Quantification of L-DOPA, lupeol and β-sitosterol from leaves of *Clerodendrum phlomidis* by TLC

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Summary

Clerodendrum phlomidis Linn. f. of the family Lamiaceae is an important and well known medicinal plant in Ayurveda and Siddha system of medicines. To ensure identity, quality of the plant material and considering the wide therapeutic application of L-DOPA, lupeol and β-sitosterol, the present study was planned to quantify these marker constituents by TLC method. The amount of L-DOPA, lupeol and β-sitosterol quantified from the leaves of *C. phlomidis* were 0.06806, 0.01733 and 0.06324 % w/w, respectively. This TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for this plant or its derived products.

Key words: *Clerodendrum phlomidis, L-DOPA, lupeol, β-sitosterol, TLC*

INTRODUCTION

In India, Ayurvedic, Siddha and other, herbal derived products which are used either as active ingredients or as adjuvants hold paramount importance as alternative medicines but their quality evaluation poses a great challenge to practitioners and consumers. Correct identification of these drugs is often problematic in entire form as well as in powder form, since these medicinal plants are known by a variety of vernacular names and frequently many medicinal plants are known by one vernacular name. *Clerodendrum phlomidis* Linn. f. (syn. *Clerodendrum multiflorum*

(Burm.f) O. Kuntze) of the family Lamiaceae is an important and well known medicinal plant in Ayurveda and Siddha system of medicines. It is commonly known as *Thalludhalai*, *Agnimantha*, *Arani* and a constituent of more than 50 indigenous drug formulations.

Co-TLC with marker compounds can be used to standardize the herbal raw materials. The clinical efficacy and pharmacological effects of plant material depends strongly on the quantity of its biological active constituents. These must be quantified if plant material is to be characterized. Moreover, TLC is often used as an alternative to other chromatographic techniques for quantification of plant products due to its simplicity, accuracy, cost effectiveness and rapidity. β-sitosterol has been reported earlier in *C. phlomidis* leaves [1]. Although β-sitosterol has shown an amazing array of scientifically acknowledged benefits for key areas of health in immune dysfunctions, inflammatory disorders, rheumatoid arthritis [2, 3], hypercholesterolemia [4], colon cancer [5], breast cancer [6] and prostate cancer [7], there is no report on the quantification of β-sitosterol. Preliminary phytochemical screening, TLC finger printing and co-TLC studies (with marker compounds) of *C. phlomidis* leaves revealed the presence of L-DOPA and lupeol for the first time. Furthermore, their presence was confirmed by R_F comparison, multi-wavelength scanning and spectral overlay. L-DOPA is used in the treatment of Parkinson's disease [8] and it is considered by many clinicians as a drug of choice in the management of idiopathic parkinsonian syndrome. Lupeol is reported for anti-inflammatory [9], antioxidant [10], antiarthritic [11] and antiplasmodial activity [12]. Considering the wide range of therapeutic applications of these chemical constituents and to ensure the identity, quality of the plant material, the present study was planned to quantify these marker constituents by TLC method.

EXPERIMENTAL

Chemicals and reagents

Pure L-DOPA, lupeol and β-sitosterol were obtained from Himedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Chemicals (Bangalore, India) and Acros Organics (NJ, USA), respectively. Other solvents and chemicals were of analytical grade. Silica gel $60F_{254}$ TLC plates were purchased from Merck (Darmstadt, Germany).

Plant material

Leaves of *Clerodendrum phlomidis* were collected from out fields of Trichy city, Tamilnadu, India and were authenticated from 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.

Chromatography

A Camag TLC system equipped with Camag Linomat V an automatic TLC sample spotter, Camag glass twin trough chamber (20 X 10 cm) were used for the analysis. Chromatography was performed using pre-activated (60°C for 5 min) silica gel 60F_{254} TLC plates (20 X 10 cm; layer thickness 250 μm). Samples and standards were applied on the plate as 8 mm wide bands with an automatic TLC sampler under a flow of N_2 gas, 10 mm from the bottom and 10 mm from the side and the space between two spots were 15 mm of the plate. The linear ascending development was carried out in a Camag twin trough chamber saturated with 20 mL mobile phase for 20 min at room temperature (25±2°C and 40% relative humidity). The plates were developed up to 8 cm under chamber saturation conditions. Subsequently to the development, TLC plates were dried in current air with the help of a hair dryer. After the post chromatographic derivatization, quantitative evaluations of the plates were performed with CAMAG scanner 3 (win CATS 4.0 integration software). Densitometric scanning was performed in the absorption-reflection/fluorescence mode using a slit width of 6 X 0.45 mm and data resolution 100 μm step and scanning speed 20 mm/s with a computerized Camag TLC scanner.

Method validation and specificity

The method was validated for precision, accuracy and repeatability [13]. Specificity was determined by analyzing the standard compounds with the samples. The spot for standard in the sample was confirmed by comparing the R_F and spectra of the spot with that of the standard. The peak purity was assessed by comparing the spectra at three different levels, i.e., peak start, peak apex and peak end position of the spot.

Quantification of L-DOPA

Preparation of sample and standard solution

Accurately weighed 5 g of the coarse powder of *C. phlomidis* leaves were extracted with water:methanol (7:3) (4 X 50 mL) under reflux (30 min each time) in a water bath. The combined extracts were filtered, concentrated and transferred to a 50 mL volumetric flask and the volume was made up with same mixture of solvents. A stock solution of L-DOPA 1 mg/mL was prepared in water:methanol (7:3). Working solutions were prepared by appropriate dilution of the stock solution with the same mixture of solvents. All solutions were protected from light and stored in refrigerator at 2 to 4°C.

Calibration curve for L-DOPA

Standard L-DOPA solutions in the range of 100 to 500 ng $spot^{-1}$ were applied on TLC plate for preparation of calibration curve of peak area versus concentration.

Quantification of L-DOPA in test sample

Quantification was performed by external standard method, using pure L-DOPA as standard [14]. 5 μL of the sample solution was applied in triplicate on the TLC plate and developed with the mobile phase acetone: chloroform: n-butanol: glacial acetic acid: water (60:40:40:40:35, v/v/v/v/v). The post chromatographic derivatization was carried out with ninhydrin 0.5% in ethanol placed in a dipping chamber (CAMAG) followed by heating for 5–10 min under observation [15]. Densitometric scanning was performed in absorption-reflection mode at 497 nm. Peak areas were recorded and the amount of L-DOPA was calculated using the calibration curve.

Quantification of lupeol

Preparation of sample and standard solutions

Accurately weighed 5 g of the coarse powder of *C. phlomidis* leaves were extracted with methanol (4 X 50 mL) under reflux (30 min each time) in a water bath. The combined extracts were filtered, concentrated and transferred to a 50 mL volumetric flask and the volume was made up with methanol. A stock solution of lupeol 100 μg/mL was prepared in methanol. Working solutions were prepared by appropriate dilution of the stock solution with the same solvent.

Calibration curve for lupeol

Standard lupeol solution in the range of 50 to 250 ng spot^{-1} was applied on TLC plate for preparation of calibration curve of peak area versus concentration.

Quantification of lupeol in test sample

Quantification was performed by external standard method, using pure lupeol as standard [16]. 5 μL of the sample solution was applied in triplicate on the TLC plate and developed with the mobile phase toluene: chloroform: ethyl acetate: glacial acetic acid (10:2:1:0.03, v/v/v/v). The post chromatographic derivatization was carried out with freshly prepared antimony trichloride reagent (20% solution of antimony III chloride in chloroform) placed in a dipping chamber (CAMAG) followed by heating in an oven at 110°C for 5-6 min [15]. Densitometric scanning was performed in fluorescence mode at 366 nm. Peak areas were recorded and the amount of lupeol was calculated using the calibration curve.

Quantification of β-sitosterol

Preparation of sample and standard solutions

Accurately weighed 5 g of the coarse powder of *C. phlomidis* leaves were extracted with methanol (4 X 50 mL) under reflux (30 min each time) on a water bath.

The combined extracts were filtered, concentrated and transferred to a 50 mL volumetric flask and the volume was made up with methanol. A stock solution of β-sitosterol 100 μg/mL was prepared in methanol. Working solutions were prepared by appropriate dilution of the stock solution with the same solvent.

Calibration curve for β-sitosterol

Standard β-sitosterol solution in the range of 100 to 600 ng $spot^{-1}$ was applied on TLC plate for preparation of calibration curve of peak area versus concentration.

Quantification of β-sitosterol in test sample

Quantification was performed by external standard method, using pure β-sitosterol as a standard [17]. 5 μL of the sample solution was applied in triplicate on the TLC plate and developed with mobile phase toluene: chloroform: methanol (4:4:1, v/v/v). The post chromatographic derivatization was carried out with anisaldehyde-sulphuric acid placed in a dipping chamber (CAMAG) followed by heating in an oven at 100°C for 5–10 min [15]. Densitometric scanning was performed in absorption-reflection mode at 527 nm. Peak areas were recorded and the amount of β-sitosterol was calculated using the calibration curve.

RESULT AND DISCUSSION

TLC separation optimization

The leaves of *Clerodendrum phlomidis*, when subjected to TLC as per the methodology described above, showed the presence of L-DOPA, lupeol and β-sitosterol peaks. A comparison of the spectral characteristics of the peaks for standard compounds and that of the sample further confirmed the identity of L-DOPA, lupeol and β-sitosterol present in the samples. Good resolutions with symmetrical and reproducible peaks were obtained.

System suitability test

Linearity and detection limit

The peak area versus concentration plots were found to be linear in the range of 100-500 ng $spot^{-1}$ for L-DOPA, 50-250 ng $spot^{-1}$ for lupeol and 100-600 ng $spot^{-1}$ for β-sitosterol. The regression equation and correlation coefficient for L-DOPA, lupeol and β-sitosterol indicated good linearity (tab. 1). The limit of detection for L-DOPA, lupeol and β-sitosterol were 2.88, 5.31 and 6.16 ng respectively. The limit of quantification was 8.73, 16.08 and 18.66 ng for L-DOPA, lupeol and β-sitosterol respectively (tab. 1).

Table 1.

Linearity regression data for quantification of L-dopa, lupeol and β-sitosterol using proposed TLC densitometric method

Linearity regression data			
parameter	L-dopa	lupeol	β-sitosterol
R_F	0.37	0.40	0.55
dynamic range (ng $spot^{-1}$)	100–500	50–250	100–600
equation	$Y=2503.286+25.433x$	$Y=675.100+26.008x$	$Y=324.473+9.729x$
slope	25.433	26.008	9.729
intercept	2503.286	675.100	324.473
limit of detection	2.88 ng	5.31 ng	6.16 ng
limit of quantification	8.73 ng	16.08 ng	18.66 ng
linearity (correlation coefficient)	0.99775	0.99959	0.99788
specificity	specific	specific	specific
amount of compound quantified[a] % w/w)	0.06806	0.01733	0.06324

[a] plant dry weight basis

Precision studies

Instrumental precision was checked by repeated scanning of the same spots of standards three times and % RSD values were calculated (tab. 2). To determine the precision of the methods, standards were analyzed three times inter-day and intra-day (tab. 2).

Table 2.

Precision and recovery studies data for quantification of L-dopa, lupeol and β-sitosterol using proposed TLC densitometric method

precision studies				
TLC method	**concentration [ng $spot^{-1}$]**	**instrumental precision [% RSD]**	**Method precision (% RSD)**	
			Intra-day	**Inter-day**
L-DOPA	100	0.32	0.51	1.23
	500	0.42	0.49	0.66
lupeol	50	0.72	1.18	1.31
	250	0.41	0.65	0.71
β-sitosterol	100	0.47	0.74	0.94
	600	0.57	0.58	0.75

recovery studies				
	amount in the sample [μg]	amount added [μg]	amount found [μg]	recovery (%)
L-DOPA	68	54.4	119.5	97.63
	68	68	130.2	95.74
	68	81.6	142.8	95.45
lupeol	17.3	13.8	30.6	98.39
	17.3	17.3	35.2	101.73
	17.3	20.8	39.1	102.62
β-sitosterol	63.2	50.6	110.6	97.18
	63.2	63.2	124.6	98.57
	63.2	75.8	141.3	101.65

Sample analysis and recovery studies

The amount of L-DOPA, lupeol and β-sitosterol quantified from the leaves of *C. phlomidis* were 0.06806, 0.01733 and 0.06324 % w/w (plant dry weight basis) respectively (tab. 1). For the examination of recovery rates 80, 100 and 120% of pure standards were added to preanalysed samples and quantitative analysis were performed (tab. 2).

The TLC methods are rapid, simple, specific and effective in quantification of L-DOPA, lupeol and β-sitosterol, should prove to be a useful alterative under circumstances where the other slower and more costly chromatographic methods are not appropriate. This TLC procedure may be used effectively for identity, quality evaluation as well as quantitative determination for this plant or its derived products.

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OCENA ILOŚCIOWA ZAWARTOŚCI L-DOPA, LUPEOLU I β-SITOSTEROLU W LIŚCIACH *CLERODENDRUM PHLOMIDIS* METODĄ TLC

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S t r e s z c z e n i e

Clerodendrum phlomidis Linn. z rodziny Lamiaceae jest ważną i dobrze znaną rośliną leczniczą stosowaną w systemach Ayurvedic i Siddha. By upewnić się co do identyfikacji i jakości materiału, a także wiedząc o wielu zastosowaniach terapeutycznych L-DOPA, lupeolu i β-sitosterolu, w niniejszej pracy zaplanowano ocenę ilości tych składników za pomocą metody TLC. Ilość L-DOPA, lupeolu i β-sitosterolu w liściach *C. phlomidis* wyniosła odpowiednio 0,06806, 0,01733 i 0,06324 % w/w. Opisana procedura z zastosowaniem TLC może być skuteczna w identyfikacji, ocenie jakościowej, a także ocenie ilościowej składników tej rośliny lub produktów z niej otrzymanych.

Słowa kluczowe: *Clerodendrum phlomidis, L-DOPA, lupeol, β-sitosterol, TLC*