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Research Article

DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE DETERMINATION OF ANDROGRAPHOLIDE FROM *ANDROGRAPHIS PANICULATA* (WHOLE PLANT)

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Andrographolide in different samples of *Andrographis paniculata* (Whole Plant). The alcoholic extract of *Andrographis paniculata* (Whole Plant) samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF_{254} and developed using Toluene : Ethyl acetate : Formic acid (5:4.5:0.5) 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 235 nm. Content of marker compound in the samples were found similar.

Key words: Andrographolide, *Andrographis paniculata* (Whole Plant), Kalmegh, Bhunimba, Chiretta, King of Bitter, HPTLC.

INTRODUCTION

Andrographis paniculata (Burm. f.) wall. ex. Nees Syn.Justica paniculata (Burm. f.) is traditionally known as Kalmegh or Bhunimba. It is vernacularly known as Kirata, Chrayetah, Kiryat, Nila vembu, Karyatu, Kreata, Nelaberu, Mahatita, Chiretta, Creat, King of Bitter etc. It is chiefly found in the plains throughout India from Himachal to Assam and Mizoram, West Bengal and all over south India. It is an erect and branched, annual herb with 4-angled branches and about 30-90 cm in height.

Kalmegh is used mainly for liver disorders and jaundice. A decoction or infusion of leaves is used in general debility and dyspepsia and a tincture of the root as a tonic, stimulant and aperients. The macerated leaves and juice, together with carminative spices such as cardamom, clove and cinnamon, may be made into pills and prescribed for gripe and other stomach ailments in infants. The leaves and roots also find use as an adjunct in the treatment of diabetes, malaria, cholera dysentery, enteritis, gastritis, pneumonia, pyelonephritis and even rabies. [1-6]

The juice of the stem and the whole plant are used to treat diarrhea, Newcastle disease and respiratory problems in poutry. The plant, especially the leaves, has been used to treat dhatoora (datura) poisoning, maggots in wounds, worms in the eyes and abdomen, liver fluke, glossitis, holes in the hard palate, constipation, tuberculosis, leeches in the nostrils, contagious abortion, retention of placenta, tetanus and scabies. [7]

The plant has been subjected to a variety of pharmacological investigations with a special emphasis on liver. The hepatoprotective activity has been reported from several research centres using a variety of experimental *in vivo* and *in vitro* models and a number of biochemical parameters. Its antigastric ulcer activity, antibacterial action and being a bitter may be responsible for its use in these conditions. Its antipyretic, anti-inflammatory and weak antimalarial actions justify its use in a variety of febrile conditions. A major chemical constituent 'Andrographolide' has an antihepototoxic activity. [8]

The plant contains bitter glucosides: andrographolide, neoandrographolide, panaculoside, flavnoids, andrographonin, panicalin, apigenin 7-4-dimethyl ether. The plant contains diterpenoids- 14-deoxy-11-oxo- andrographolide; 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, neo-androgra-pholide and andrographolide. The roots gave flavones-apigenin-7,4'-di-O-methyl ether, 5-hydroxy-7,8,2'3'-tetramethoxy-flavone, andrographonin and panicolin and α-sitosterol. Leaves contain homoandrographolide, andrographosterol and andrographone. Whole plant, leaves and root contains a furonoid diterpine Andrographolide; 2',5-dihydroxy-7,8-dimethoxyflavone-2'-o-β-(D)-glucoside,3β-hydroxy-5-stigmasta -9(11),22(23)-diene, andrographin, panicolin, diterpene glucoside-neoandro grapholide, flavone-5-hydroxy-7,8,2',3'tetramethoxyflavone,5-hydroxy-7,8-flavano ne, α-sitosterol, apigenin, 7,4-dioxymethylether, mono-oxymethyl-wigthin, 5-hydroxy-7,8-dimethoxyflavone, 5-hydroxy-3,7,8,2'-tetramethoxyflavone, 7-o-meth ylwogonin, apigenin-7,4'-di-o-methyl ether, flavone glucoside A,B,C,D,E & F (root), β-sitosterol glucoside, bitter substances, deoxyandro-grapholide-19β-D-glucoside, neoandrographolide, caffeic, chlorogenic, dicaffeoylquinic acids, panico- lide, myristic acids, carcrol, eugenol, hentriacontane, tritricontane, andrographone, homoandro-grapholide, α-β-unsaturated lactone (leaves); 3, 14-dideoxyandrographo- lide, 14-deoxyandrographiside, andro-graphiside, ent-14 β-hydroxy-8(17),12-labadien-16,15-olide-3 β19-oxide (aerial part); oroxylin A, wogonin, andrograpanin, andropanoside, 14-deoxy-12-methoxyandrographolide, 14-deoxyandrographolide, neoandro-grapholide, 2',5-dihydroxy 7,8-dimethoxyflavone, 5-hydroxy-2',7,8-trimethoxyflavone, 14-deoxy-11-oxoandrographolide, 14-deoxy-11,12-didehydro androgra-pholide, andrographside, 14-deoxy-andrographoside (plant). A new diterpine glucosideisolated from leaves and characterized as deoxyandrographolide -19β-D-glucoside. [9-12]

Literature survey reveals that the TLC, HPLC and HPTLC methods are reported but no method as yet is reported for the determination of Andrographolide in *Andrographis paniculata* (whole plant). A simple, rapid, economical, precise and accurate HPTLC method has been established for the determination of Andrographolide in *Andrographis paniculata* whole plant powder. This method can be used for phytochemical profiling of *Andrographis paniculata* whole plant and quantification of Andrographolide.

MATERIALS AND METHODS

Plant material

The Kalmegh whole plant was collected from the Local area Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad. One genuine sample also taken from the Museum of Pharmacopoeial Laboratory for Indian Medicine, 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.

Chemicals

Analytical grade; Alcohol, Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Anisaldehyde, Sulphuric acid and n-

Hexane were used; obtained from S.D. Fine Chem. Ltd. (Mumbai, India). TLC Aluminium pre coated plate with Silica gel 60 GF_{254} (10X10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India). Reference standard Andrographolide procured from Natural Remedies Pvt. Ltd., Bangalore (CAS No. 5508-58-7).

EXPERIMENTAL

Sample preparation

1g of coarsely powdered drug samples were extracted with 10 ml absolute alcohol for 24 hours by cold extraction method. The extracts were filtered by Whatmann no. 42 filter paper and make up to 10 ml in a volumetric flask.

Standard preparation

5mg of standard Andrographolide dissolved in 5ml of absolute alcohol and made up to 5ml in standard volumetric flask.

Chromatography

Procedure

TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20x10 cm^2; 0.2 mm thick) was used with Toluene : Ethyl acetate : Formic acid (5:4.5:0.5) V/V as mobile phase. alcoholic extract of samples and Andrographolide 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 plate was scanned before derivatization using Camag TLC Scanner III at wavelength 235nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data.

Table 1

Sr. No.	Detection/ visualization	Kalmegh whole plant (Track T1, T2, T3 & T4)		Standard-Andrographolide (Track S1, S2 & S3)	
		R_f. values	Colour of band	R_f. values	Colour of band
		0.10	Grey		
1.	Under UV	0.38	dark grey	0.38	dark grey
	254 nm	0.47	grey		
		0.52	grey		
		0.59	grey		
		0.73	grey		
		0.87	grey		
2.	Under UV	0.10	green		
	366 nm	0.45	bright sky blue		No significant band
		0.52	blue		
		0.59	blue	-	
		0.64	red		
		0.73	red		
		0. 82	red		
		0.90	red		
3.	After derivatization				
		0.21	light violet		
		0.38	dark violet	0.38	dark violet
		0.52	blue		
		0.64	violet		
		0.73	violet		
		0.82	violet		

Table 2

Sr. No.	Track no.	Volume applied on plate	Quantity applied on plate	Quantity of Andro-grapholide via graph	Linearity & regression coefficient and standard deviation via graph
1.	T1	9µl	900µg	6.944µg	
2.	T2	9µl	900µg	6.769µg	
3.	S1	10µl	10µg	10.000µg	Y = -104.162 + 11.098 * X + -0.003 * X2
4.	S2	5µl	5µg	5.000µg	r = 0.99999 sdv = 0.00%
5.	S3	2.5µl	2.5µg	2.500µg	
6.	T3	(9+1)µl	900µg +1µg	7.939µg -1µg = 6.939µg	
7.	T4	(9+1)µl	900µg +1µg	7.764µg -1µg = 6.764µg	

T1- Alcoholic extract of Local collected Sample, Ghaziabad, T2- Alcoholic extract of Museum Sample of PLIM, Ghaziabad, S1- Andrographolide standard solution (1mg/ml), S2- Andrographolide standard solution (1mg/ml), S3- Andrographolide standard solution (1mg/ml), T3- Alcoholic extract (spiked with std. solution) of Local collected sample, Ghaziabad, T4- Alcoholic extract (spiked with std. solution) of Museum Sample of PLIM, Ghaziabad

Table 3

Sr. No.	Sample from	Local collected sample, Ghaziabad	Museum sample PLIM, Ghaziabad
1.	Quantity of Andrographolide in 1g	7.7133mg	7.5189mg
2.	% Andrographolide	0.77133 % w/w	0.75189 % w/w
3.	% Recovery	99.92% w/w	99.93% w/w

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 Andrographolide was added (ratio 9:1 v/v) and total amount of standard Andrographolide were determined. Percent recovery was calculated from the amount of Andrographolide found via graph (Table No. 3).

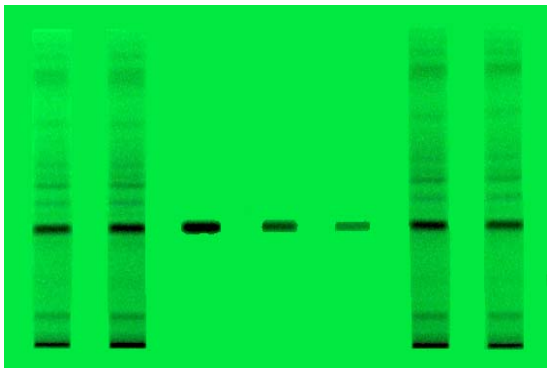
Linearity of detector response, assay and recovery

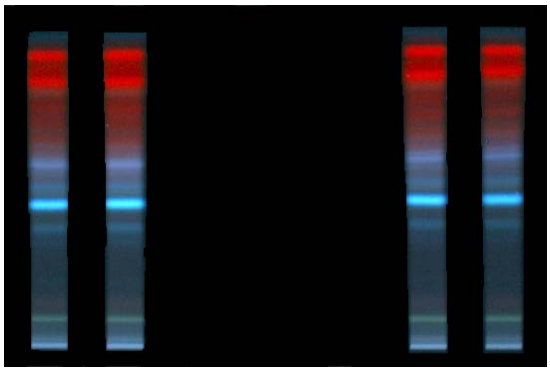
In order to establish linearity, standard solution of Andrographolide (1mg/ml) applied on TLC Aluminium pre coated plate with Silica gel60 GF_{254} (20X10 cm^2; 0.2 mm thick), 10µl, 5µl, 2.5µl on Track No. S1, S2 & S3 respectively and for assay, 9µl of alcoholic extract of both samples applied on Track No. T1 & T2 and for recovery study, the alcoholic extract of both samples were spiked with standard Andrographolide 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 scanned immediately before derivatization using Camag TLC Scanner III at wavelength 235nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that Andrographolide appeared at R_f. 0.38 (dark grey 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 Andrographolide, linearity, standard deviation & regression coefficient found via graph (Table No. 2) and calculated quantity of Andrographolide & % recovery were given in Table No. 3

RESULTS AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Toluene : Ethyl acetate : Formic acid (5:4.5:0.5) v/v and the active principle Andrographolide resolved as a dark grey colour band at R_f. 0.38 very efficiently from the other components in alcoholic extract of *Andrographis paniculata* (whole plant) (Fig.1). Sharp peaks of Andrographolide (Standard and samples) were obtained when the plate was scanned at wavelength 235nm (Fig.2). Quantity of Andrographolide found in samples were obtained automatically (Table No. 2) via graph (Fig.3) and % Andrographolide found in samples and % recovery were calculated (Table No.3). Quantity of Andrographolide found in Local collected Sample, Ghaziabad (U.P.) is 7.7133mg in 1g drug sample (0.77133 % w/w) and quantity of Andrographolide found in Museum Sample of PLIM, Ghaziabad is 7.5189mg in 1g drug sample (0.75189 % w/w). The % recovery of Andrographolide in Local Market Sample, Ghaziabad (U.P.) is 99.92% w/w and 99.93%w/w in Museum Sample of PLIM, Ghaziabad (U.P.). The mean % recovery was 99.925%.

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.





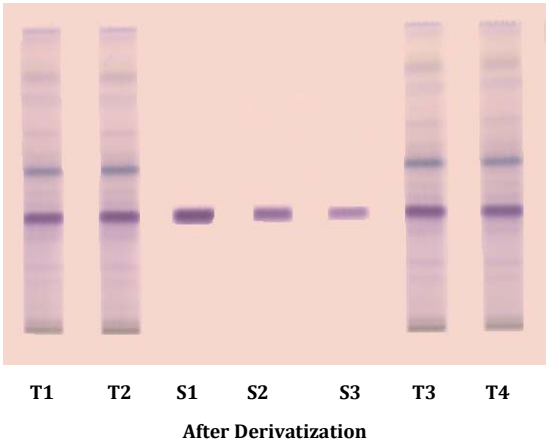
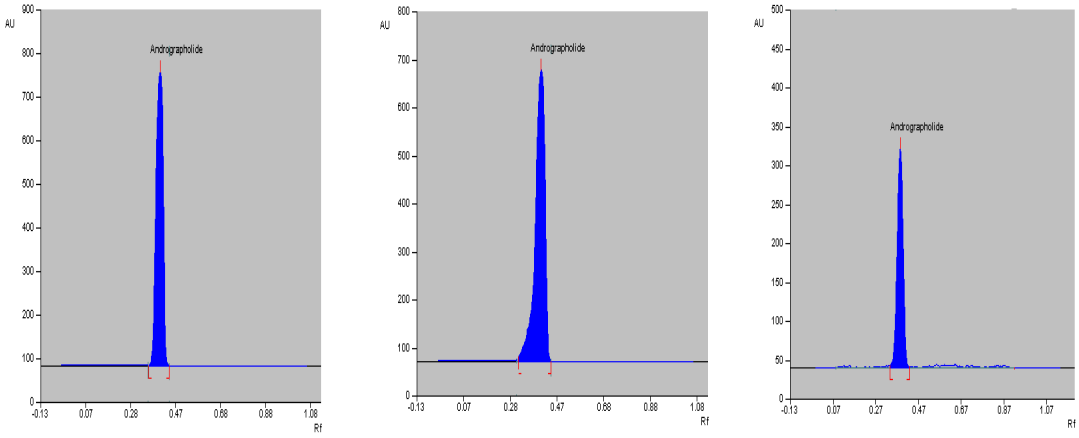
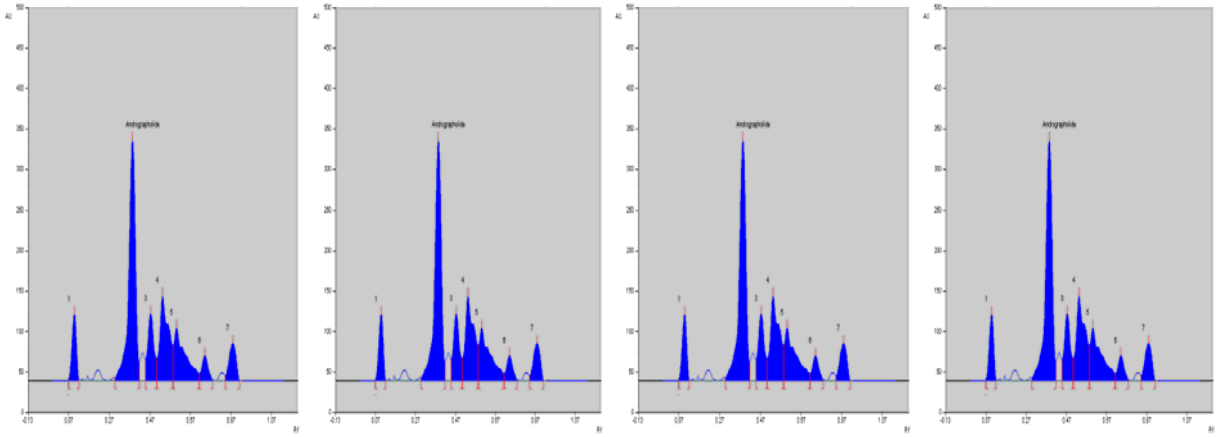


Fig. 1: H.P.T.L.C. finger print of *Andrographis paniculata* (whole plant)



(Track S1) **(Track S2)** **(Track S3)**

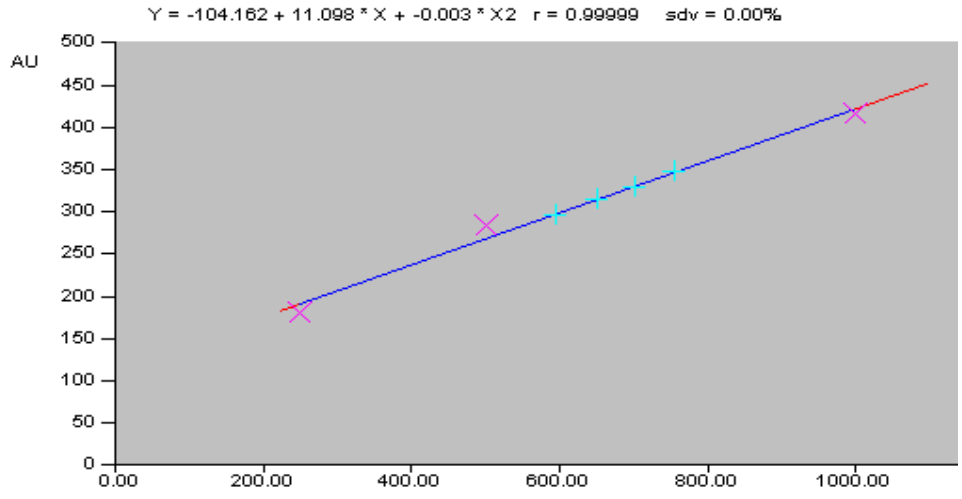
Peaks of Andrographolide(@235nm



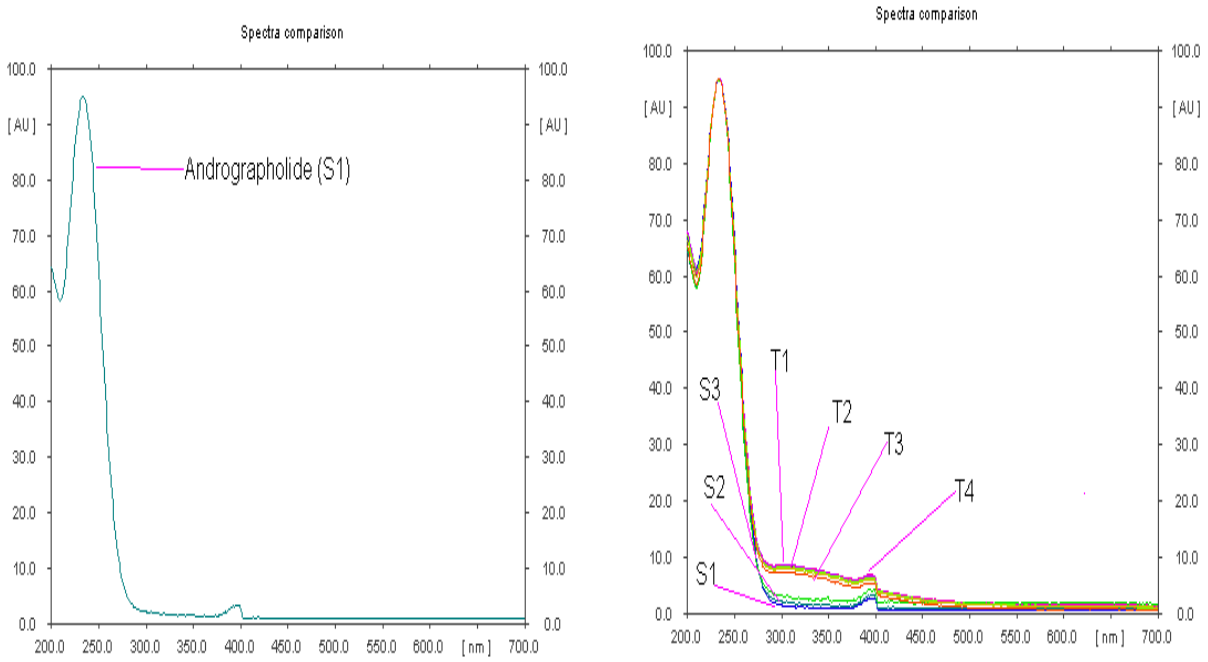
(Track 1) **(Track 2)** **(Track 3)** **(Track 4)**

Peaks of Andrographis paniculata alcoholic extract @235nm

Fig. 2: Peaks of *Andrographis paniculata* (whole plant) in all Tracks



Conc. (µg)



pectra of Andrographolide @ 235nm **Spectra of Andrographolide in all trcks@ 235nm**

Fig .3: Graph and spectrra of *Andrographis paniculata* (whole plant)

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 Andrographolide 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 *Andrographis paniculata* (whole plant) powder and quantitative determination of Andrographolide in whole plant powder.

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