonging to the family Fabaceae and is an important medicinal plant of India and has been largely used in the traditional Indian system of medicine (ayurveda) for bronchitis, whooping cough, rheumatic arthritis, and diabetes [2]. A review investigation by Chopade et al., [3] reveals that there is presence of karanjin, pongamol, pongagalabrone,, pongapine, pinnatine and kanjone which are responsible for various pharmacological activity. Karanjin is a furanoflavone, of this plant possesses insecticidal and antibacterial properties and is highly toxic to fish, shows activity against both Gram positive and Gram negative bacteria [4]. Standardization based on the pharmacological active chemical is an essential aspect of assessing the quality of the drugs and it is also well known that different batches of collected plant material may vary both in their chemical constituents [5]. So uniformity is required in the manufacture of herbal medicines. Further, the increasing demand of the population and the chronic shortage of authentic raw material have increased the importance of standardization [6]. Nowadays HPTLC is a routine analytical technique. It has been well reported that several samples can be run

simultaneously by use of a smaller quantity of mobile phase than in HPLC [7, 8, 9]. It has also been reported that mobile phases of pH 8 and above can be used for HPTLC. Another advantage of HPTLC is the repeated detection (scanning) of the chromatogram with the same or different conditions. Consequently, HPTLC has been investigated for simultaneous assay of several components in a multi-component formulation [10-12]. In this study, therefore, a quantitative HPTLC method was established for analysis of karanjin content in *Pongamia pinnta* obtained from tribal belt of Chhattisgarh.

MATERIALS AND METHODS

Collection of Samples

Pongamia pinnta sample root bark was collected from dense forest of Achanakmar foothills located at Chhattisgarh State (India) and authenticated by Dr N Shiddamallayya of Regional Research Institute (Ay.), Bangalore (India), where Voucher Specimen (No. *RRCBI/Mus/15*) of the plant had been submitted.

Chemicals

All chemicals were of analytical grade and obtained from Sigma Aldrich Mumbai and used with out further purification. The HPTLC plates Si 60F254 (20 cm × 20 cm) were purchased from E. Merck (Darmstadt, Germany) supplied by Anchrom Technologies, Mumbai, India).

Preparation of extract for analysis of Karanjin in Samples

To determine the content of karanjin, 2g of powder was extracted with ethanol. To ensure complete extraction of the drug, it was sonicated for 30 min and volume was made up to 30 ml. The resulting solution was centrifuged at 3000 rpm for 5 min and supernatant was analyzed for drug content. The filtered solution was applied on TLC plate followed by development and scanning. Analysis was repeated in triplicate [13].

Preparation of standard solution

A stock solution of 1mg/10ml of karanjin was prepared in ethanol, and different amounts 1,5,10 and 12 µl were spotted in triplicate on TLC plate, using Camag Linomat V sample applicator (Camag, Muttenz, Switzerland) and a 100µl Hamilton syringe for preparing four point calibration curve.

HPTLC instrumentation

The HPTLC system (Camag, Muttanz, Switzerland) consisted of (i) TLC scanner connected to a PC running WinCATS software under MS DOS; (ii) Linomat IV sample applicator using 100µl syringes and connected to a nitrogen tank. Each plate accommodated 8 tracks of samples and standards, applied according to following settings: band width 5 mm; distance between bands 10 mm; application volume 1-12 µl; gas flow 150 nl/s. The plates were developed to 7 cm in a twin trough glass chamber presaturated with the upper layer of mixture ethanol-ethyl acetate (7:3, v/v). The scanner was set for maximum light optimization and with the following settings: slit dimension, 5.00 mm × 0.45 mm, micro; scanning speed, 20 mm/s; data resolution, 100µm/step; scanning wave length, 260 nm in absorbance reflectance mode. All remaining measurement parameters were left at default settings. Regression analyses and statistical data were generated by the WinCATS Planar chromatography version 1.1.5.0 software.

Development of the optimum mobile phase

The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity.

Calibration plot of Karanjin

A stock solution of 1mg/10ml of Karanjin was prepared in ethanol. Different volumes of stock solution, 1,5,10 and 12 µl were spotted in triplicate on TLC plate to obtain concentrations of 0.1, 0.5, 1 and 1.2 µg spot^{-1} of Karanjin, respectively. The data of peak area versus drug concentration were treated by linear least-square regression.

Purity of Spot in chromatogram

The spot obtained on the chromatogram were analysed at wavelength 260 nm at three points in the standard as well as in sample i.e. in the point start to middle, middle and finally in the middle to end.

VALIDATION OF THE METHOD

Linearity, limits of detection and quantification

The linearity of the detector response for the prepared standards was assessed by means of linear regression regarding the amounts of each standard, measured in µg, and the area of the corresponding peak on the chromatogram (n=4). Linearity was also confirmed for '*Pongamia pinnata*' extract. After chromatographic separation, the peak areas obtained were plotted against the extract concentrations by linear regression. Limits of detection and quantification were determined by calculation of the signal to noise ratio. Signal-to-noise ratios of approximately 3:1 and 10:1 were used for estimating the detection limit and quantification limit, respectively, of the method.

Accuracy

Recovery studies were carried out to check accuracy of the method. Recovery experiments were performed by adding three different amounts of karanjin i.e., 25, 50 and 75% of the amount of karanjin analysed from different formulations and result was analysed (n=6).

Precision

The intra-day precision was evaluated by analysing karanjin repeatedly at concentration range of 5-50 µg/spot (n=5). The inter-day precision was evaluated by analysing karanjin at concentration range of 5-50 µg/spot over a period of 10 days (n=5).

RESULTS AND DISCUSSION

The mobile phase ethanol: ethyl acetate (7:1, v/v) provided good resolution with R_F value 0.17 for karanjin but typical peak nature was missing. Finally, the mobile phase consisting of ethanol-ethyl acetate (7:3, v/v) provided a sharp and well defined peak at R_F value 0.69 **(Fig 1B).** The well defined spot was obtained when the chamber was saturated with the mobile phase for 15 min at room temperature.

Karanjin showed a good linear relationship over the concentration range 0.1µg-1.2µg per spot with respect to peak area (n=3). The linearity was observed with the regression coefficient being 0.9986 with Standard error of the mean (SEM) of 0.0044. No significant differences were observed in the slopes of standard curve. Purity of each spot which is scanned at wave length 260 nm with value of r (S, M) within the range, 0.988-0.989 and r (M, E) within the range, 0.987-0.997**(Fig 1A).**

Fig-1A

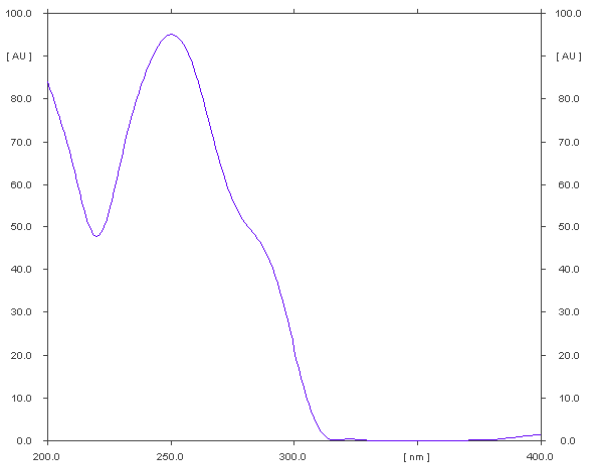

Fig-1B

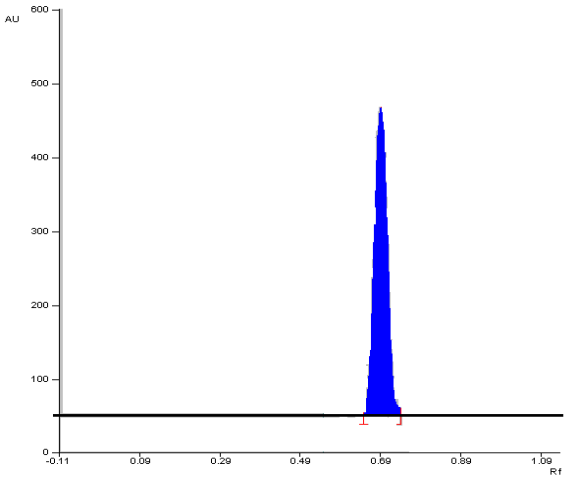

Fig-1A Peak purity spectra of karanjin at 260 nm, Fig-1B Typical HPTLC Chromatogram of karanjin

LOD, LOQ, accuracy and precision were evaluated for quantitative purposes. LOD (0.0101µg) and LOQ (0.0739 µg) suggests high efficiency of the process. Accuracy in terms of % recovery (97.19±1.204) which shows high extraction efficiency of karanjin. The % coefficient of variance for intra-day and inter-day precision was found to be 4.2 and 5.2 respectively which is comparable and within the limits. Hence, the proposed method can be used for estimation of karanjin in *Pongamia pinnata* root bark. The karanjin content in *Pongamia pinnata* studied was found to be 2.37±0.066 in µg /g sample. Which clearly indicates that there is very less quantity is present, it may be due to varied geographical locations where these plants grow. The proposed HPTLC technique is found to be precise and accurate. Further, the method is sensitive for the analysis of karanjin in crude drugs as well as pharmaceutical formulations containing Pongamia pinnata. With the growing demand of herbal drugs in the herbal drug market and with the increased belief in the usage of green medicine (herbal drugs), this standardization tool will help in maintaining the quality and batch to batch consistency of this important Indian medicinal plant.

CONCLUSION

The HPTLC method developed here for the quantification of karanjin in *Pongamia pinnata* is simple, rapid, cost-effective and easily adaptable for screening and quantitative determination than any other analytical technique.

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