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Original Article

Histo-Chromatographic Finger Printing Profiles of the Root of *Plumbago zeylanica* Linn and Quantification of Marker Compound, Plumbagin

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ABSTRACT

Background: Plumbagin, a plant-derived naphthoquinone is known to be biosynthesized by polyacetate-malonate pathway. The ayurvedic drug Chitraka is obtained from the medicinal plant *Plumbago zeylanica* Linn, which belongs to the family Plumbaginaceae. The Chitraka is found used in the traditional forms of medicine for the treatment of various illnesses, since ancient times. **Aim**: The present study concerns the microscopic, powder and quantitative microscopic characteristics of the root of *Plumbago zeylanica* Linn. **Materials and Methods**: Delimiting the morpho-histological profile of the root using digital, stereo and polarized microscopic techniques and to develop the chromatogram of the extract of the root of *Plumbago zeylanica* Linn using High performance thin-layer chromatographic (HPTLC) method. **Results**: The presence of cortical deposition of plumbagin pigment as yellowish tints in the surface view of the sections of root, arrangement of starch grains as bunches of grapes in cortical tissues and ray parenchyma, crystal idioblasts containing calcium oxalate embedded in the intervening walls of the cortical cells, distinct band or patch of sclreids at the pericyclic region of the phloem, wood with non-storied cambium, distinct growth ring boundaries, paratracheal axial parenchyma, chains of vessels in radial multiplies of three to four, uniseriate to biseriate medullary rays with homogeneous cells, crowding of vessels at the central portion of secondary xylem, wood plugged with tylosis and low mesomorphy ratio for wood were the anatomical features characteristics of the taxon. The HPTLC profile of the methanol extract of root developed using the mobile phase, n-hexane:ethyl acetate (8:2 v/v) had revealed four phytoconstituents. The R_f value for plumbagin ($C_{11}H_8O_3$) was found to be 0.86. Densitometric scanning had shown λ_{max} of plumbagin at 270 nm. Spectral matching by overlaying the spectra of both standards and extract of root sample were confirmed the specificity of λ_{max} at 270 nm for the marker compound. The calibration curve was found to be linear in the concentration range of 2.00 to 10.00 µg/ band with the polynomial calibration equation $Y=178.8+91.61*X+-4.825*X^2$ and estimated that 5 µL of methanol extract of roots contained 1.326 µg of plumbagin. Thus the content of marker constituent (plumbagin) present in shade-dried roots of *Plumbago zeylanica* Linn (a Kerala habitant) was estimated as 0.179%. **Conclusion**: The present study suggests that the delineated characteristics of the roots of *Plumbago zeylanica* Linn could tag as the identifying parameters to substantiate and authenticate the raw drugs from the spurious/adulterants materials and developed HPTLC method could be effectively used for the regulatory perspectives and quality assessment of plumbagin in the polyherbal formulation/finished products of traditional medicine.

Key words: Axial Parenchyma, Mesomorphy Ratio, Tylosis, Plumbagin, Calibration Curve.

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INTRODUCTION

Plumbagin, a plant-derived naphthoquinone (IUPAC: 5-hyroxy-2-methyl 1,4 naphthoquinone or 5-hydroxyl-2-methyl-naphthalene-1,4-dione) pigment is obtained from a few taxonomically related families of the order Charyophyllales, which include Droseraceae (eg., *Aldrovanda*, *Drosera*, *Dionaea*) Plumbaginaceae (eg., *Ceretostigma*, *Plumbago*), Ancistrocladaceae (eg., *Ancistrocladus*), Dioncophyllaceae (eg., *Dionocophyllum*, *Triphyophyllum*), Nepenthaceae (eg., *Nepenthus*) and also from the genus *Diospyros*, an unrelated Ebenaceae family.[1] Members of these families are in common having the production of bioactive 1,4 naphthoquinones (1,4-NQs), a group of highly reactive small molecules scaffolding several potential pharmacological and therapeutic actions. The plant-derived 1,4-NQs are known to be biosynthesized from several metabolic pathways, and their 1,4-naphthalenoid ring often bearing one or more methyl, hydroxyl and/or methoxy substitutions in diverse species. Plumbagin was first isolated in 1828 from the plumbago species, in a fairly pure condition by Dulong[2] and it was named so, after the genus from

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which it isolated. The empirical formula of plumbagin ($C_{11}H_8O_3$) was established in 1934 by A. Madinaveitia and Gallego. Plumbagin is known to be biosynthesized by polyacetate-malonate pathway.[3,4] It is synthesized from two carbon units derived from activated acetates in the form of acetyl CoA and malonyl CoA and form six acetates units, one carbon is lost. Subsequent cyclization and aromatization of the poly acetate chain yielding eleven carbon skeleton with two six membered ring, a benzene moiety fused with cyclic diketone (**Figure 1**).

Plumbagin is the major active principle of Plumbago species. It is a powerful irritant and toxin. The toxic effects are associated with its interaction as pro-oxidant; it can transfer electrons from a biological substrate to oxygen, causing the cellular production of ROS (reactive oxygen species) by reducing oxygen. Plumbagin can also react as electron-deficient electrophile, forming covalent bonds with nucleophilic functional groups of cellular metabolites, causing alteration in gene expression. ROS molecules like superoxide, hydroxyl radical, and hydrogen peroxide cause oxidative damage to protein, DNA and phospholipids.[5,6] Thus having the properties of being a reactive oxygen species (ROS) generator and apoptosis inducing agent, plumbagin has recently been implicated with altering multiple cancer –signaling pathways and reported to have shown immense potentials in anticancer (such as breast, prostrate, ovarian, lung, liver, pancreatic, renal, cervical and skin cancer, also in myeloma and leukemia)[7-14] therapy and in radiation therapy as the chemosensitizer and radiosensitizer.[15-17]

Genus Plumbago(Plum-Bay-go)of the family Plumbaginaceae is comprised of about 17 species.[18] They are found distributed mostly in semi-arid regions of Medititerranan and Central Asia and grow in salty, calcareous, rocky and other dry habitat. Three species of Plumbago are found distributed in India. They are P. *zeylanica* Linn, P.*indica* Linn and P.*capensis* Thunb. The root is the official part from which the drug Chitraka is obtained. *Plumbago zeylanica* Linn and *P.indica* Linn are espoused as source for the drug, Chitraka. Of these white plumbago (*Plumbago zeylanica* Linn.) is being used by the physician of Northern India. Whereas, in Sourthern States including Kerala preferred, the red flowered plumbago (P.*indica* Linn.) as the source plant for Chitraka.[19] Since ancient times, the root and whole plant of *Plumbago zeylanica* were found used in several traditional systems of medicine including Ayurveda, Chinese, sub-Saharan, Kempo, for the treatment of rheumatic pain, diarrhea, dyspepsia, pustule, skin diseases and as a diaphoretic, appetite stimulant, vesicant and contraceptive agent.[19,20,21] The pulp of the whole plant or roots of *P. zeylanica* have found used in folklore as abortifacient agent by inserting it into the female genitalia. Chitraka is one of the major ingredients in many Ayurvedic formulation including Citrakasavam, Dasamularistam, Gulguluktakam kasayam, and Yogarajachurnam.[19]

Since it is an important traditional remedy for several ailments, *P. zeylanica* Linn have extensively been investigated by various workers for the phytochemical,[22,23] microbiological,[24,25] *in vitro* microprogation,[26] pharmacological[27] and physiological studies[28] Many recent experimental studies have shown that plant having multiple therapeutic potentials including antioxidant, anti-inflammatory, anti-leishmanial, antifeedent, antibacterial, antifungal, antiatherogenic activities and also have cardiotonic, hepatoprotective, neuroprotective and central nervous system stimulating effects.[29,30] However, the botanical standards specified for appraisal of officinal part (ie, root) of *P. zeylanica* seemed to be too scanty in literature[31-33] and the available literatures are not exhaustive enough to extricate the crude raw material from the adulterants and substitutes. Therefore, the present study was undertaken with the objectives of elaborating the morphological and histological characteristics of the root of *P. zeylanica* using the techniques of digital, polarizing and stereo microscopy. It also aims to delineate and establish pharmacongostic markers and chromatographic finger print of the root extract of *P. zeylanica*, which could serve some of the important target regarding the regulatory aspects of quality control measures and as a rapid method for, quantify the marker compound plumbagin in the traditional herbal formulation/ finished product.

MATERIALS AND METHOD

Materials

The plant is a perennial spreading shrub, often grows in salty, dry places or coastal habitat. Stem is greenish, cylindrical, striated, slender and weak. Sprawling stem with profuse branches often appeared intertwined and branches attain a height up to 1- 1.5 m (**Figure 2**). Leaf are simple, membranous, alternate, lamina is broadly cordate at the base, acuminate at apex with entire margin. Leaves contained the calcium salt or chalk secreting glands. Flowers are pentamerous, occur in cluster in spike like racemes at the tip of branches. Flowers are white in color, calyx is persistent, tubular with five lobes and sticky, covered by many mucilage secreting emergences on their surfaces. Fruit is linear acheme. Seed is capsule; small in size contains hairs on their surfaces, which stick to the fur of animals' and aid in long distance distribution.

Methods

Delineation of Microscopic markers

Plumbago zeylanica Linn. for the present study was collected from the natural habitat of Thiruvananthapuram District of State of Kerala and identification and authentication were done using Gamble's Flora of Presidency of Madras. Fine hand sections of transverse longitudinal and radial planes of the roots were taken using the standard procedures. Sections were stained with aqueous Safranin 1% and mounted in glycerin. The investigation on macroscopic, micromorpho diagnostic profile, and powder microscopic properties of the specimens were undertaken. Microphotographs of sections and powder analysis were made by using Olympus Microscope (Model CX 41; Tokyo, Japan) with CCD camera 2 mega pixel and quantitative measurements were taken using Olympus Image-Pro Plus, version 5.1 software. The descriptive terms of the anatomical features used here as per Metcalfe and Chalk[34] and Sudhakaran.[35] The mesomorphy values ratio for wood was estimated based on Carlquist & Boggs method.[36] The shade-dried roots were pulverized and passed through an 85–mesh sieve. The root powder was cleared with absolute alcohol and mounted on glass slides for powder analysis. The pulverized powder was kept in a labeled, air tight glass container for chromatographic analysis.

Development of Chromatogram by High-Performance Thin-Layer Chromatography (HPTLC)

Chemicals and reagents

Aluminum plate (20×10 cm) pre-coated with silica gel 60 F254 (Merck) of uniform thickness was used as adsorbent. Analytically pure standard Plumbagin procured from Sigma and solvents of HPLC/ Chromatographic Grade procured from Merck and Qualigens Fine Chemicals, India were used.

Optimization of Chromatographic Conditions

The chromatographic separation was achieved, using different ratios of solvents of varying polarity and the mobile phase consisted of n-hexane:ethyl acetate (8:2 v/v) was found suitable for resolved separation of the analytes and quantification of marker compound.

Standard stock solution

The standard stock solution 10.5 mg/mL of plumbagin was prepared in HPTLC-grade methanol. One ml of this stock solution was diluted with 10 ml of methanol and a working solution of 1.05 mg/mL concentration was prepared and used for the HPTLC analysis.

Sample preparation

Accurately weighed 1.5 gms of root powder of *P. zeylanica* Linn was refluxed in 25 ml of Methanol. Extract obtained was filtered using Whatman filter paper and transferred to a volumetric flask, and volume was made upto 10 ml with methanol.

Instrumentation

CAMAG HPTLC System (Switzerland) equipped with CAMAG Linomat V Automatic Sample Spotter with syringe (100 µl), Twin trough glass chamber, UV cabinet with dual wavelength, and the densitometer consisted of TLC scanner 3 linked to WINCATS software were used.

HPTLC Methods

For the HPTLC analyses, five different application volumes of the working solution were used in five different tracks on the pre-coated plate of Silica Gel and methanol root extract of the test sample in one track (track no.3). The spotting volume of standard working solution were 1,3,5,7 and 9 µl with concentrations ranged from 1.05 to 9.45 µl /band respectively and the volume of root extract on the plate was 5 µl (ie., five standards plus one methanol extract of the root sample). The plate (20×10cm) was developed in Twin trough glass chamber, saturated with the mobile phase n-hexane: ethyl acetate (8:2 v/v). Densitometric scanning of the plate at 254 nm and 270 nm were performed using CAMAG TLC Scanner 3, and operated by the WINCATS software. Calibration graph was plotted using the peak height vs concentration of the standard marker compound. The content of plumbagin present in the methanol extract of the root was estimated using polynomial regression equation. The developed HPTLC method was validated for the parameters like specificity, linearity, limit of detection (LOD) and limit of quantitation (LOQ).

RESULTS AND DISCUSSION

Microscopic Evaluation of Root

Root is elongated cylindrical shaped, straight or slightly twisted and curved at places. Bark appeared brownish- yellow in color and surface appeared smooth (**Figure 3**). When cut, the root appeared starchy white internally. Transverse section (T.S) of the root showed somewhat circular outline with external bordering cork cells were interrupted at places and margin appeared irregular (**Figure 4 & 4c**). The tissue organization of the root consisted of an outer narrow zone of cork, followed by middle cortex and inner most zone of wood. Of these, wood was the comparatively larger area comprised of about 60% of the volume, followed by cortex (about 30%).Conspicuous cork was composed of 6–7 layers (**Figure 4a & 4b**) of regularly arranged rectangular cells. The outer 2–3 layers of cork were compactly arranged, thick walled, cells were square or rectangular in shape and filled with blackish brown contents, followed by inner 3-4 layers of cells which often filled with colored substances. Phellogen was distinct and consisted of one to two rows of cells. Cortex formed a narrow zone, which consisted of 14-16 layers of cells. The outer cortex contained 5-6 layers of elongated thick-walled, large parenchymatous ells. Cells were polygonal in shape filled with reddish brown content. Inner cortex consisted of 7-9 layers, composed of relatively small cells, square or rectangular in shape. The intervening walls between the cortical cells often embedded with crystal idioblasts, which contained prismatic crystals of calcium oxalate. The crystals varied in size, and ranged from

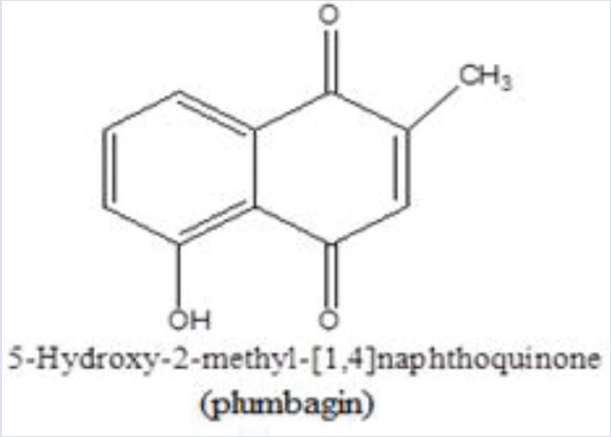

Figure 1: Structure of plumbagin

Figure 2: *Plumbago zeylanica* Linn.

Figure 3: *Plumbago zeylanica* Linn. Root (stereo microscopic view x 0.8)

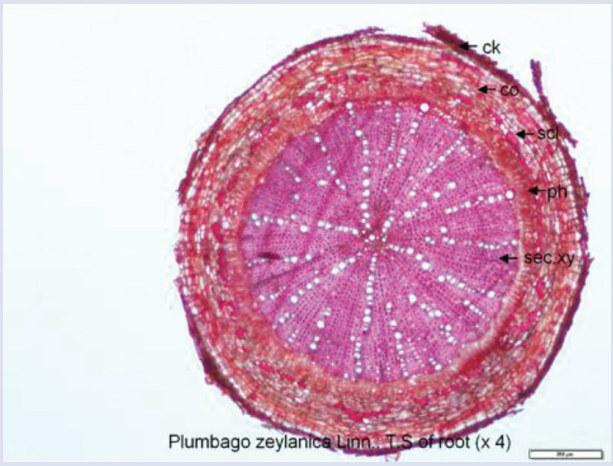

Figure 4: *Plumbago zeylanica* Linn. T.S of root (x 4)

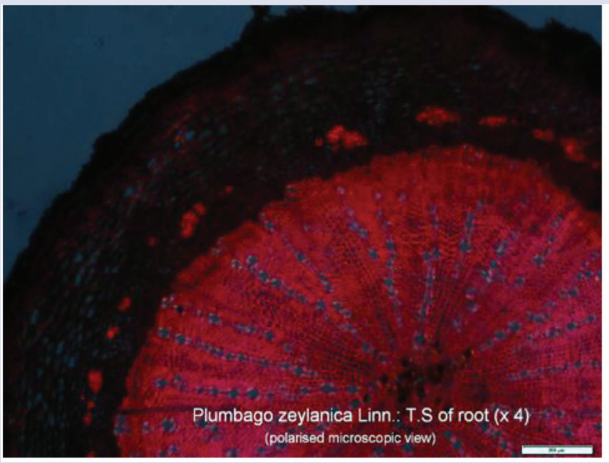

Figure 4c: *Plumbago zeylanica* Linn. T.S of root: (polarized microscopic view x 4)

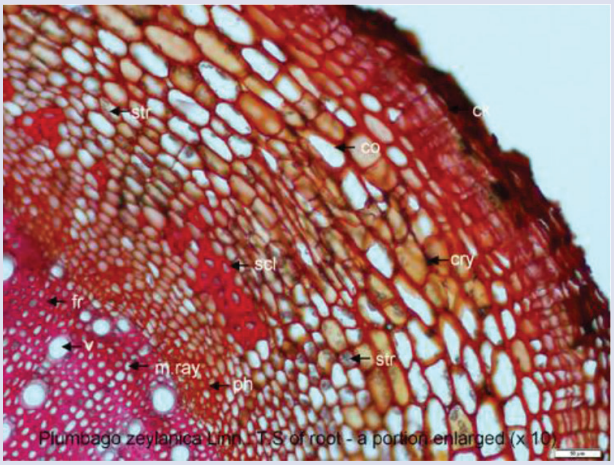

Figure 4a: *Plumbago zeylanica* Linn. T.S of root: a portion enlarged (x 10)

Figure 4d: *P. zeylanica* Linn. T.S of root: cortical cells contained starch grains (x 40)

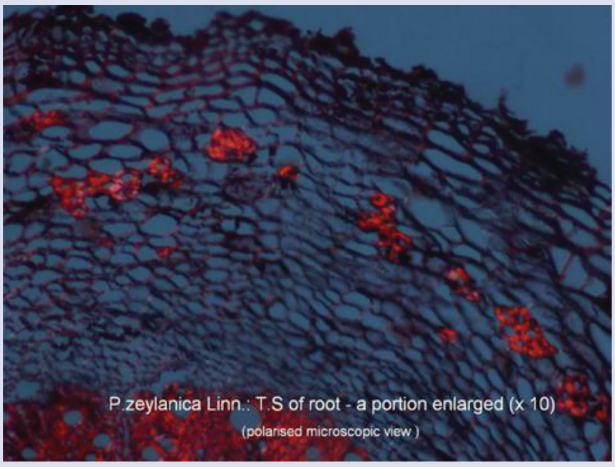

Figure 4b: *Plumbago zeylanica* Linn. T.S of root: a portion enlarged (x 10)

Figure 4e: *P.zeylanica* Linn. T.S of root: cortical cells with starch grains & sclereids (x 40)

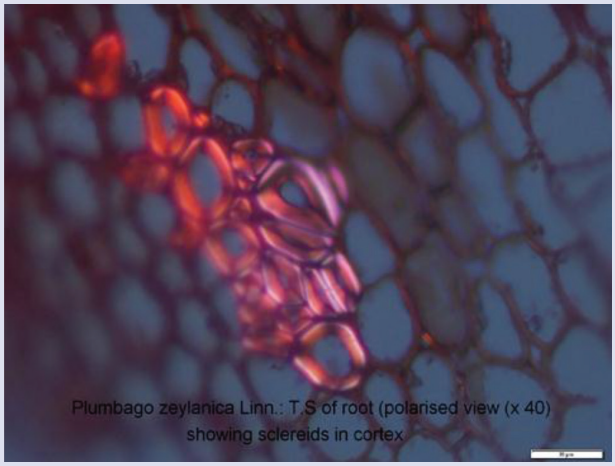

Figure 4f: *P. zeylanica* Linn. T.S of root: showing sclereids in cortex (polarized view x 40)

Figure 4i: *P. zeylanica* Linn. T.S of root: central portion enlarged (x 10)

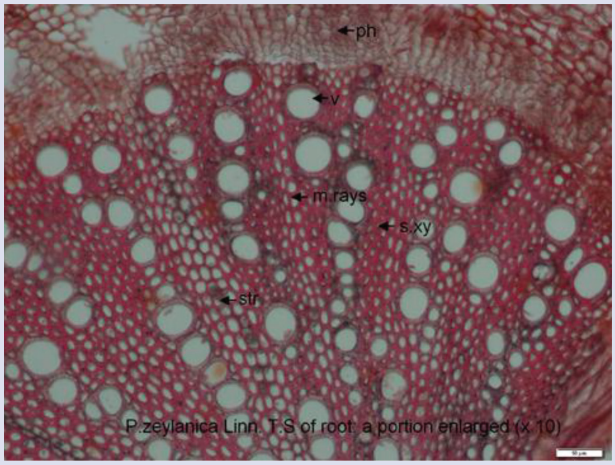

Figure 4g: *P. zeylanica* Linn. T.S of root: a portion enlarged (x 10)

Figure 4j: *P. zeylanica* Linn. T.S of root: secondary xylem (polarized view x 40)

Figure 4h: *P.zeylanica* Linn. T.S of root: central portion enlarged (x 10)

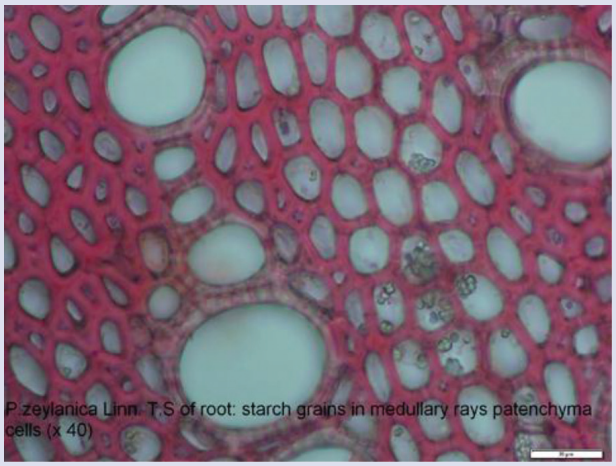

Figure 4k: *P. zeylanica* Linn. T.S of root: starch grains in medullary ray cells (x 40)

20µm to 34µm in length and 10 to 13µm in diameter. The cortical cells also contained abundant deposition of starch grains. Starch grains were of both simple and compound types; however, the latter became the predominant type and seemed to be arranged in the form of bunches of grapes (Figure 4d). Starch grain was oval to elliptical in shape, and individual grain ranged in size from 4.5 µm to 8.1µm in diameter. The cut surface of the root specimens, when exposed to air and viewed under the microscope plumbagin pigment was found to be oozed out from the cortical tissues, which could be very discernible in microphotograph as yellowish tints (Figure 5). Solitary sclereid or three to four rows of sclereids (Figure 4f & 4g) were found aligned as distinct band or patch at the pericyclic region of the phloem. Several such small patches of sclereids were found distributed throughout, so as to form a more or less continuous ring across the inner cortical tissues of root, leaving a broad area of parenchyma between the bands of sclerids. The secondary walls of sclereids were heavily lignified with wide lumen. The lignified sclereids of the cortex could be distinctively discernible (Figure 4e & 4f) under polarized light and appeared birefringent (Figure 4g), because their wall constituents of microfibrils contain crystalline cellulose, which could appear as bright in microphotographs (Figure 4g). Wood occupied the major portion of the root; growth ring boundaries were distinct on the secondary xylem. The bulk of the wood was constituted by libriform fibers (Figure 4d) and vessels distributed in radial rows (Figure 4h & 4j). The secondary wall of libriform fibers appeared thin to thick walled leaving prominent lumens in the center, which were conspicuously wide opened (Figure 4j). Wood was formed from non-storied cambium (Figure 5a & 5b), lacking the longitudinal series of cell arrangements of rays, axial parenchyma or libriform fibres on tangential surface (Figure 5b). Distribution of axial parenchyma in wood appeared to be of paratracheal (Figure 4j). Medullary rays were very conspicuous and the abundance of ray parenchyma cells between xylem strands was discernible in cross section. Medullary rays were of uniseriate and biseriate (Figure 5b) types and rays lacking vessel contacts. Medullary rays were homogeneous, consisted of mostly upright cells (Figure 5b). At places the accumulation of starch grains was found in medullary ray parenchyma cells (Figure 4k). Vessels were mostly occurred in radial chains, vessels grouping of radial multiple of three to six (Figure 4k) were common. Solitary vessel was found to be ovoid or polygonal in shape (Figure 4i & 4j). The diameter of the vessels was found to be small, which ranged between 24.4 µm to 50.6 µm with a mean diameter of about 34 µm. The length of the xylem elements was ranged between 127.4 µm to 132.6 µm with a mean length of about 130µm. When the Xylem was scanned for vessel density measurement, the mean vessel density was found to be 301 per mm^2 and mesomorphy ratio for *P.zeylanica* averaged was found to be 9.8. Mesomorphy ratio values obtained for *P.zeylanica* was notably low compared to woods growing in most moist regions.[37] However, the value obtained in the present study is found to be in agreement with mesomorphy ratio values for Plumbaginaceae family in general[37] with the concept that Plumbaginaceae plants characteristically grow in dry and or saline habitat. At the center portion of secondary xylem showed somewhat a crowding of vessels and most vessels at the center of the wood seemed to be plugged with tylosis. The presence of tylosis in the vessels (Figure 4i & 4j) and the latter contained the deposition of secretary substances were common features of the wood of *P. zeylanica*. Radial longitudinal section (RLS) of the root had shown that vessels having bordered pits with prominent margo and outer part of the secondary wall shaved by sectioning had shown the grooves interconnecting pit apertures (Figure 5c). Intervessel pitting or vessel to vessel pitting was found to be multiseriate and in alternate (Figure 5c) position. Pits were moderately sparse and the diameter of the pit aperture was found to be about 6.3µm (Figure 5c).

Figure 5: *P. zeylanica* Linn. TLS of root: deposition of plumbagin (yellow color) in cortex (x 10)

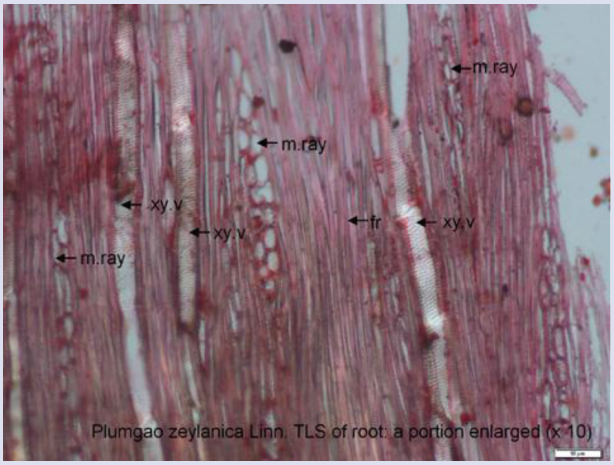

Figure 5a: *P. zeylanica* Linn. TLS of root: a portion enlarged (x 10)

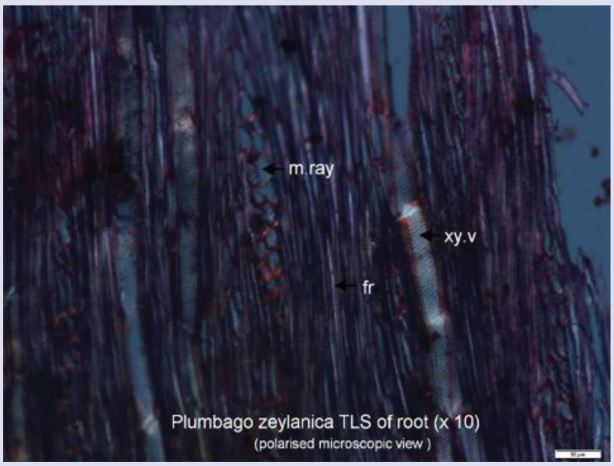

Figure 5b: *P. zeylanica* Linn. TLS of root: polarized view (x 10)

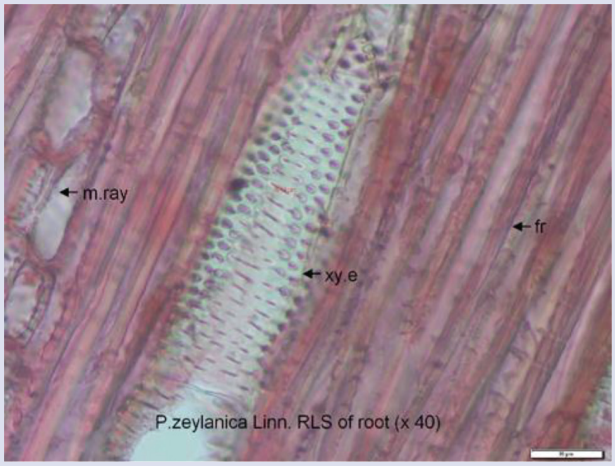

Figure 5c: *P. zeylanica* Linn. TLS of root(x 40)

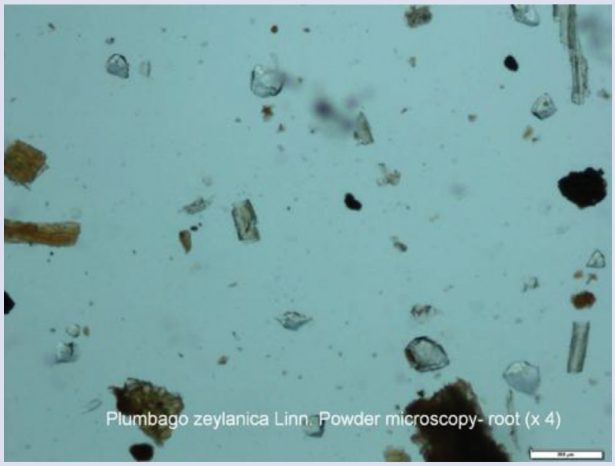

Figure 6b: *P. zeylanica* Linn. Powder microscopy of root- prismatic crystals (x 4)

Figure 6: *P. zeylanica* Linn. Powder microscopy of root- libriform fibers (x 10)

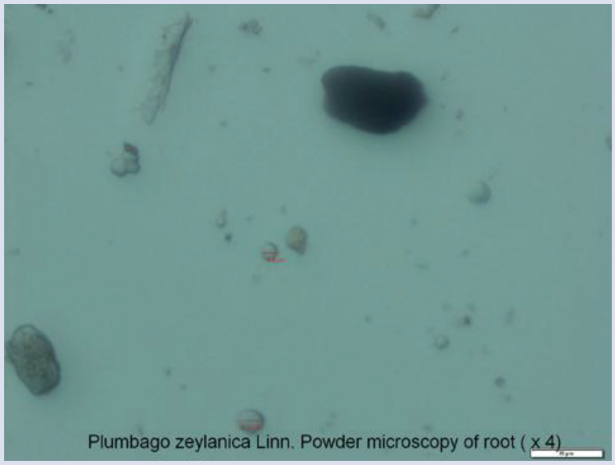

Figure 6c: *P. zeylanica* Linn. Powder microscopy of root- starch grains (x 4)

Figure 6a: *P. zeylanica* Linn. Powder microscopy of root- xylem elements (x 10)

Figure 6d: *P. zeylanica* Linn. Powder microscopy of root- parenchyma (x 10)

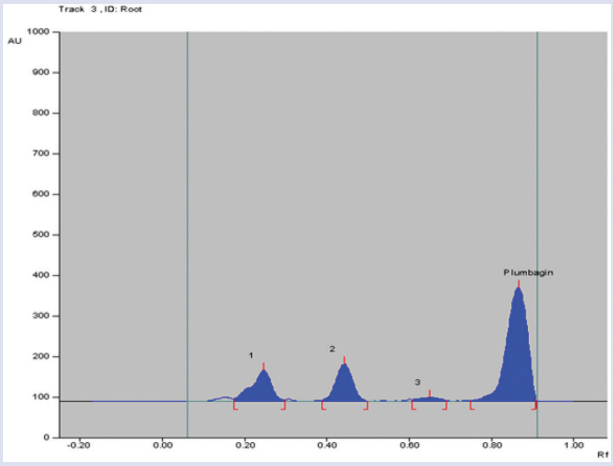

Figure 7: HPTLC chromatogram of methanol extract of *P. zeylanica* root

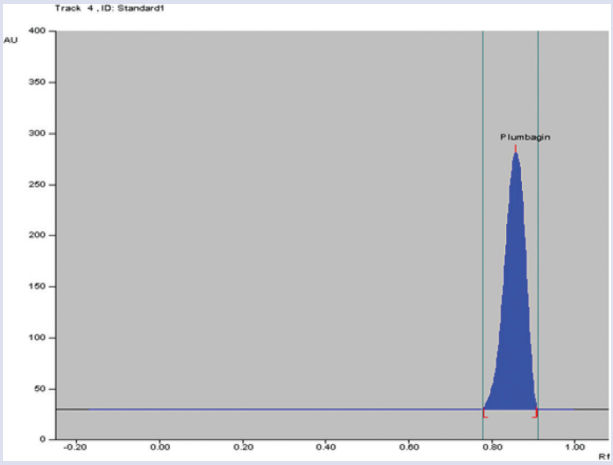

Figure 8: HPTLC chromatogram of plumbagin (1.05 µl/spot) with R_f=0.86

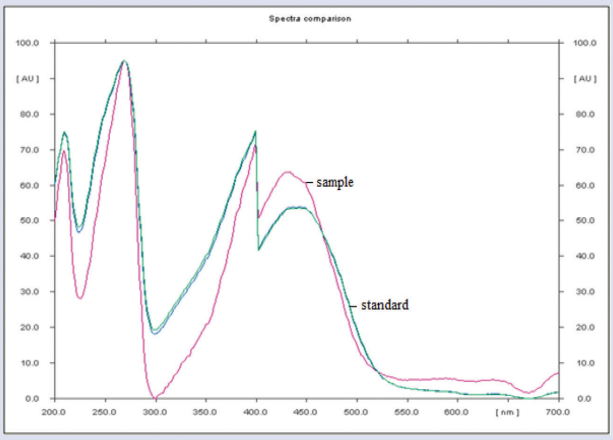

Figure 9: Overlay spectra (λ_{max} 270 nm) standard plumbagin (green colors) with methanol extract of root (pink color) of P.zeylanica Linn.

Powder microscopy

The dried roots of *P. zeylanica* were analyzed for powder characteristics. Root powder was yellowish brown in color and has no characteristic taste and odor. Microscopic examination of the root powder showed presence of pyramidal calcium oxalate crystals (Figure 6b); lignified libriform fibers with pointed ends (Figure 6a) simple and compound starch grains (Figure 6c). Fragments of parenchymatous tissues of the cortex (Figure 6d) and xylem elements (Figure 6a) were also observed.

HPTLC Finger print

The spotted samples of methanol extract of root of *P. zeylanica* and standard marker compound (plumbagin) containing on the silica gel plates were developed in the Twin trough chamber with the solvent system n-hexane:ethyl acetate 8:2 (v/v) to a distance of 8 cm. All tracks in the plate were scanned at 254 nm and individual R_f values and the resolved bands were recorded. The Chromatogram of the methanol extract of root had revealed four phyto-constituents (Figure 7). Data of peak area, peak height, R_f value of each resolved band were recorded in track no.3 and the expressed pattern of chemical ingredient's distribution were also depicted in Figure 7. Out of these bands, one band (R_f= 0.87) generated had matched with the R_f value (0.86) of the marker compound (Figure 8) and the densitometric scanning had obtained the same λ_{max} (270 nm) as that shown by standard marker, Plumbagin.

Quantitative Estimation

Quantitative investigation of the marker compound (plumbagin) was carried out by densitometric reflection/absorption mode at 270 nm (Figure 8). Spectral matching by overlaying the absorption spectra of standard marker compound (9.00-11.00 µg/band) with the absorption spectrum of marker present in the extract of root sample (ie., plumbagin in the root) was confirmed the specificity of λ_{max} at 270 nm (Figure 9).

Calibration curve

When the concentrations of plumbagin ($C_{11}H_8O_3$) and their respective peak areas were subjected to regression analysis by polynomial method, calibration curve was found to be linear in the concentration range of 2.00 to 10.00 µg/band with the calibration equation Y=178.8+91.61*X + -4.825*X2 and regression coefficient, r=0.99735 and sdv=3.02%. Calibration graph showing the acceptable correlation (r=0.997) between the y and x values had revealed a good linearity response for the method developed. The calibration data depicted in Table 1 had revealed that 5 µL of methanol extract of leaves contained 1.326 µg of plumbagin and the content of marker constituent (plumbagin) present in dried root sample of *P.zeylanica* Linn (a Kerala habitant) was estimated as 0.1768%. The finding of the present study in consonant with the other reported estimates[38-41] for the plumbign from the root samples of *P.zeylanica* Linn.

Sensitivity of the HPTLC method

The sensitivity of an instrument in an analytical procedure is a measure of its ability to discriminate between small differences in analyte concentration. According to Srivastava[42] a signal-to-noise ratio (S/N) of 3.3 and 10 were generally be considered as limits of detection (LOD) and limits of quantitation (LOQ) respectively for HPTLC. The LOD and LOQ values of plumbagin calculated from the calibration regression equation were found to be 98.89 ng and 329.66 ng respectively. This suggests that the developed HPTLC method exhibits a good sensitivity for the quantitation of plumbagin. The developed active constituent based on HPTLC method has the advantage of simple, specific and easy identification of plumbagin in the root samples and could be applied for the regulatory perspective as routine quality analysis for the Plumbago species in the polyherbal formulation/finished products.

Table 1: Calibration data of plumbagin at 270nm by HPTLC

Track	Vial	R_f	Amount Fraction	Height	(X calc)	Area	(X calc)	Remark
4	4	0.86	1.050 µg	263.04		9741.38		Std
5	4	0.86	3.150 µg	435.87		17259.88		Std
6	4	0.86	5.250 µg	517.31		21626.18		Std
7	4	0.86	7.300 µg	587.69		25662.02		Std
8	4	0.85	9.450 µg	617.11		27783.92		Std
3	3	0.87		291.81	1.326 µg	10514.46	1.213 µg	Sample root

CONCLUSION

Men in health profession and consumers alike are justifiably apprehensive about the quality of herbal medicine, batch to batch inconsistency and inefficacious marked products. Though the herb contains many phyto-constituents, standardization protocols for herbal medicine reliably lays in the development of finger print profiles. Delineation of anatomical markers and chemical profile of therapeutically important marker (s) with quantification of principle active component would appear to serve the only sensible ways towards establishing quality control parameters, which could reliably deliver appreciable health benefits. The results of the present study suggest that, the documented morphological descriptors, delineated anatomical markers and developed active constituent based on HPTLC finger print profile of the root of *P.zeylanica* Linn may supplement in the regulatory perspective of the routine quality control analysis of the crude drug and also for the Plumbagin in the formulation/ finished products.

CONSENT

Not applicable.

CONFLICT OF INTEREST

Author has declared that no conflict of interest exists

ABBREVAITIONS USED

CK:Cork; CO:Cortex; CRL, CRY: Crystal; FR: Fiber; PH: Phloem; M.RAY: Medullary ray; SS: Secretary substance; SCL:Sclereids; STR:Starch grain; S.XY:Secondary xylem; XY:Xylem

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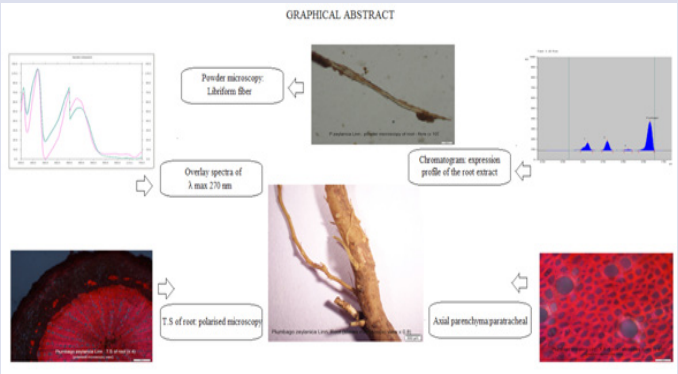
GRAPHICAL ABSTRACT



SUMMARY

- Plumbagin is the major active principle of Plumbago species. It is a powerful irritant and redox cycling compound. Plumbagozeylanica Linn.is espoused as source plant for the Ayurveda drug, Chitraka. It is used for the treatment in rheumatic pain, diarrhea and skin diseases and as an appetite stimulant,vesicantand contraceptive agent.
- Distinct band or patch of sclreids at the pericyclic region of the phloem of the root, wood with non-storied cambium, paratracheal axial parenchyma, chains of vessels in radial multiplies of three to six, wood plugged with tylosis and low mesomorphy ratio values for wood were the anatomical features characteristics of the taxon.
- Densitometric scanning of plambaginhad shown the characteristic λ_{max} at 270 nm by HPTLC method.The calibration graph plotted was found to be linear with the polynomial calibration equation Y=178.8+91.61*X+-4.825*X2 and the percentage of plumbagin present in the shade-dried root of P. zeylanica(a Kerala habitant)was estimated to be 0.179 w/w.
- The anatomical markers, quantitative microscopic characteristics of the root,and developed HPTLC method could be effectively used for the regulatory perspectives of the quality assessment.

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