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PHYSICO-CHEMICAL STANDARDISATION AND DEVELOPMENT OF HPTLC METHOD FOR THE DETERMINATION OF PLUMBAGIN IN CHITRAK HARITAKI – AN AYURVEDIC FORMULATION

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for determination of plumbagin in Ayurvedic formulations of Chitrak Haritaki of different manufactures. The chloroform extract of Chitrak Haritaki and Chitrak root samples were applied on TLC Aluminium plate pre coated with Silicagel60 GF254 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 270nm.

Keywords: Chitrak Haritaki, Chitrak Root, Plumbagin, Standardisation, HPTLC.

1. INTRODUCTION

Chitrak haritaki is a very famous Ayurvedic medicine used in treating chronic respiratory conditions. It is in herbal jam form. It is also known as Chitrak haritaki Avaleha, Chitraka Haritaki etc.Avaleha suggests that it is a herbal jam. Chitraka and haritaki are two herbs, which are the main ingredients of this product.

Chitrak Haritaki Uses

It is used in the treatment of chronic respiratory conditions, Ashtma, bronchitis, rhinitis and

tuberculosis.

It is also used to improve digestion power and to treat bloating and intestinal wor

Chitrak Haritaki Ingredients

A 4.8 liters of decoction is prepared with each of -

Chitraka – Plumbago zeylanica,

Amalaki-Embellica officinallis

Guduchi – Tinospora cordifoliasand Dashamoola

It is added with 4.8 kg of jaggery and 3.072 kg of Haritaki – Terminalia chebula.

This mixture is heated till semi solid consistency.

It is added with **Trikatu** – pepper, long pepper and ginger – 96 g each

Cinnamon – 96 g

Tejpatra – Cinnamomum tamala – 96 g

Yavakshara – 24 g

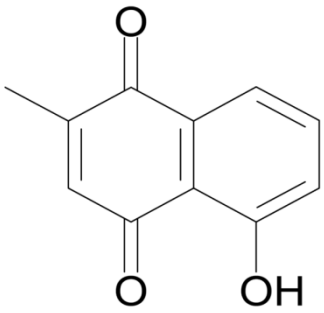
And 384 grams of honey.

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 one of the main ingredient of this formulations and 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 leaves are up to 8 cm long, simple, glabrous, alternate, ovate or oblong with an entire or wavy margin, an acute apex and a short petiole. The flowers are white in terminal spikes with a tubular calyx, a slender, glandular, hairy corolla tube with five lobes and five stamens, a slender style and a stigma with five branches. The fruit is a membranous capsule enclosed with the persistent calyx. The dried roots occur as cylindrical pieces of varying length, less than 1.25 cm in width, reddish brown in colour with a brittle, fairy thick, shriveled, smooth or irregularly fissured bark. The roots have a short fracture an acrid and biting taste and disagreeable odour.[1-13]

The root and root bark are bitter, stomachic carminative, astringent to bowels, anthelmintic, piles bronchitis, itching, diseases of lever, consumption and ascities. The root is bitter, laxative, expectorant, tonic, abortifacient, alexipharmic, good appetizer, useful in laryngitis, rheumatism, diseases of spleen, ringworm and 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 antiperiodic. It acts as a powerful sudorific. Leaves are caustic, versicant aphrodisiac and good for scabies.[5]

Plants contains number of naphthaquinone derivatives viz. plumbagin, 3-chloroplumbagin, 3,3'-biplumbagin, elliptinone, chitranone, zeylinone, isozeylino ne, 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 and its compound formulations.

2. MATERIAL AND METHOD

(1) The Chitrak haritaki of three different manufactures was procured from the Local Market Ghaziabad. It was identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad and coded for further study.

(i) CH1DB (ii) CH2BY (iii) CH3ZB

(2) The Chitrak root was procured from the Local Market, Ghaziabad and also identified and authenticated by the Botanists of Pharmacopoeial Laboratory for Indian Medicine, Ghaziabad and coded as SD1 for study.

2.1 Determination of Physico-Chemical constants

The following Physico-Chemical constants has been analysed for both samples and results are given in Table No. 1.

Table No. 1: Physico-Chemical constants of Chitrak haritaki

S. No.	Name of Physico-chemical constants	CH1DB	CH2BY	CH3ZB
1.	Moisture content	4.76% w/w	4.07% w/w	3.42% w/w
2.	pH (of 5% aq. Solution)	5.2	4.7	4.9
3.	Total ash	2.98% w/w	2.57% w/w	2.84% w/w
4.	Acid in-soluble ash	0.43% w/w	0.37% w/w	0.33% w/w
5.	Water soluble ash	1.23% w/w	1.08% w/w	1.61% w/w
6.	Water soluble extractives	37.21% w/w	39.07% w/w	38.88% w/w
7.	Ethanol soluble extractives	17.54% w/w	16.98% w/w	17.06% w/w
8.	Chloroform soluble extractives	9.43% w/w	9.32% w/w	8.74% w/w
9.	Hexane soluble extractives	8.72% w/w	8.01% w/w	8.44% w/w

2.2 H.P.T.L.C. (High Performance Thin Layer Chromatography)

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; Toluene, ethyl acetate, Formic acid, Chloroform, Methanol, Alcohol, 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). Reference standard Plumbagin procured from Aldrich Chem. Co. Milw, WI 33201 (414-273-3850/19,064-0481-42-5).

Sample & Standard preparation

Sample preparation: 1g of coarsely powdered crude drug and Citrak Haritaki samples were extracted with 10 ml Chloroform 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. Filtrate was concentrated to 2 ml and used for H.P.T.L.C.

Standard Preparation: 5mg of standard Plumbagin dissolved in 5ml of Chloroform and made up to 5ml in standard volumetric flask.

2.3 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 (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.

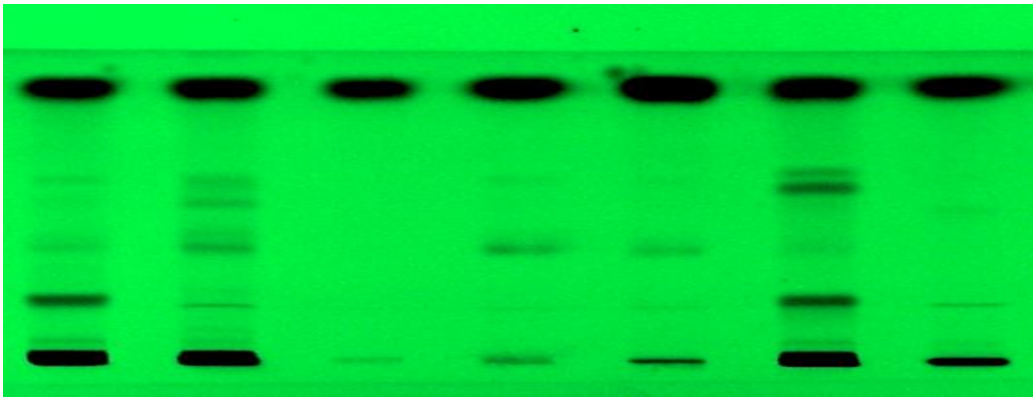
2.4 Linearity of Detector Response, Assay and Recovery

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, 12µl of Chloroform extract of samples applied on Track No. T1 T2 & T3 on the same plate. TLC plate 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. 0.88 (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 was given in Table No. 4.

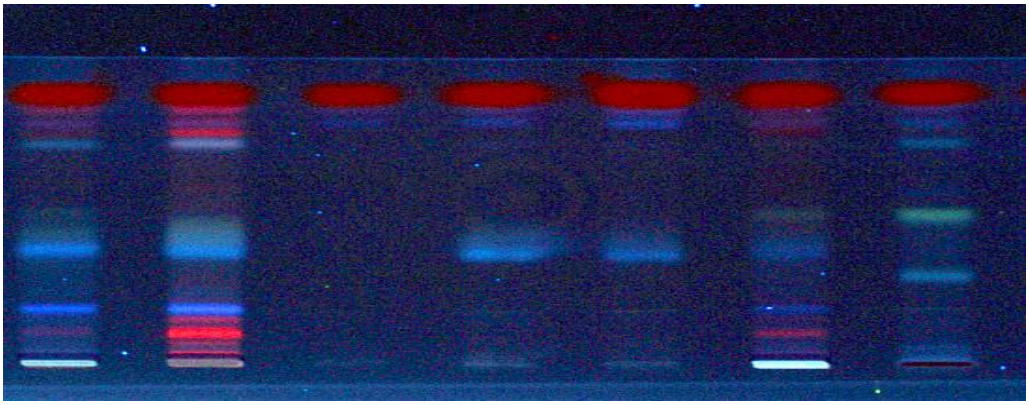
Table No. 2: HPTLC details of chloroform extract of Citrak Haritaki

Sr. No.	Detection/ visualization	Citrak Haritaki (Track T1, T2 & T3)		Standard- Plumbagin (Track S1, S2 & S3)		Chitrak Root (Track SD1)	
		R_f. values	Colour of band	R_f. Values	Colour of band	R_f. values	Colour of band
1.	Under UV 254 nm	0.22 0.38 0.51 0.60 0.88	grey grey grey grey dark grey	0.88	dark grey	0.22 0.38 0.51 0.88	grey grey grey dark grey
2.	Under UV 366 nm	0.07 0.15 0.22 0.38 0.48 0.71 0.76 0.88	sky blue red sky blue sky blue green sky blue red bright red	0.88	bright red	0.07 0.32 0.41 0.48 0.71 0.88	sky blue sky blue sky blue green sky blue bright red
3.	After derivatization	0.21 0.38 0.51 0.71 0.88	greenish grey violet violet violet grey	0.88	grey	0.38 0.51 0.58 0.88	violet violet violet grey



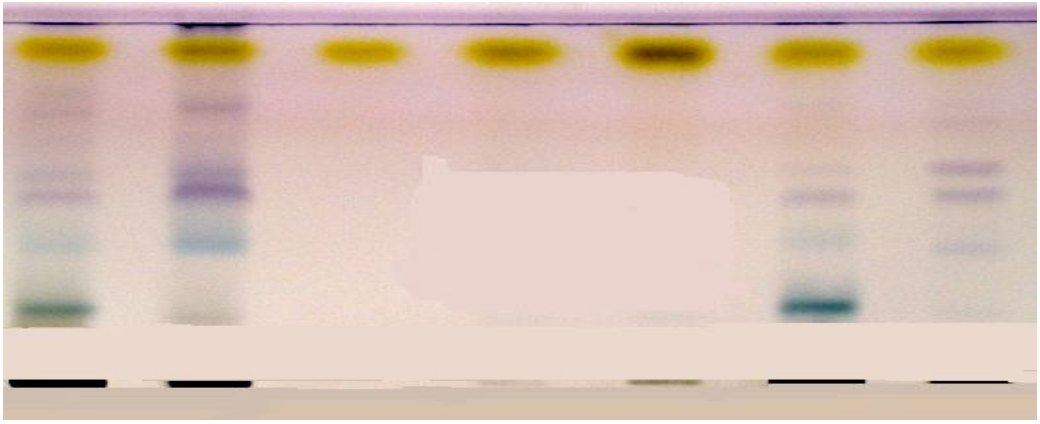
T1 T2 S1 S2 S3 T3 SD1

UV-254 nm



T1 T2 S1 S2 S3 T3 SD1

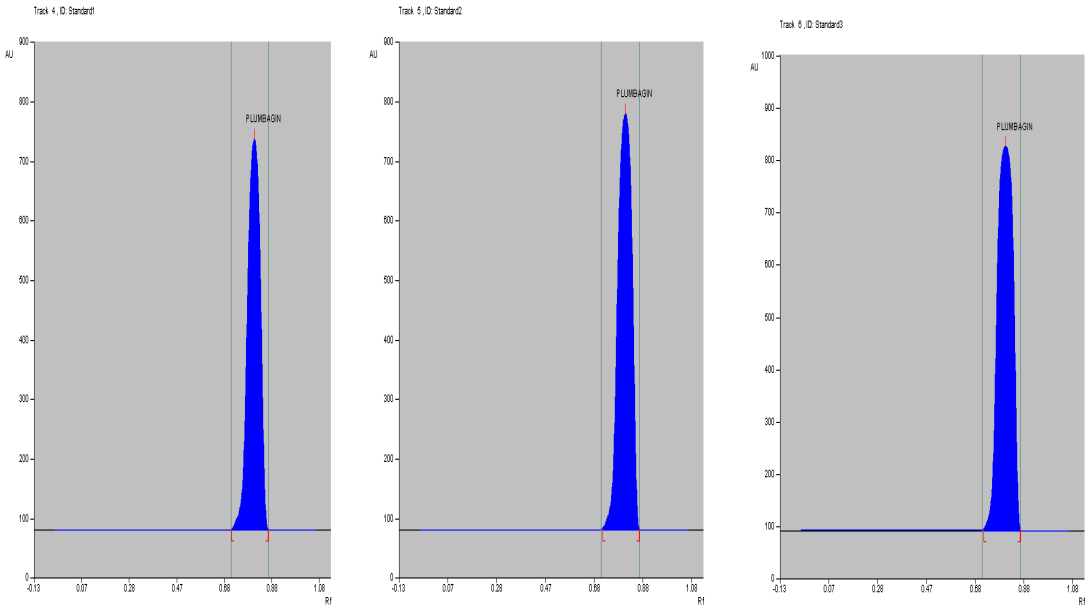
UV-366 nm



T1 T2 S1 S2 S3 T3 SD1

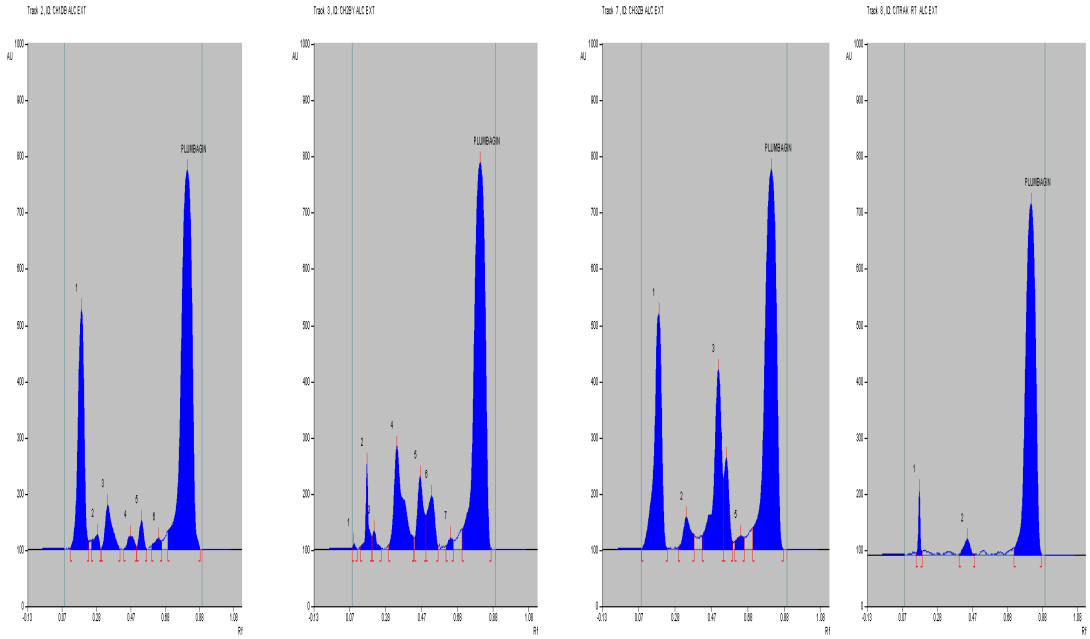
After derivatization

Fig.- 1: H.P.T.L.C. Finger print of Citrak Haritaki



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

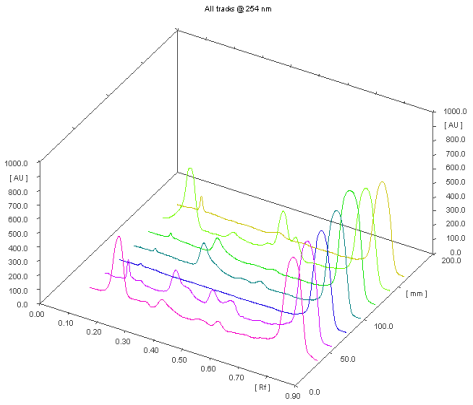
Peaks of Plumbagin @ 270nm



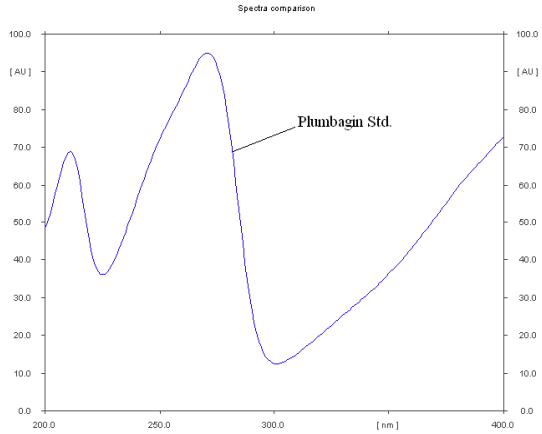
(Track T1) **(Track T2)** **(Track T3)** **(SD1)**

Peaks of Citrak Haritaki and Citrak Root CHCl3 Extract @ 270nm

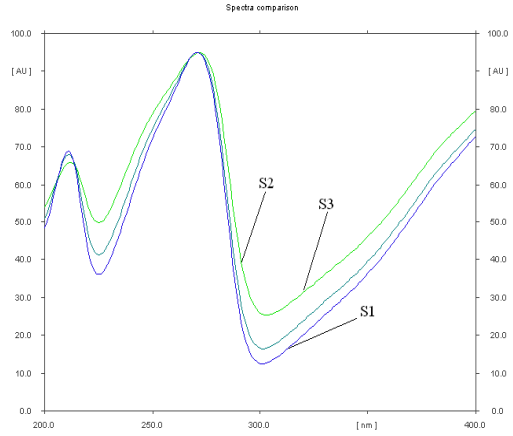
Fig. 2: Peaks of Citrak Haritaki in all Tracks



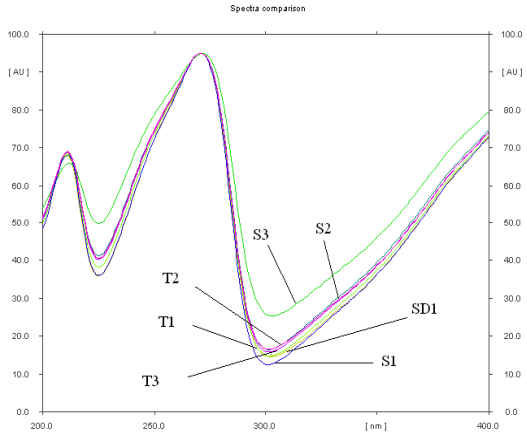
3D representation of Plumbagin



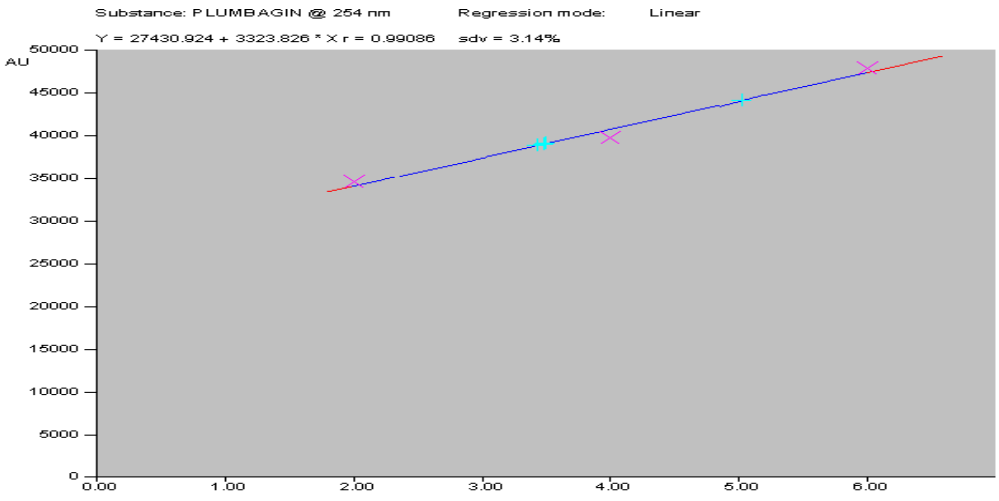
Spectra of Plumbagin @ 270nm



Spectra of Plumbagin in all Std.@ 270nm



Super Imposable UV Spectra of Plumbagin in all tracks @ 270nm



Graph Area vs AU

Fig. 3: 3D representation, Spectrra and Graph of Citrak Haritaki

Table No.3 Quantity applied on plate and values found via graph

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	12µl	6000µg	3.567µg	Y = 27430.924 + 3323.826 * X -63.331 * X2 r = 0.99086 sdv = 3.14%
2.	T2	12µl	6000µg	3.642µg	
3.	S1	2µl	2µg	2.000µg	
4.	S2	4µl	4µg	4.000µg	
5.	S3	6µl	6µg	6.000µg	
6.	SD1	3µl	1500µg	5.217µg	
7.	T3	12µl	6000µg	3.689µg	

T1 - Chloroform extract of CH1DB
T2 - Chloroform extract of CH2BY
S1 - Plumbagin Std. Chloroform solution (1mg/ml)
S2 - Plumbagin Std. Chloroform solution (1mg/ml)
S3 - Plumbagin Std. Chloroform solution (1mg/ml)
SD1 - Chloroform extract of Chitrak Root
T3 - Chloroform extract of CH3ZB

Table No.4: Summary of results

Sr. No. ↓	Sample from →	CH1DB	CH2BY	CH3ZB	Citrak Root
1.	Quantity of Plumbagin in 1g	0.595mg	0.607mg	0.615mg	3.478mg
2.	% Plumbagin	0.0595%w/w	0.0607%w/w	0.0615%w/w	0.3478%w/w

3. RESULT 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. 0.88 very efficiently from the other components in Chloroform extract of Citrak Haritaki (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 was calculated (Table No.4). Quantity of Plumbagin found in TC1TH is 0.595mg in 1g drug sample (0.0595% w/w), in TC2VP is 0.607mg in 1g drug sample (0.0607%w/w), in TC3DV is 0.615mg in 1g drug sample (0.0615%w/w) and Quantity of Plumbagin found in Citrak Root is 3.478mg in 1g drug sample (0.3478%w/w).

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.

4. CONCLUSION

The proposed HPTLC method is simple, rapid, accurate, reproducible, selective and economic and can be used for routine quality control analysis of *Plumbago zeylanica* Linn. (root) powder and quantitative determination of Plumbagin in Chitrak Haritaki.

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