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SEASONAL VARIATION AND STORAGE STUDIES IN CHEMICAL CONSTITUENTS
OF *PLUMBAGO ZEYLANICA* LINN BY HPTLC

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Abstract

The plant *Plumbago zeylanica* Linn. of Plumbaginaceae known as Chitraka/ Chitramoolam is a popular drug in Ayurveda and Siddha. The root of this plant finds place in several of the compound formulations of these systems of medicine, as one of the ingredients. The root being a vital component in these formulations, its quality and consistency is of prime importance. Plumbagin being the major bioactive chemical was used as a reference standard with which the HPTLC chromatogram obtained for each sample was compared. Plumbagin present in each sample was quantified using a calibration graph of the reference standard. The present study deals with the study of the presence of any variation in the plumbagin content due to change in season and storage. Sufficient quantities of root was collected during the month of July 2008 and stored at room temperature for the assay of Plumbagin. From August onwards every month, fresh root samples were also collected till June 2009. Simultaneous comparison of the fingerprints of n-hexane extract of the roots as well as estimation of plumbagin was done on the samples collected and the stored sample at room temperature using HPTLC technique. The plumbagin content showed a sharp decline from July 2008 to June 2009 on the stored root samples. The study established more concentration of Plumbagin constituent in February followed by January. The content was found to be very less in April. Hence the root may show more efficacy if collected in February. The technique is simple and cost effective.

Key words: *Plumbago zeylanica*, HPTLC, plumbagin, seasonal variations, storage changes.

Introduction

In the Ayurveda and Siddha formulations plants are used as raw material due to the presence of bioactive chemical entities. Duration of the activity of the formulations depends upon the stability of the secondary metabolites available in the plants. In classical texts of Ayurveda, it is mentioned that 'Viriya Kalavadi' is a certain period within which the drug is to be used in the preparation of formulations. Generally plant materials are either collected from wild or cultivated. At present, majority of the plant species are collected from natural resources. In normal practice, the excess quantities of the material or one which is required after some time is stored for subsequent use. Under such situations, stability and potency of the plant materials may vary due to the change in their bioactive chemical contents. It is necessary to check the quality of the material by estimating the phytomarkers available in the plant parts.

The plant *Plumbago zeylanica* of Plumbaginaceae known as Chitraka/ Chitramoolam is a popular drug in Ayurveda and Siddha [1]. The root of this plant is used as ingredient in several of the formulations of these systems of medicine [2,3]. The root and root bark are bitter, stomachic, carminative, and astringent to the bowels, anthelmintic, alterative, cure intestinal troubles, dysentery, inflammations, piles, bronchitis. Root is vesicant, laxative, expectorant, diuretic, tonic, abortifacient, alexipharmic and useful in laryngitis, rheumatism, diseases of the spleen, leucoderma, ringworm and scabies. Root is said to increase the digestive power, to promote the appetite, and to be useful in dyspepsia, piles, anasarca, diarrhoea and skin diseases. For external administration, it is made into a paste with milk, vinegar or salt and water. It may be applied externally in leprosy and other skin diseases of obstinate characters and be allowed to remain until a blister has formed. A tincture of the root bark has been employed as an antiperiodic and it acts as a powerful sudorific. The roots of this species is recognized due to the presence of naphthaquinones namely plumbagin and its derivatives [4,5]. The roots of *P.rosea* and *P.zeylanica* have been evaluated for biological activities [6, 7]. *P. zeylanica* had been studied in greater detail [8, 9, 10] and number of other benzoquinones, including dimers of plumbagin, have been reported [11]. The percent content of plumbagin was found to be in the decreasing order of *P. rosea* (0.17), *P. capensis* (0.04) and *P. zeylanica* (0.01) [12]. The roots of *P. zeylanica* have been used extensively. Though the studies on its

medicinal utility are well established, there are no criteria for exact period of collection. The promising pharmacological activities encouraged to know the season that would be suitable for the collection and how long these active principles remain in the crude material (roots) of this plant. Therefore the present paper deals with the seasonal variation and storage of *P. zeylanica* roots with respect to its plumbagin content by HPTLC.

Materials and Methods

Collection of plant material

Roots of *P. zeylanica* was collected from Chengalpet, Tamil Nadu and authenticated at Captain Srinivasa Murthi Drug Research institute for Ayurveda (CCRAS), Chennai. The voucher specimen of this plant (00518) is deposited at the herbarium of this institute. The plant material was washed quickly, shade dried, coarsely powdered using a rotary grinder, stored in air tight containers for HPTLC analysis.

Preparation of sample solution

Coarsely powdered roots (1 to 4 g) were extracted using n-hexane (3X50ml) for 16-20 hrs. The combined n-hexane extract was filtered and concentrated on a water bath. The extract was transferred quantitatively into a 10ml volumetric flask and made up to the mark. From this stock solution 1ml was pipetted out and diluted to 2 ml with n-hexane and used for analysis.

Preparation of standard solution

Standard plumbagin was purchased from M/S Sigma chemicals. 10mg of standard plumbagin were accurately weighed into 10ml volumetric flask, dissolved in n-hexane and the volume was adjusted with the same solvent up to the mark. From this stock solution, 1ml was pipetted out into 10ml standard flask and made up to the mark(1 μ l =0.1 μ g).

HPTLC Analysis

Chromatographic conditions

All chemicals and solvents used were analytical and HPLC grade (E.Merck, Mumbai, India).

Sample and Standard solutions were spotted in the plate as 6 mm wide bands with TLC applicator Linomat V

with N₂ flow (CAMAG, Switzerland), 10 mm from the bottom, and 13 mm space between two bands were identical, for all the analysis performed. Various volumes of extract ranging from 5 to 10 µl were spotted on aluminum plates precoated with silica gel 60 F₂₅₄ plates 10x 10 cm of 0.2 mm thickness (Merck).

Detection of plumbagin

The HPTLC plates were developed using a CAMAG twin trough glass tank which was pre-saturated with the mobile phase Toluene: Ethyl acetate (8:2) for 20 minutes and each plate was developed to a height of 8cm. The composition of mobile phase was optimized by using the different mobile solvent of varying polarity. The HPTLC runs were done in laboratory condition of 25 ± 2°C and 50 ±5 % relative humidity, after development the plate was withdrawn and dried. Spots were visualized under short wavelength UV 254 nm light (UV Cabinet, CAMAG, and Switzerland).

Plumbagin was quantified with CAMAG TLC scanner 3 equipped with WINCATS software version 1.3.4 and computer under the following conditions: slit width 6 x0.40mm, wavelength 265nm UV (Deuterium lamp) absorption – reflection detection mode.

Calibration graph

5-9µl of the standard solution containing 0.5µg to 0.9µg of plumbagin was applied on the HPTLC plate and developed in the same solvent and scanned at 265nm. Calibration graph of plumbagin was constructed by plotting concentration versus spot area of the compound [13, 14]. The calibration graph as obtained was used to quantify the plumbagin. The relative standard deviation, regression coefficient were then calculated. The software employed for the analysis was Wincats.

Result and Discussion

Seasonal variations

The HPTLC profile of the extract of *P.zeylanica* collected during the month of July 2008 is shown in Fig. 1. Similar fingerprinting profiles were observed for the roots of extract of *P. zeylanica* collected during every month though quantity of the chemical constituents differs. The difference in constituents among the extracts of roots

collected in different months studied showed similar bands. The plumbagin standard had R_f at 0.77. The identified band of plumbagin in the sample extract was confirmed by overlaying its absorption spectrum with that of standard using CAMAG TLC Scanner 3. The UV spectra of all the extracts were recorded and then overlaid with that of standard plumbagin (Fig 2). The exact overlay of the UV spectra of the samples with that of the reference standard as well as the display of the λ max of each of the overlaid spectra at λ 265nm indicated that all of the bands at R_f 0.77 were those belonging to plumbagin. Thus it could be concluded that plumbagin is present in the root in all seasons. The purity of plumbagin band in the sample extract was confirmed by comparing the absorption spectra at start, middle and end position of the band. The calibration graph of plumbagin was linear in the range of 0.5 μ g to 0.9 μ g (Fig 3) $Y=109.709 + 0.124 * X$; $r = 0.99411$; $sdv = 2.24$ (Fig 3). The amount of plumbagin content per 100g of stored sample and fresh sample are shown in Table 1. Samples collected during the period from July 2008 to June 2009 showed variation in the concentration of plumbagin. The amount of plumbagin started increasing gradually from July 2008 and maximum amount was observed in February followed by January 2009. There was again decrease in the concentration of plumbagin observed during the month of March to May 2009. This indicates that the content of plumbagin vary due to climate. The present study reveals that the suitable period of collection for the roots of *P. zeylanica* is January / February.

Storage variations

The study of storage of roots of *P. zeylanica* revealed that the HPTLC finger print profile of the extracts were similar in all the months. The bands were very few in number in some cases. The prominent bands observed in all the extracts were those at R_f 0.77. The bands at R_f 0.77 in all the samples matching with that of plumbagin reference standard were subjected to UV spectral analysis. The λ max of each month extract was found to be at 265nm, which matched with that of the reference plumbagin. This indicated the presence of plumbagin in the root extracts of every month. The content of plumbagin was quantified each month. The amount of plumbagin in each sample quantified on the basis of calibration graph was found to decrease gradually starting from the fresh collection (0.1168g / 100g, July 2008) till the end of study (0.102g/ 100g, June 2009) (Table 1). The present

study on storage of roots of *P. zeylanica* revealed that the storage conditions light/ heat/ humidity affect the phytochemicals present in the plant, thereby suggesting that the root of *P.zeylanica* can not be stored for a longer time.

Table 1 Data showing the plumbagin content in root of *P.zeylanica*

S.No	Month and Year	Amount of plumbagin content in root (g per 100g)	
		Sample Stored at room temperature	Fresh Sample
1	July 2008	0.1168	0.1168
2	August 2008	0.0969	0.1298
3	September 2008	0.1047	0.1644
4	October 2008	0.0908	0.1656
5	December 2008	0.0871	0.1700
6	January 2009	0.0861	0.1811
7	February 2009	0.0761	0.1958
8	March 2009	0.0599	0.1475
9	April 2009	0.0281	0.0825
10	May 2009	0.0112	0.0950
11	June 2009	0.0102	0.1028

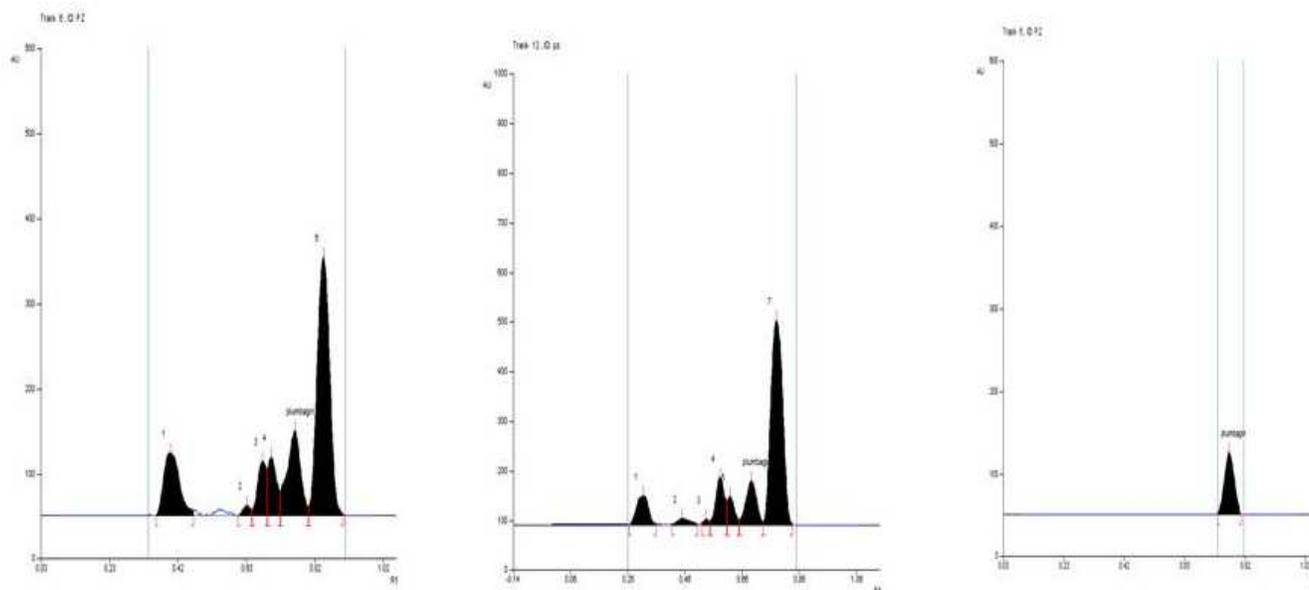


Fig.1 HPTLC finger print profile of *Plumbago zeylanica* root extract June 2008

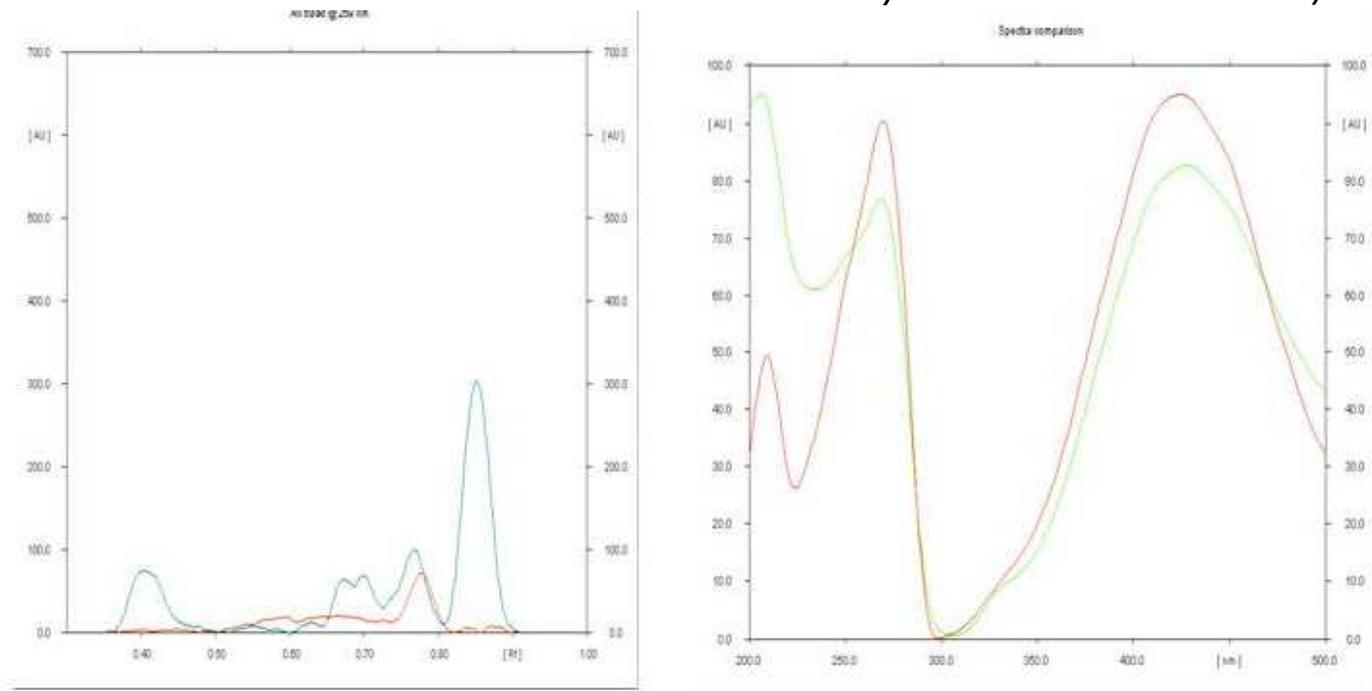


Fig.2 Super imposable spectra of Marker compound and sample

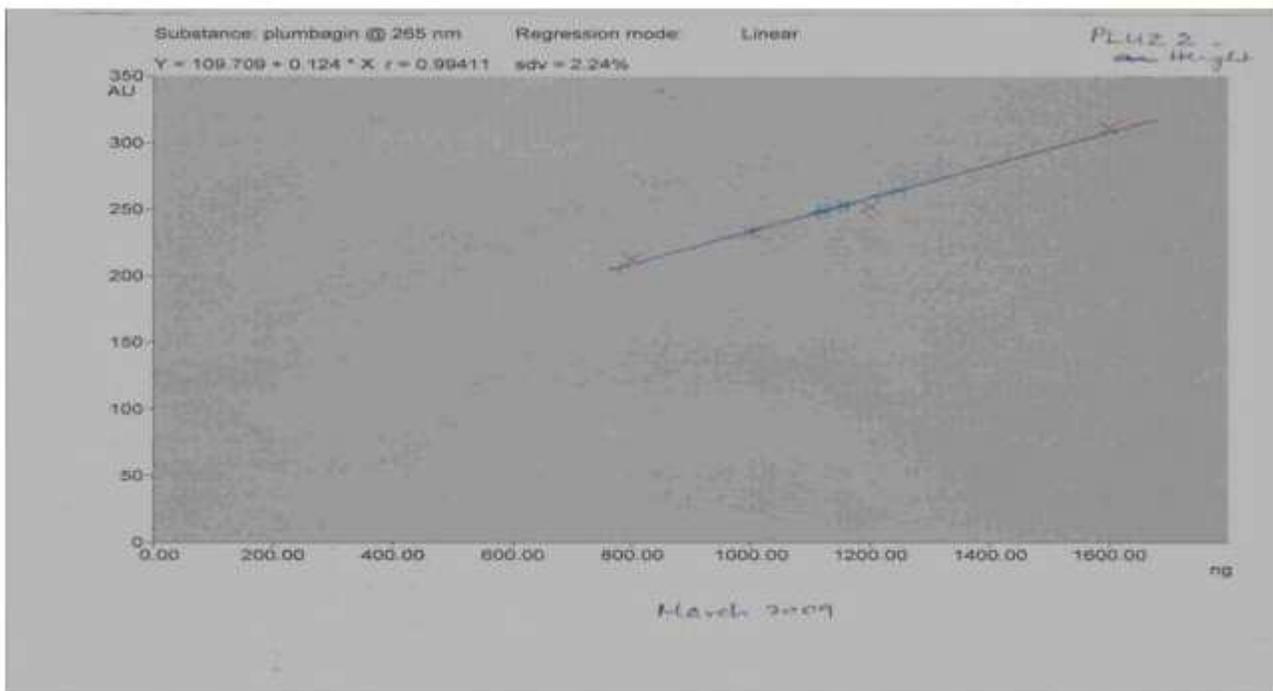


Fig 3 Calibration plot obtained by chromatography of marker compound. Regression via Area regression mode = Linear

Conclusion

The HPTLC study on seasonal variations and storage studies of *P.zeylanica* revealed that there was a

continuous decrease in absorbance value of plumbagin at 265nm in case of storage sample as compared to fresh sample *P.zeylanica*. This indicates that the plumbagin is not very stable undergoes some chemical changes, thereby indicating that the percentage reduces due to environmental conditions. The present investigation supports the use of fresh *P. zeylanica* plant and enlightens that sample stored for long period possess less chemical and biological properties. The best harvesting time was observed to be the month of February to get maximum plumbagin content. So similar analysis need to be carried out for all other plants to find its consistency before they are used to prepare formulation.

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