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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE DETERMINATION OF CATECHIN FROM *SMILAX PERFOLIATA* LOUR ROOT

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Catechin in different samples of *Smilax perfoliata* Lour. (Ramdatun) root. The ethanolic extract of *S. perfoliata* (root) samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF_{254} and developed using Chloroform : Methanol (8:2) v/v as a mobile phase. The plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and detection and quantification were carried out densitometrically using an UV detector at wavelength of 455 nm. Content of marker compound in the samples was found similar.

Keywords: Catechin, *Smilax perfoliata* Lour, Ramdatun root, HPTLC.

INTRODUCTION

Smilax Perfoliata Lour., Syn. Smilax prolifera Roxb., Smilax zeylanica L. (Family - Smilacaceae) [1,5] is well known as Ramdatun is distributed in tropical western Himalaya, West Bengal, Madhya Pradesh and Bihar. It is a robust more or less strongly armed climber, almost coextensive with S. ovalifolia and frequently confounded with it; its vernacular names are also likely to be the same. Roots are used as anticancer, blood purifier, anti-dysenteric and in urinary complaints. In some parts of India roots of this plant are used as a substitute for sarsaparilla in the treatment of venereal diseases; they are also applied in rheumatism[2-5]. Catechin, proanthocyanidins, steroidal saponins, flavonoids, phenolic glycosidal compounds, flavones and tannins are the major constituents of this plant. Catechin is an active constituents and used as a marker. Literature survey reveals that the TLC and HPTLC methods are reported but no method as yet is reported for the determination of Catechin in *Smilax perfoliata* Lour. (Ramdatun) root [5].

With increasing demand for herbal products in medicines and cosmetics there is an urgent need for standardization. So the aim of the work is to develop a simple, rapid, selective and cost effective HPTLC method for the determination of Catechin in *Smilax perfoliata* Lour. root.

MATERIALS AND METHODS

Plant material

The Ramdatun root was collected from the forest of District-Chhindwara (M.P).) and dried under the shade. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad. One sample also taken from the Local Market, Ghaziabad.

Equipment

A Cammag (Switzerland) HPTLC system equipped with a sample applicator Linomat V, Twin trough glass Chamber (20x10 cm^2) with SS lid, TLC Scanner III, Reprostar III and Wincats an integrated Software 4.02 (Switzerland), Rotavapour.

Chemical & reagents

Analytical grade; Alcohol, Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Sulphuric acid, were used; obtained from S.D. Fine Chem. Ltd.(Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF_{254} (20x10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard- Catechin procured from Natural Remedies Pvt. Ltd., Bangalore, India.

Sample & standard preparation

Sample preparation: 1g of coarsely powdered drug samples were extracted with 10 ml alcohol for 24 hours by cold extraction method. The extracts were filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask. Filtrates were concentrated to 5 ml on Rotavapour at 40^0C and used for H.P.T.L.C. work.

Standard preparation: 5mg of standard Catechin dissolved in 3ml of absolute alcohol and made up to 5ml in standard volumetric flask.

Chromatography

TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Chloroform : Methanol (8:2) V/V as mobile phase. Alcoholic extract of samples and Catechin standard solution applied on plate by using Linomat V applicator. Cammag Twin Trough Glass Chamber (20x10 cm^2) with SS lid was used for development of TLC plate. The Twin Trough Glass Chamber was saturated with mobile phase for 30 minutes. TLC plate was developed to 8 cm distance above the position of the sample application. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^0C for 10 minutes and HPTLC finger print profile was snapped by Cammag Reprostar III, before deivatization under UV 254 nm, 366 nm and after derivatization (Fig.1). The derivatized plate was scanned immediately using Camag TLC Scanner III at wavelength 455nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

Method validation and recovery study

To study the accuracy and precision of the proposed method, recovery experiment was carried out. To a fixed amount of alcoholic extract of samples, the standard solution of Catechin was added (ratio 9:1 v/v) and total amount of standard Catechin were determined. Percent recovery was calculated from the amount of Catechin found via graph (Table No. 3).

Linearity of detector response, assay and recovery

In order to establish linearity, standard solution of Catechin (1mg/ml) applied on TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20X10 cm^2; 0.2 mm thick), 2µl, 4µl, 6µl on Track No. S1, S2 & S3 respectively and for assay, 9µl of alcoholic extract of both samples applied on Track No. T1 & T2 and for recovery study, the alcoholic extract of both samples were spiked with standard

Catechin solution (ratio 9:1v/v) and applied 10µl on Track No. T3 & T4 on the same plate. TLC plates was developed to 8 cm distance above the position of the sample application and removed from the chamber and air dried at room temperature. This HPTLC finger print profile was snapped by Cammag Reprostar III, before derivatization under UV Light 254 nm, 366 nm and after derivatization (Fig.1).

The plate was derivatized with Anisaldehyde- Sulphuric Acid reagent followed by heating at 110^{0}C for 10 minutes and scanned immediately using Camag TLC Scanner III at wavelength 455nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that Catechin appeared at R_f. 0.33 (brown colour). The peaks, graph and spectra obtained were given in Fig.2 and 3 and R_f. values, colour of bands (Table No.1), quantity of Catechin, linearity, standard deviation & regression coefficient found via graph (Table No. 2) and calculated quantity of Catechin & % recovery were given in Table No. 3

RESULTS AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Chloroform : Methanol (8:2) v/v and the active principle Catechin resolved as a brown colour band at R_f. 0.33 very efficiently from the other components in ethanolic extract of *Smilax perfoliata* Lour. root (Fig.1). Sharp peaks of Catechin (Standard and samples) were obtained when the plate was scanned at wavelength 455nm (Fig.2). Quantity of Catechin found in samples were obtained automatically (Table No. 2) via graph (Fig.3) and % Catechin found in samples and % recovery were calculated (Table No.3). Quantity of Catechin found in sample collected from forest of Distt.- Chhindwara (M.P.) is 1.498mg in 1g drug sample (0.1498% w/w) and quantity of Catechin found in Local Market, Ghaziabad is 1.546mg in 1g drug sample (0.1546%w/w). The % recovery of Catechin in sample collected from forest of Distt.- Chhindwara, (M.P.) is 99.77%w/w and 99.85%w/w in Local Market, Ghaziabad, (U.P.). The mean % recovery was 99.81%.

Table 1: TLC details of alcoholic extract of *Smilax perfoliata* Lour. (root)

Sr. No.	Detection/ visualization	Ramdatun root (Track No. T1, T2, T3 and T4)		Standard- Catechin (Track No. S1, S2 and S3)	
		R_f. values	Colour of band	R_f. values	Colour of band
1.	**Under UV 254 nm**	0.04	grey		
		0.08	grey		
		0.10	grey		
		0.33	grey	0.33	grey
		0.46	grey		
		0.60	grey		
		0.64	grey		
		0.68	grey		
		0.88	dark grey		
2.	**Under UV 366 nm**	0.04	blue		
		0.20	blue		
		0.35	blue		
		0.46	blue		No significant band
		0.60	blue	-	
		0.64	blue		
		0.68	blue		
		0.88	blue		
3.	**After derivatization**	0.10	dark grey		
		0.16	brown		
		0.33	brown		
		0.46	brown	0.33	
		0.53	brown		Brown
		0.60	brown		
		0.71	violet		
		0.77	violet		
		0.78	violet		
		0.85	violet		
		0.88	violet		

Table 2: Quantity applied on plate and values found via graph

Sr. No.	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of Catechin via graph	Linearity & regression coefficient and standard deviation via graph
1.	T1	9µl	1800µg	2.699µg	
2.	T2	9µl	1800µg	2.785µg	
3.	S1	2µl	2.000µg	2.000µg	
4.	S2	4µl	4.000µg	4.000µg	Y = -11941.196 + 8958.045 * X + -719.377 * X2
5.	S3	6µl	6.000µg	6.000µg	
6.	T3	(9+1)µl	1800µg +1µg	3.693µg -1µg = 2.693µg	r = 0.99999 sdv = 0.00%
7.	T4	(9+1)µl	1800µg +1µg	3.781µg -1µg = 2.781µg	

T1- Alcoholic extract of sample collected from Distt. -Chhindwara M.P.
T2- Alcoholic extract of Local Market sample, Ghaziabad
S1- Catechin standard solution (1mg/ml)
S2- Catechin standard solution (1mg/ml)
S3- Catechin standard solution (1mg/ml)
T3- Alcoholic extract (spiked with std. solution) of sample collected from Distt. –Chhindwara, M.P.
T4- Alcoholic extract (spiked with std. solution) of Local Market sample, Ghaziabad

Table No. 3: Summary of results

Sr. No.	Sample from	Collected Sample (Chhindwara M.P.)	Market Sample
1.	Quantity of Catechin in 1g	1.498mg	1.546mg
2.	% Catechin	0.1498% w/w	0.1546%w/w
3.	% Recovery	99.77%w/w	99.85%w/w

T1 T2 S1 S2 S3 T3 T4

UV-254 nm

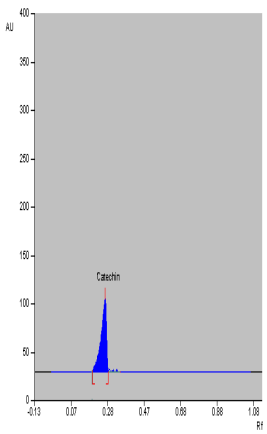
T1 T2 S1 S2 S3 T3 T4

UV-366 nm

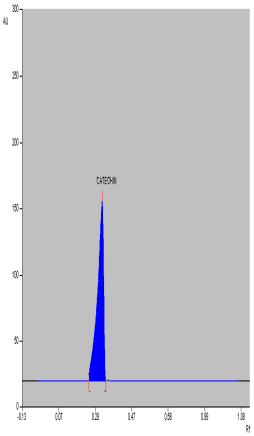
T1 T2 S1 S2 S3 T3 T4

After Derivatization

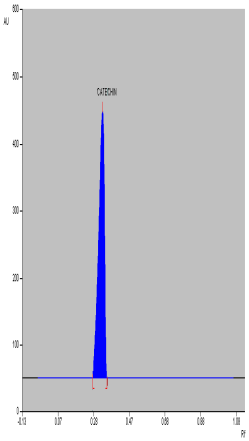
Fig. 1: H.P.T.L.C. Finger print of *Smilax perfoliata* Lour. (Root)



(Track S1)

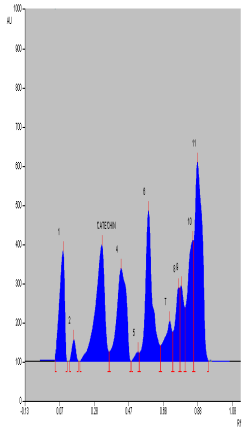


(Track S2)

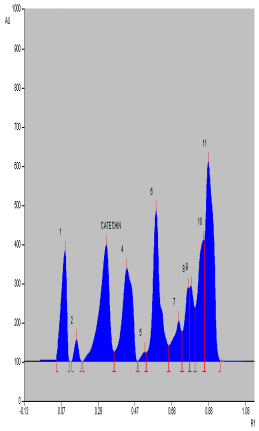


(Track S3)

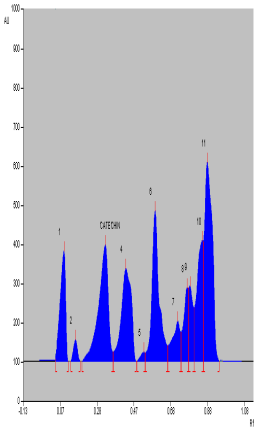
Peaks of Catechin @455nm



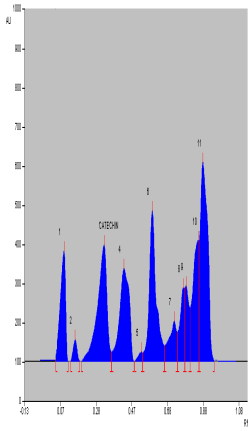
(Track T1)



(Track T2)



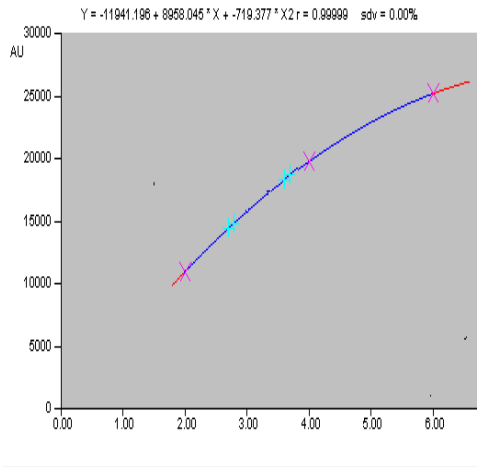
(Track T3)



(Track T4)

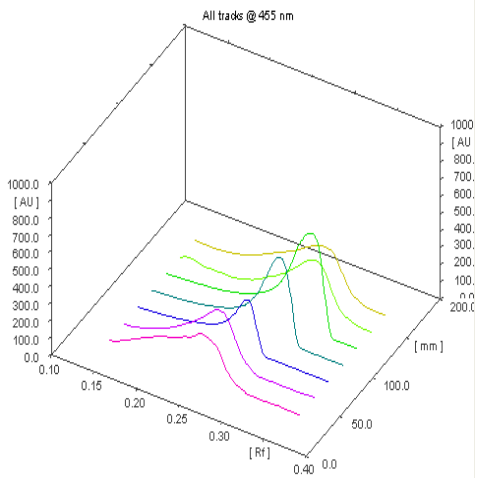
Peaks of *Smilax perfoliata* Lour. (Root) extract @ 455nm

Fig. 2: Peaks of *Smilax perfoliata* Lour. (Root) in all Tracks



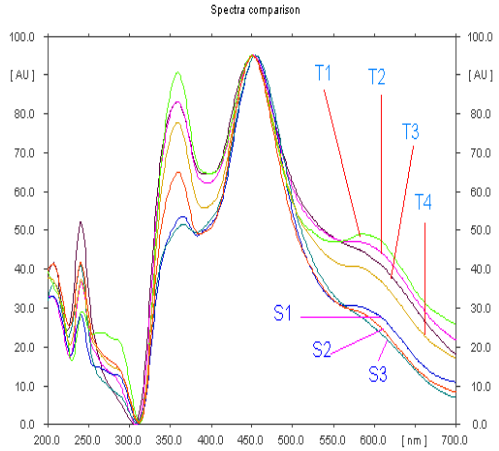
Conc. (mg)

Graph- Conc. vs AU



3D Representation of Catechin

Spectra of Catechin @ 455nm



Super Imposable UV Spectra of Catechin in all tracks@ 455nm

Fig. 3: Graph, 3D representation and spectra of *Smilax perfoliata* Lour. (Root)

The accuracy and reproducibility of the method was established by means of recovery experiment. The mean recovery was close to 100% which indicates the accuracy of the method.

The robustness of the method was studied, during method development, by determining the effect of small variation, of mobile phase composition (±2%), chamber saturation period, development distance, derivatization time, and scanning time (10% variation of each). No significant change of R_f. or response to Catechin was observed, indicating the robustness of the method.

CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Smilax perfoliata* Lour. root powder and quantitative determination of Catechin in root powder.

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