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Research Article

PHYSICO-CHEMICAL STANDARDISATION AND DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF PLUMBAGIN IN KALMEGH NAVAYAS LOHA- AN AYURVEDIC FORMULATION

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of Plumbagin in different samples of *Plumbago zeylanica* Linn. root and its ayurvedic formulation-Kalmegh Navayas Loha with Physico-chemical standardization. The chloroform extract of *Plumbago zeylanica* Linn. root and its ayurvedic formulation-Kalmegh Navayas Loha samples were applied on TLC Aluminium plate pre coated with Silica gel60 GF_{254} and developed using Toluene : Ethyl acetate (3:1) 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 270 nm. Content of marker compound- Plumbagin found in the *Plumbago zeylanica* Linn (Root) and its formulation-Kalmegh Navaya Loha were 0.4836% w/w and 0.04989 % w/w respectively.

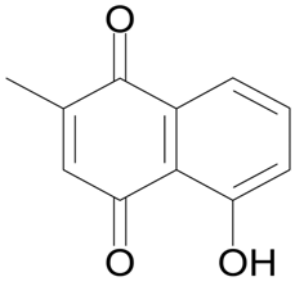
Keywords: Plumbagin, *Plumbago zeylanica* Linn. Root., Chitrak root, Chitrakmul, HPTLC, Kalmegh Navayas Loha, Ayurvedic Formulation.

INTRODUCTION

Plumbago zeylanica Linn Syn. *Plumbago rosea* Linn (Family- Plumbaginaceae) known vernacularly as Chitrak, Chitra, Chitraka, Chitrakmul, Agni, Pathi, Ushana, Chita, Chitramulam, Ceylong Leadwort or white Leadwort is found wild in the tropics, subtropics and throughout India including West Bengal, Bihar and peninsular India. It is also widely cultivated as an ornamental plant. It is a much branched shrub with long tuberous root and a striate stem.

The root and root bark are bitter, stomachic carminative, astringent to bowels, anthelmintic, piles bronchitis, itching, diseases of lever, consumption, ascetics**.** The root is bitter, laxative, expectorant, tonic, abortifacient, alexipharmic, good appetizer, useful in laryngitis, rheumatism, diseases of spleen, ringworm, scabies. Paste of root with milk, vinegar or salt and water is applied in leprosy and other skin diseases externally. Tincture of root bark is used as an antipatriotic . it acts as a powerful sudorific. Leaves are caustic, vesicant aphrodisiac and good for scabies. [5]

Plants contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3'-biplumbagin, elliptinone, chitranone, zeylinone, isozeylinone, droserone, plumbagic acid, plumbazeylanone, naphthelenone and isoshinanolone[5]. Fructose, glucose, invertase and protease isolated from root bark. 3,3'-bisplumbagin, chitranone (binaphthaquinone), droserone, elliptinone, isozeylinone, catechol tannin 8]. Amino acids; β-(2, 3 dihydroxybenzoyl)-butyric acid (plumbagic acid), vanillic acid, 1,2(3)-tetra hydro-3,3'-bisplumbagin, isoshinanolone, dihydrosterone and β- sitosterol also islated from plant [3,8]. Plumbagin shows as anticancer and antitumor activity [5,9]. Aspartic acid, tryptophan, tyrosine, threonine, alanine, histidine, glycine, methionine, hydroxyproline, were isolatedfrom the aerial parts [9, 15].Lupeol and lupenyl acetate have been isolated from the root [9, 16].



Structure of Plumbagin

Literature survey reveals that the TLC, HPLC and HPTLC methods are reported but no method as yet is reported for the determination of Plumbagin in *Plumbago zeylanica* Linn., root. A simple, rapid, economical, precise and accurate HPTLC method has been established for the determination of plumbagin in *Plumbago zeylanica* Linn., root powder. This method can be used for phytochemical profiling of *Plumbago zeylanica* Linn., root and quantification of Plumbagin.

MATERIALS AND METHODS

Material

(i) Chitrak root was procured from the Local Market, Ghaziabad.

(ii) An herbal product KALMEGH NAVAYAS LOHA, B. No. Nil containing *Plumbago zeylanica* Linn. (Root) was procured from the Local Market, Ghaziabad.

Label claimed- Each pills contains:

Badi Haran (Terminalia chebula), Bahera (Terminalia belerica), Kalimirch (Piper nigrum), Pippali (Piper longum), Sonth (Zingiber officinale), Amla (Embelica officinalis), Chitrak (Plumbago zeylanica), Baybidang (Embelica ripes), Nagarmotha (Cyprus rotundus) each equal parts; Kalmegh Kwath (andrographis paniculata) Q.S. and Loha Bhasma(Farrum-calcined) – 9parts.

DETERMINATION OF PHYSICO-CHEMICAL CONSTANTS

The following Physico-chemical constants has been analyzed and result given in Table No. 1

H.P.T.L.C. (HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY)

Equipment

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 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} (20x10 cm^2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India).

wReference standard Plumbagin procured from Aldrich Chem. Co. Milw, WI 33201 (414-273-3850/19,064-0481-42-5).

Sample & standard preparation

Sample preparation

(1) 1g of coarsely powdered drug sample (Kalmegh Navayas Loha) was extracted with 10 ml Chloroform for 24 hours by cold extraction method. The extract was filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask. Filtrate was concentrated to 2 ml and used for T.L.C.

(2) 1g of coarsely powdered drug sample (Citrak root) was extracted with 10 ml Chloroform for 24 hours by cold extraction method. The extract were filtered by Whatmann filter paper and make up to 10 ml in a volumetric flask.

Standard preparation: 5mg of standard Plumbagin dissolved in 5ml of Chloroform 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 Toluene : Ethyl acetate (3:1) V/V as mobile phase. Chloroform extract of samples and Plumbagin 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 270nm. 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 chloroform extract of samples, the standard solution of Plumbagin was added (ratio 9:1 v/v) and total amount of standard Plumbagin were determined. Percent recovery was calculated from the amount of Plumbagin found via graph (Table No. 4).

Linearity of detector response, assay and recovery

In order to establish linearity, standard solution of Plumbagin (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, Chloroform extract of both samples applied on Track No. T1 & T2 and for recovery study, the Chloroform extract of both samples were spiked with standard Plumbagin solution (ratio 9:1v/v) and applied 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 270nm. Wincats an integrated Software 4.02 was used for the detection as well as for the evaluation of data. It was observed that Plumbagin appeared at R_f. 0.84 (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.2), quantity of Plumbagin, linearity, standard deviation & regression coefficient found via graph (Table No. 3) and calculated quantity of Plumbagin & % recovery were given in Table No.4.

RESULTS AND DISCUSSION

Of the various mobile phases tried, the mobile phase containing Toluene : Ethyl acetate (3:1) v/v and the active principle Plumbagin resolved as a dark grey colour band at R_f. 0.84 very efficiently from the other components in Chloroform extract of *Plumbago zeylanica* Linn. (root) and Kalmegh Navayas Loha (Fig. 1).

Sharp peaks of Plumbagin (Standard and samples) were obtained when the plate was scanned at wavelength 270nm (Fig. 2). Quantity of Plumbagin found in samples were obtained automatically (Table No. 3) via graph (Fig. 3) and % Plumbagin found in samples and % recovery were calculated (Table No.4). Quantity of Plumbagin found in Local Market Sample, Ghaziabad (U.P.) is 4.836mg in 1g drug sample (0.4836% w/w) and quantity of Plumbagin found in Kalmegh Navayas Loha is 0.4989 mg in 1g drug sample (0.04989%w/w). The % recovery of Plumbagin in Local Market Sample, Ghaziabad (U.P.) is 99.91% w/w and 99.73%w/w in Kalmegh Navayas Loha. The mean % recovery was 99.82%.

Table 1:

S. No.	Name of Physico-chemical constants	KALMEGH NAVAYAS LOHA	Local Market, Ghaziabad
1.	Moisture content	6.86% w/w	4.33% w/w
2.	pH (of 5% aq. Solution)	5.23	5.82
3.	Total ash	27.04% w/w	1.63% w/w
4.	Acid in-soluble ash	3.23% w/w	0.45% w/w
5.	Water soluble ash	17.05% w/w	0.24% w/w
6.	Water soluble extractives	19.78% w/w	13.23% w/w
7.	Ethanol soluble extractives	6.34% w/w	15.05% w/w
8.	Chloroform soluble extractives	2.97% w/w	16.95% w/w
9.	Hexane soluble extractives	0.656% w/w	2.01% w/w
10.	Disintegration Time	5 minutes	-
11.	Average weight of 20 pills	0.1334 gm	-
12.	Weight variation with respect to average weight of 20 pills	Minimum variation - 0.75% w/w Maximum variation - 12.29% w/w	-

Table 2:

Detection/ Visualization	Citrak Root (Track No. T1 and T3)		Standard-Plumbagin (Track No. S1, S2 and S3)		Kalmegh Navayas Loha (Track No. T2 and T4)	
	R_f. values	Colour of band	R_f. values	Colour of band	R_f. Values	Colour of band
					0.05	dark grey
	0.05	grey			0.08	dark grey
Under	0.10	grey			0.10	dark grey
UV 254 nm	0.46	grey			0.15	dark grey
	0.84	dark grey	0.84	dark grey	0.19	grey

					0.26	dark grey
					0.28	dark grey
					0.35	dark grey
					0.40	dark grey
					0.46	dark grey
					0.63	grey
					0.71	dark grey
					0.84	dark grey
					0.93	dark grey
					0.05	blue
	0.05	light green			0.08	blue
Under	0.10	brown			0.10	blue
UV 366 nm	0.25	bright green			0.15	blue
	0.46	bright sky blue			0.19	brown
	0.70	sky blue			0.26	blue
	0.84	red	0.84	red	0.28	blue
					0.35	green
					0.40	pink
					0.46	light blue
					0.58	red
					0.63	blue
					0.71	red
					0.80	pink
					0.84	red
					0.93	blue
					0.05	dark violet
	0.05	violet			0.10	dark violet
After derivatization	0.10	violet			0.15	brown
	0.15	violet			0.26	blue
	0.46	dark violet			0.35	violet
	0.57	light violet			0.46	violet
	0.65	light violet			0.63	brown
	0.73	light violet			0.80	violet
	0.84	yellow	0.84	yellow	0.84	yellow
					0.93	dark violet

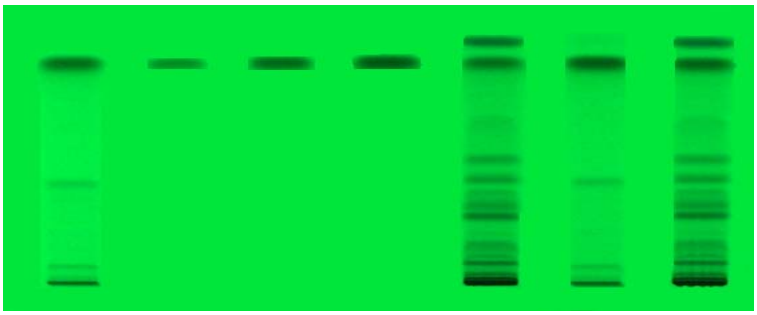
Table 3:

Sr. No.	Track No.	Volume applied on plate	Quantity applied on plate	Quantity of Plumbagin via graph	Linearity & regression coefficient and standard deviation via graph
1.	T1	9µl	900µg	4.354µg	
2.	S1	2µl	2µg	2.000µg	
3.	S2	4µl	4µg	4.000µg	Y = 16247.523 + 5397.956 * X + -255.343 * X2
4.	S3	6µl	6µg	6.000µg	r = 0.99999 sdv = 0.00%
5.	T2	9µl	4500µg	2.248µg	
6.	T3	(9+1)µl	900µg +1µg	5.350µg -1µg = 4.350µg	
7.	T4	(9+1)µl	4500µg +1µg	3.242µg -1µg = 2.242µg	

T1- Chloroform extract of Citrak Root, Local Market sample, Ghaziabad
S1- Plumbagin standard solution
S2- Plumbagin standard solution
S3- Plumbagin standard solution
T2- CHCl3 Extract of Kalmegh Navayas Loha, Local Market Sample, Ghaziabad
T3- Chloroform extract of Citrak Root, Local Market sample, Ghaziabad
T4- CHCl3 Extract of Kalmegh Navayas Loha, Local Market Sample, Ghaziabad

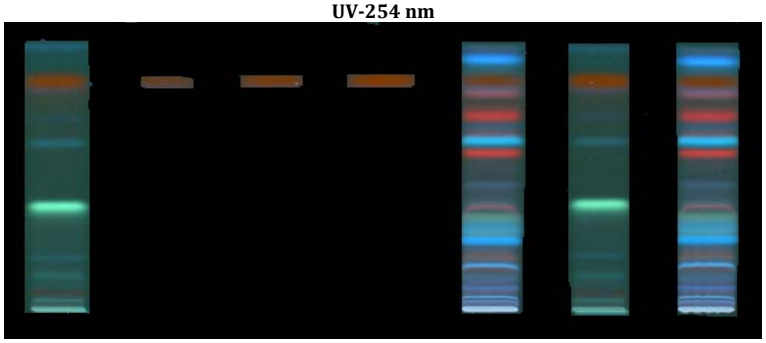
Table 4:

Sr. No.	Sample (local market sample, Ghaziabad)	Citrak Root	Kalmegh Navayas Loha
1.	**Quantity of Plumbagin in 1gm**	4.836mg	0. 4989 mg
2.	**% Plumbagin**	0.4836% w/w	0.04989 % w/w
3.	**% Recovery**	99.91% w/w	99.73% w/w



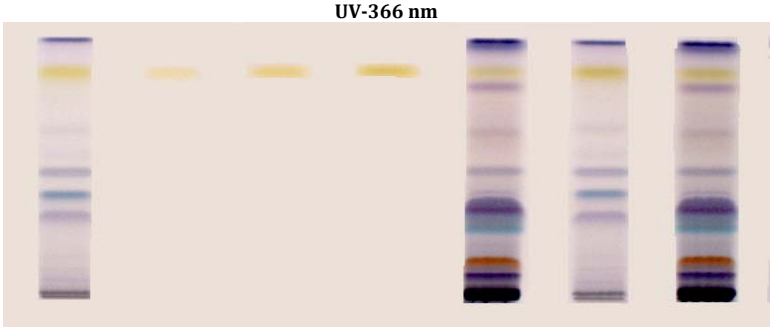
T1 S1 S2 S3 T2 T3 T4

UV-254 nm



T1 S1 S2 S3 T2 T3 T4

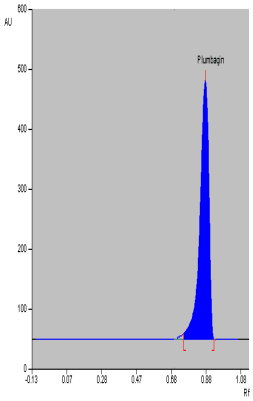
UV-366 nm



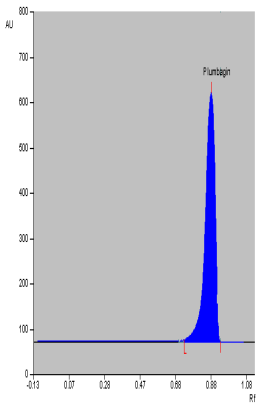
T1 S1 S2 S3 T2 T3 T4

After derivatization

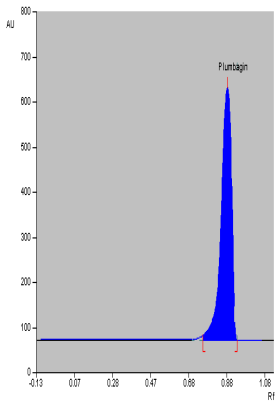
Fig. 1: H.P.T.L.C. Finger print of KALMEGH NAVAYAS LOHA



(Track S1)

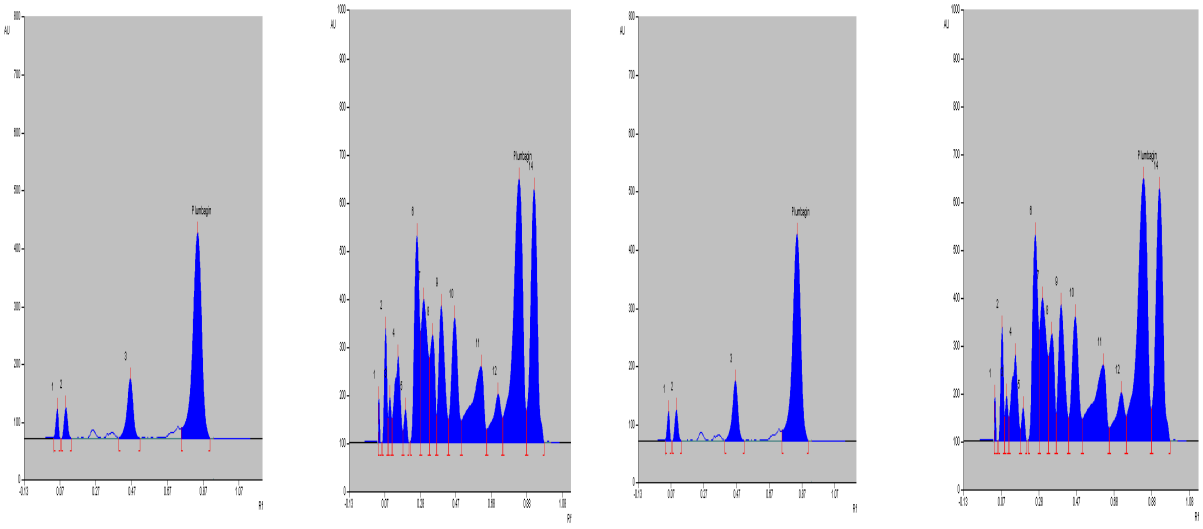


(Track S2)



(Track S3)

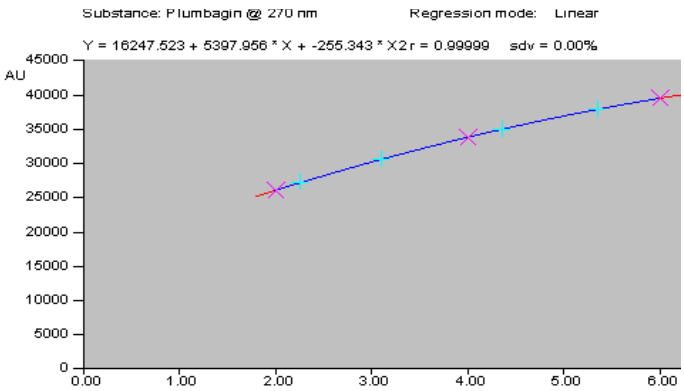
Peak of Plumbagin @ 270nm



(Track T1) **(Track T2)** **(Track T3)** **(Track T4)**

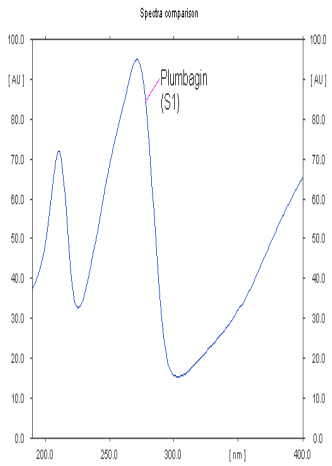
Peak of Citrak (root) extract @ 270nm (Track T1& T3) **Peak of Kalmegh Navayas Loha extract @ 270nm (Track T2& T4)**

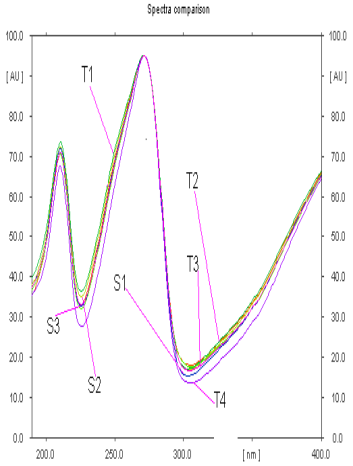
Fig. 2: Peaks of Kalmegh Navayas Loha in all tracks



Area

Graph Area vs AU





Spectra of Plumbagin@ 270 nm **Spectra of Plumbagin in all tracks @ 270 nm**

Fig. 3: Graph and spectrra of Kalmegh Navayas Loha

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 Plumbagin 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 Kalmegh Navayas Loha powder and quantitative determination of Plumbagin in Kalmegh Navayas Loha powder.

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