Short Report 短篇报道

Identification and quantification of adrenaline from the leaves of *Clerodendrum phlomidis* using thin-layer chromatography

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In the Ayurvedic, Siddha and other medical systems of India, products derived from herbs, used either as active ingredients or as adjuvants, hold paramount importance as alternative medicines. However, their standardization poses a great challenge. Correct identification of these drugs is often problematic in both their complete form as well as in powder form, since these medicinal plants are known by a variety of vernacular names and frequently many medicinal plants share the same name. Comparative thin-layer chromatography (co-TLC) with chemical or biological marker compounds can be used to standardize raw materials. Moreover, due to its simplicity, accuracy, cost effectiveness and rapidity, TLC is often used as an alternative to other chromatographic techniques for quantifying plant products.

Clerodendrum phlomidis Linn. f. (syn. *Clerodendrum multiflorum* (Burm. f) O. Kuntze, abbr. *C. phlomidis*) of the Lamiaceae family is an important and well-known medicinal plant in the Ayurvedic and Siddha systems of medicine. It is commonly known as *Thalludhalai*, *Agnimantha*, or *Arani* and is a constituent of more than 50 indigenous medicinal formulations. Popular uses include the treatment of inflammation, diabetes, nervous disorders, asthma, rheumatism, digestive disorders, and urinary disorders and also as a bitter tonic[1]. Non-clinical investigations have revealed anti-inflammatory, hypoglycemic, immunomodulatory, antidiarrhoeal and antiplasmodial properties[1].

Preliminary phytochemical screening and co-TLC studies of *C. phlomidis* leaves in this study revealed the presence of adrenaline for the first time. Chemical substances that play an essential role in the peripheral and central neurotransmission of animals, such as acetylcholine and biogenic monoamines, have been demonstrated in the plant kingdom[2, 3]. Adrenaline or 4-[1-hydroxy-2-(methylamino) ethyl] benzene-1, 2-diol, is a biogenic monoamine belonging to the catecholamine group. Adrenaline, noradrenaline, dopamine and

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their derivatives have been detected in many plant families[4]. In contrast to the considerable knowledge concerning the role and action of adrenaline in mammals, very little is known of the role of adrenaline in plants. Several reports suggest that adrenaline is a precursor for alkaloids[5] while others suggest that it may interact with plant hormones[6, 7]. At present, high-performance liquid chromatography (HPLC) methods are typically used for identification and quantification of catecholamines using fluorescence or electrochemical detection[8-10]. Considering the wide physiological significance of adrenaline and also its potential as a marker for standardization of raw material, a simple, sensitive and accurate TLC method was developed for the quantification of adrenaline in *C. phlomidis* leaves.

1 Materials and methods

1.1 Reagents and chemicals Pure adrenaline was procured from Himedia Laboratories Pvt. Ltd. (Mumbai, India), other solvents and chemicals were of analytical grade. Silica gel 60 F_{254} TLC plates were purchased from Merck (Darmstadt, Germany). A Camag TLC system equipped with Camag Linomat V and a computerized Camag TLC scanner (for densitometric scanning and UV-Visible spectra) was used.

1.2 Plant materials Leaves of *C. phlomidis* were collected from multiple plants in the outskirts of Trichy city, Tamilnadu, India and were authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, Tamilnadu, India. A voucher specimen (Pharmacy/HDT/CP/08-09/15/MKM) has been deposited in the Pharmacy Department of The M. S. University of Baroda, Vadodara, Gujarat, India.

1.3 Preparation of sample and standard solution Accurately weighed 2.5 g of coarse powder of *C. phlomidis* leaves was extracted separately with 1% glacial acetic acid in water (4×50 mL) under reflux (30 min each time). The combined extracts were filtered, concentrated and transferred into a 25 mL volumetric flask and the volume was made up with the same solvent. A standard stock solution of adrenaline (100 μg/mL) was prepared in 1% glacial acetic acid in water. Working solutions were prepared by appropriate dilution of the stock solution (10 μL each) to obtain 100, 200, 300, 400 and 500 ng/spot in order to prepare a five-point calibration curve of peak area versus concentration.

1.4 Thin-layer chromatography A Camag TLC system equipped with a Camag Linomat V, an automatic TLC sample spotter and a Camag glass twin trough chamber (20 cm×10 cm) was used for analysis. Chromatography was performed by using preactivated (60 ℃ for 5 min) silica gel 60 F_{254} TLC plates (20 cm×10 cm; layer thickness 250 μm). Samples and standards were applied to the plate as 8 mm wide bands by using an automatic TLC sampler under a flow of nitrogen; 10 mm from the bottom of the plate, 10 mm from the side and leaving a space between the spots of 15 mm. The linear ascending development was carried out in a Camag twin trough chamber saturated with 20 mL mobile phase (acetone : chloroform : n-butanol : glacial acetic acid : water (60 : 40 : 40 : 40 : 35, v/v/v/v/v)) for 20 min at room temperature ((25±2) ℃ and 40% relative humidity). The plates were developed up to 8 cm under chamber saturation conditions. Subsequent to the development, TLC plates were dried with the help of a hair dryer. Adrenaline oxidizes rapidly hence post chromatographic derivatization was carried out in potassium hexacyanoferrate (Ⅲ)-ethylenediamine reagent followed by heating at 80 ℃ for 15 min[11]. Qualitative identification of adrenaline was carried out by comparing the retardation factor (R_f) value and the UV-Visible spectra of a standard adrenaline spot and an identical spot in the leaf extract. Quantification of adrenaline in the leaf extract was performed by the external standard method. Quantitative evaluations of the plates were performed with Camag scanner 3 (CATS 4.0 integration software). Densitometric scanning was performed in the absorption-reflection mode at 407 nm by using a slit width of 6 mm × 0.45 mm and data resolution 100 μm step and a scanning speed 20 mm/s with a computerized Camag TLC scanner.

1.5 Quantification of adrenaline in test sample A total of 10 μL sample solution was applied to a TLC plate, developed and scanned as above. Peak areas were recorded and the amount of adrenaline was calculated by using the calibration curve.

1.6 Specificity Specificity of the method was determined by analyzing the adrenaline standard and the leaf extract samples. The spot for adrenaline in the leaf extract was confirmed by comparing its R_f and UV-Visible spectra with that of the standard adrenaline spot. The peak purity of adrenaline was assessed by comparing the spectra at the peak start, peak apex and peak end positions of the spot.

1.7 Method validation The method was validated for precision, accuracy and repeatability[12]. Instrumental precision was checked by repeated scanning of the same standard spot 100 and 500 ng three times and was expressed as the coefficient of variance (relative standard deviation, % RSD). Method precision was studied by analyzing the standards 100 and 500 ng/spot under the same analytical procedure and laboratory conditions, on the same day and on different days (interday precision), the results were expressed as % RSD. Accuracy of the method was tested by performing recovery studies of pre-analyzed samples with the adrenaline standard at three levels (23.6, 26.3

and 29.0 μg/mL), and the percentage of recovery was calculated.

2 Results and discussion

2.1 TLC separation optimization Different compositions of the mobile phase were tested and the desired resolution of adrenaline with a symmetrical and reproducible spot was achieved by using acetone : chloroform : n-butanol : glacial acetic acid : water (60 : 40 : 40 : 40 : 35, v/v/v/v/v) with 20 min of chamber saturation. The standard peak for adrenaline was seen at R_f = 0.45. The leaf extract of *C. phlomidis*, when subjected to TLC, as per the methodology described above, showed the presence of adrenaline. A comparison of the spectral characteristics of the peaks for the adrenaline standard and that of the sample peak confirmed the presence of adrenaline. Peak purity was assessed by comparing UV-Visible spectra of both the adrenaline standard and the adrenaline spot in leaf extract track.

2.2 Linearity and detection limit Linearity was confirmed by applying standard solutions of adrenaline at five different concentration levels. A calibration curve was drawn in the concentration range of 100 to 500 ng/spot. The equation for the calibration curve of adrenaline is $y = 311.08 + 7.41x$ and the correlation coefficient of the calibration curve is 0.998, indicating good linearity. Detection and quantification limits were determined as per ICH Guidelines Q2(R1) (1996/2005)[12]. Results of regression analysis on the calibration curve and detection limits are presented in Table 1.

2.3 Precision studies Instrumental precision was checked by repeated scanning of the same spots (100 and 500 ng/spot) of standard adrenaline three times and the %RSD values were 0.94 and 0.80 respectively. To determine the precision of the developed assay method, 100 and 500 ng/spots of adrenaline standard were analyzed three times within the same day to determine the intraday variability. The %RSD values were 0.96 and 0.78 for the 100 and 500 ng/spot spots, respectively. Similarly, the interday precision was tested on the same concentration levels on two consecutive days and the % RSD values were 1.28 and 0.87, respectively (Table 2).

2.4 Quantitative analysis and recovery studies This developed TLC method was subsequently applied for the quantitative analysis of adrenaline in the leaf extract of *C. phlomidis*. The adrenaline content of the leaves by this proposed method collected from multiple plants were found to be 0.013% w/w. For the examination of recovery rates, 10.60, 13.30 and 16.00 μg/mL of pure adrenaline were added to pre-analyzed samples and quantitative analysis was performed. The recoveries were between 97.03% and 99.19% (Table 3).

Table 1 Linearity regression data for quantification of adrenaline using proposed thin-layer chromatography densitometric method

Parameter	Results
R_f	0.45
Dynamic range (ng/spot)	100 to 500
Equation	$y=311.08+7.41x$
Slope	7.41
Intercept	311.08
Limit of detection (ng)	18.33
Limit of quantification (ng)	61.11
Linearity (correlation coefficient)	0.998

Table 2 Precision data for quantification of adrenaline using proposed thin-layer chromatography densitometric method

Concentration (ng/spot)	Instrumental precision (%RSD)	Method precision (%RSD)	
		Intraday	Interday
100	0.94	0.96	1.28
500	0.80	0.78	0.87

%RSD: relative standard deviation.

Table 3 Recovery data for quantification of adrenaline using proposed thin-layer chromatography densitometric method

Amount of adrenaline in the sample (μg)	Amount of adrenaline added (μg)	Amount of adrenaline found (μg)	Recovery (%)
13.00	10.60	23.41	99.19
13.00	13.30	25.52	97.03
13.00	16.00	28.37	97.82

3 Conclusion

Thin-layer chromatography is a useful alternative under circumstances where the other slower and more costly chromatographic methods are not appropriate. It is a suitable method to standardize raw herbs, active constituent-enriched extracts and their formulations. The TLC method developed for the quantification of adrenaline in *C. phlomidis* leaves is simple, specific, precise, accurate, rapid and cost-effective. This TLC procedure may be used effectively for the qualitative and quantitative determination of adrenaline, allowing for the standardization of adrenaline in this plant and its derived products.

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薄层色谱法检测海州常山桐树叶中肾上腺素的含量

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