Fingerprinting and quality control of *Plumbago zeylanica* Linn. (Chitraka) Root

[1]A.V. Dusane, [2]S.K. Patil, [3]P.R. Borkar, [4]U.R. Manerikar [5]S.A. Dusane
[1]Associtate Professor
[1]Sir Parashurambhau College, Pune, India

Abstract : Plumbago zeylanica L., (family: Plumbaginaceae) is a rambling perennial under shrub. This plant is commonly known as 'Chitraka (Sanskrit)' and 'Ceylon Leadwort (English)'. *P. zeylanica* is the rich source of various phytochemicals viz. alkaloids, steroids, flavones, saponins and tannins. Chitraka is an important indigenous root drug that is frequently used on the treatment of dyspepsia, piles, diarrhoea, skin diseases, leprosy and rheumatism. Roots of Chitraka are reported to possess antibacterial, antifungal and abortifacient properties. Chitrak is used in several herbal formulations including Chitraka swarasam, Chitraka churnam, Chitrakadi Vati, Chitraka ghritam and Chitraka Phantam. In the present investigation exomorphic, anatomical, pharmacognostic, physicochemical and chromatographic (fingerprinting) standards had been evolved for *P. zeylanica*. The root of this plant has distinct anatomical structure, circular in outline, 4-9 layered cork cells, vessels in a group of 2-8 and distinct single to multilayered medullary rays. The quantitative microscopic studies have shown that vessels and phloem fibres length were 198 µ and 560 µ respectively. The physico-chemical parameters viz. total ash, water soluble ash, acid insoluble and sulphated ash values are recorded as 15.68, 12.14, 3.40 and 6.60 % w/w respectively. The extractive values of root powder with ethanol, chloroform, acetone and water were 9.20, 4.0, 9.10 and 9.20% w/w respectively. The fluorescence pattern serves as an easy and non-destructive parameter in fingerprinting of Chitraka powder. TLC pattern developed using Benzene: Ethyl acetate (4:1) and Chloroform: Methanol (93:7) solvent systems serve as quality control for this valuable drug. The present study is important and lays down parameters for standardization and authentication of medicinal plants. The evaluation of all pharmacognostic parameters such as organoleptic characters, macroscopic study, microscopic study, powder study, physico chemical analysis (moisture content, loss on drying, ash values, extractive values), phytochemical analysis, fluorescence analysis and TLC studies helps in to prevent adulteration and substitution.

IndexTerms: Ash values, Chitraka, finger printing, quality standards, *Plumbago zeylanica*, pharmacognostic standards, physicochemical studies and Thin Layer Chromatography.

1. INTRODUCTION

Plumbago zeylanica L. (Plumbaginaceae) is a rambling perennial under shrub grown in most parts of India [1] *P. zeylanica* L. is commonly distributed in forest and cultivated in the gardens throughout India. This plant is commonly known as 'Ceylon Leadwort' in English and 'Chitraka' in Sanskrit. It is used in many Ayurvedic formulations such as Chitraka swarasam, Chitraka churnam, Chitrakadi vati, Chitraka ghritam and Chitraka Phantam [2]. The root of this plant is used as laxative, expectorant, astringent, abortifacient, diuretic and abortifacient. This plant is a good remedy for piles, cough, worms, enlarged abdomen, anaemia, diabetes, leprosy, diarrhea, dyspepsia and elephantiasis [3] [4]. The phytochemical studies of revealed that it contains a variety of chemicals viz. plumbagin, 3-chloroplumbagin, 2, 3-biplumbagin, 6, 6-biplumbagin, zeylinone, isozeylinone, chitranone, droserone, plumbagic acid, plumbazeylanone, glucose, fructose, enzymes as protease and invertase [5]. Plumbagin is the bioactive compound mainly present in root however very little amount is present in stem [6]. World Health Organization emphasized certain quality standards and has proposed certain guidelines for the assessment and development of standard herbal products. The main aim of the present work is to study evolve the macroscopic, microscopic, pharmacognostic, physicochemical and chromatographic standards for *P. zeylanica* L. roots as the guidelines of the World Health Organization (WHO).

2.MATERIALS AND METHODS

2.1. Plant material: The plant specimens were collected from Western Ghats (Tamhini, Pune). The healthy and fully matured roots were taken for the investigation. The roots were cut suitably and thoroughly washed with water to remove the adherent impurities. The roots were dried in semi-shade condition for the further use.

2.2 Microscopic studies: Roots were cut into a desirable size and kept in FAA. Free hand sections with thickness of 10 and 12 µm were stained with phloroglucinol -hydrochloric acid (1:1) and mounted in glycerin. The Camera lucida drawings of T.S. of root were made. The permanent slides were made as per

the standard procedure [7]. The starch grains were stained with Iodine solution. Root powder was prepared using sieve mesh 60 (Sixty) for the observation of microscopical characteristics. The powdered drug was separately treated with phloroglucinol-hydrochloric acid (1:1) solution and iodine solution.

2.3 Fluorescence studies: The fluorescence analysis carried out as per the standard method [8]. The mountant medium viz. distilled water, 1 N NaOH, 1 N HCl, 1 N H_2SO_4 and 1 N HNO_3 were used, and the fluorescence at ordinary light and at under 254nm and 366nm UV light was recorded. The fluorescence of solvent extracts viz. Water, alcohol, acetone and chloroform examined under UV light and the normal light. The colour for fluorescence analysis was confirmed from 'A Mycological Colour Chart' of Rayner [9]. The details of fluorescence analysis have been tabulated in table no.6 and table no.7.

2.4 Physico-chemical analysis: The physico- chemical studies were carried out as per the WHO guidelines [10]. The parameters viz. total ash, water-soluble ash, acid-insoluble ash and sulphated values were determined. Alcohol, water, acetone and chloroform-soluble extractive values were determined. The root extractives of *P. zeylanica* L. were used for chemical analysis. The different phytochemicals tests were performed to record the phytochemicals present in the root [11].

Thin Layer Chromatography (TLC): It has been carried out as per the methods described standard methods of Stahl [12].

Solvent system (s): Benzene: Ethyl acetate (4:1) solvent was used for chloroform extract of the root. For ethanol extract Chloroform: Methanol (93:7) solvent system were used. The TLC was carried out on precoated E. Merk silica gel plates of 0.30 mm thickness. After air-drying the plate was visualized in UV 254 nm, 366 nm and iodine vapour. The Rf values recorded. To detect the alkaloids, the plates were sprayed with Dragendorff's reagent. For the final outputs of TLC plates, the standardized conditions were maintained.

2.5 Photomicrographs: Microscopic descriptions of selected tissues were supplemented with micrographs. Photographs of different magnifications were taken with Canon Microscopic unit.

3.RESULTS AND DISCUSSION

3.1Macroscopical characteristics: This plant is an undershrub with white coloured flowers (Fig.1a). The roots are 30 cm or more in length, 7 mm or more in diameter

Figure 1a Plumbago zeylanica plant 1b Root

Table no.1 Characteristics features *P. zeylanica* roots

Characteristics	Observations
Root type	Large Tap root, tapering at one end and fusiform.
Root dimensions (cm)	11.0-19.39-320 X 0.5-0.7-1.1 cm
Nature of roots	Roots are straight, thick, smooth texture, unbranched or slightly branched with or without secondary roots.
Colour of root	Light yellow when fresh and reddish-brown when dry.
Odour	Characteristic
Taste	Bitter
Texture	Smooth and uniform

Microscopic study of root: T.S. of root was circular in outline and showed 4-9 layered rows of cork cells that are light brown and cubical to rectangular. The cortex cells are with abundant round shaped with simple starch grains. The anatomical peculiarities are tabulated in Table no.2


Fig 2. T.S. *P. zeylanica* root (X100)

Table no.2. Anatomical peculiarities of *P. zeylanica* root.

Anatomical parameters	**Important peculiarities seen in *P. zeylanica L.***
Outline	Circular
Cork	4-9 layered with cubical rectangular brown coloured cells.
Cortex	It is many layered with polygonal cells with abundant starch grains.
Phloem	It is well developed with phloem fibres. It is outside xylem.
Cambium	Single layered and tangentially elongated.
Xylem	Vessels are in a group of 2-8. The vessels are polygonal to oblong in shape
Medullary rays	It is very distinct and single to multilayered containing starch grains
Pith	Less abundant.
Cell contents	Starch grains are simple and round. The prismatic crystals of calcium oxalate are present.

In view of the importance of quantitative microscopy, the details are tabulated in Table no. 3.

Table no.3. Quantitative microscopic studies of root *P. zeylanica*

Cells and cell contents	Dimensions
Vessels (with simple pits)	198.0µ (length)
Vessels (with bordered pits)	124.0µ (length)
Stone cells	30.5µ diameter
Phloem fibres	560µ (length)
Calcium crystals	Prizmatic (11.0-40.0 X 8.0-35.0 µ
Starch grains (simple)	10-35µ

Transverse section of root shows simple, round or elliptical shaped starch grains with 10-35µ in diameter. The prismatic shaped crystals have been observed. Vessels with simple pits up to 198µ in length, vessels without bordered pits , 124 µ, stone cells with 30.5 µ, sclereids with lignified 180µ in length, prismatic crystals of calcium oxalate, outer most tissue of cork consisting of 5-7 row, parenchyma containing starch grains and some cells with yellow contents, fibres scattered singly or in groups of 2-7. The phloem fibres are usually seen in a group of 2-5. The phloem fibres were pointed end up to 560µ length. Abundant simple starch grains, xylem vessels with helical to spiral thickenings, elongated phloem fibers have been recorded as diagnostic features for the root of this plant.

Powder microscopy: It showed the presence of fragments of thin walled parenchyma cells with simple starch grains. The spiral to helical elongated xylem vessels, sclerenchymatous interfasicular tissues, parenchymatous tissues had been recorded (Fig.3).

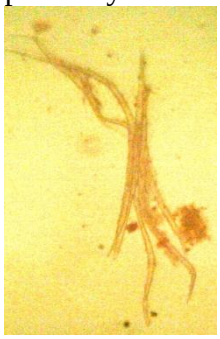
Fig. 3. Microscopic characteristics of root powder (X100).

Physico-chemicals characteristics of root: The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in

the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and silica. Total ash value of plant material indicated the amount of minerals and earthy materials attached to the plant material. The results of ash values are tabulated in Table no. 5.

Table 5. Physico-chemical parameters of *P. zeylanica* L. root.

Srno	Parameters	Results (with range)
1	Foreign matter	< 3.0 %
2	Total ash	15.68% w/w (14.40-16.30)
3	Water soluble ash	12.14% w/w (10.2-14.3)
3	Acid-insoluble ash	3.40 w/w (2.92-3.67)
5	Sulphated ash	6.6 % w/w (5.74-7.23)
6	Ethanol extractives	9.20 w/v (9.0-9.5)
7	Chloroform extractives	4.0% w/v (3.10-4.80)
8	Acetone extractives	9.10 w/v (8.80-10.5)
9	Water extractives	9.20 w/v (8.60-9.87)

Total ash was approximately, four times more than acid insoluble ash value. The earlier work showed that total ash 2.49 % w/w, acid insoluble ash 0.76 % w/w, and water-soluble ash 1.73% w/w. The present investigation shows higher ash values. The earlier workers have recorded extractive values for water 13.25 % w/w and 14.60 for ethanol. However, the extractive values recorded for water and ethanol are quite low in the present investigation. The water extract was brownish and semisolid, yellowish coloured and sticky; Chloroform shows brown sticky while ethyl acetate shown reddish brown with gummy consistency.

The fluorescence analysis is easy and effective identification tool for the detection of adulterants and to check the purity of powders.

Table no.6 Fluorescence characteristics of root powder of *P. zeylanica*

Sr no	Mountant medium	254nm	366nm	Natural day light
1	Dry powder	Vinaceous buff	Vinaceous buff	Pale vinaceous
2	P + D.W.	Dark brick	Sepia	Dark vinaceous
3	P+1N HCl	Rust	Umber	Bay
4	P+1N HNO_3	Livid vinaceous	Brown vinaceous	Fawn

Table no.7. Fluorescence characteristics of *P. zeylanica* root extracts

Sr no	**Extracts**	**256nm**	**366nm**	**Natural day light**
1	Water	Blood colour	Umber	Chestnut
2	Chloroform	Greenish black	Yellowish green	Smoke grey
3	Acetone	Greenish grey	Olivaceous black	Herbage green
4	Alcohol	Sepia	Dark brick	Dark vinaceous

Preliminary phytochemical study: Phytochemical test revealed the presence or absence of alkaloid, glycoside, saponins, flavonoids, polysaccharides, steroid and tannin. The results are given in Table no. 8.

Table no. 8. Preliminary phytochemical tests *P. zeylanica* L.

Sr no	Chemicals	Test Performed	W	C	A	EA
1	Alkaloids	Dragendorff's test	-	-	-	+
2	Flavone	Shinoda test	+	+		-
3	Steroid	Liebermann-Burchard reagent	+	-	+	-
4	Tannins	Neutral $FeCl_3$	+	-		-
5	Sugar	Molisch's test	+	-		-
6	Terpenes	Noller's test	+	+		-
7	Glycosides	Berlin-blue reaction	-	+	+	-

W= water, C= chloroform, A= acetone, EA= ethyl alcohol.

'+' sign indicates the presence of a particular phytochemical while
'-' indicates that the particular phytochemical is not detected.

Phytochemically root exhibited alkaloids, flavone, steroids, tannins, sugars, terpenes and glycosides.

In the root the alkaloids, flavone, steroids, tannins, sugar, terpenes and glycosides are present.

TLC analysis: This technique has applications in standardisation, determination of the ingredients of formulations and detection of adulterants or substitutes. TLC is also practised as a qualitative tool for the study of admixtures. In the present investigation, the chloroform extract was a workout by using Benzene: Ethyl acetate (4:1) and in ethanol extract Chloroform: Methanol (93:7) solvent system.

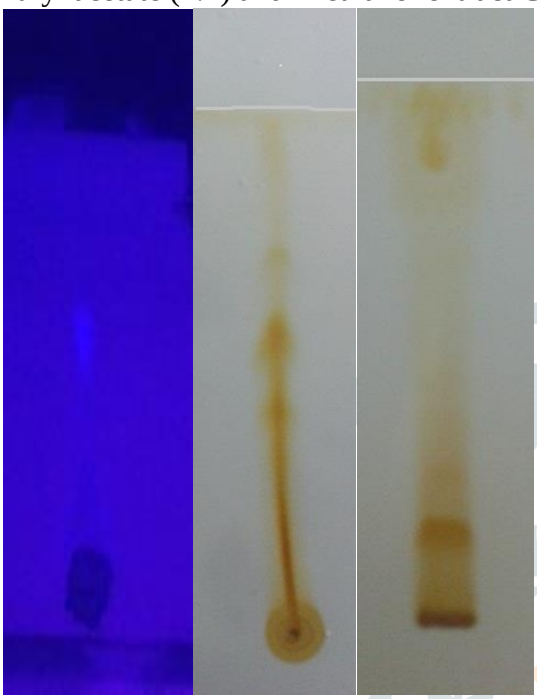

Figure 4 1. Water extract 2. Chloroform extract 3. Ethanol extract

P.zeylanica Root TLC pattern (chloroform extract)

Table no. 9. TLC pattern of various extracts of *P.zeylanica* L. root.

Sl.	Extractives	Adsorbent	Solvent system	Viewing	Rf Values (Retention factor)
1	Chloroform	Precoated silicagel	Benzene: Ethyl acetate (4:1)	Iodine vapor	0.41,0.55,0.7,0.9
2	Distilled water	Precoated silicagel	Chloroform: Methanol (93:7)	Iodine vapor	0.31,0.51
3	Ethanol	Precoated silicagel	Chloroform: Methanol (93:7)	Iodine vapour	0.17, 0.25, 0.86

Table no. 10. TLC Finger print of *P. zeylanica* L. root ethanol extract (Solvent system- Chloroform: methanol (93:7).

No. of spots	Rf value	254nm	366 nm	Iodine developer
1	0.17	Faint blue	Intense blue	Yellow
2	0.25	Faint blue	Faint blue	Yellow
3	0.86	Intense blue	Intense blue	Yellow

Table 11. TLC Finger print of *P. zeylanica* L. root ethanol extract (Solvent system- Benzene:ethyl acetate (4:1).

No. of spots	Rf value	254nm	366 nm	Iodine developer
1	0.41	Faint blue	Intense blue	Yellow

TLC studies with Toluene: Ethyl acetate: Formic acid (5:1.5: 0.5 v/v) solvent system has been worked out [13]. While in the present study chloroform:methanol (93:7) and solvent system- Benzene:ethyl acetate (4:1) the chromatography pattern for chloroform, distilled water and ethanol extract was studied so as to emerge the unique finger printing pattern of *P.zeylanica* .

CONCLUSION

Exomorphic, microscopic, quantitative microscopy, pharmacognostic, preliminary phyto-chemical and TLC studies in authentication, detection of adulteration and for quality control of *P. zeylanica*. The plant of *P. zeylanica* L. exhibited a set of diagnostic characteristics which would be helping to identify the drug in dried condition. The present study would be useful for standardization of this drug.

ACKNOWLEDGEMENT

The authors are very grateful to Principal, S.P.College Pune for providing necessary facilities for carrying out present research work.

REFERENCES

[1] Kirtikar KR, Basu BD 1993. Indian Medicinal Plants. Shiva publishers: Dehradun, India, 2, 1466-1468.

[2] Atal CK, Kapur BM 1989. Cultivation and Utilization of Medicinal Plants. Regional Research Laboratory, Council of scientific and Industrial Research: Jammu-Tawi, India.

[3] Choudhary AK, Sushanta KC, Azadkhan AK 1982. Antifertility activity of *Plumbago zeylanica* Linn. root. *Ind J Med Res;* 76: 99-101.

[4] Chetty KM, Sivaji K, Sudarsanam G, Sekar PH 2006. Pharmaceutical Studies and Therapeutic Uses of *Plumbago Zeylanica L.* Root. Ethnobotanical Leaflets; 10: 294-304.

[5] Lin LC, Yang LL, Chou CJ 2003. Cytotoxic naphthoquinones and plumbagic acid glucosides from *Plumbago zeylanica*. *Phytochemistry* 62:619-22.

[6] Israni SA, Kapadia NS, Lahiri SK, Yadav GK, Shah MB 2010. An UV-visible spectrophotometric method for the estimation of plumbagin. *Int J ChemTech Res*;2:856-9.

[7] Johanson DA 1940. Plant Microtechniques. New York: Mc Graw Hill, 1940. pp. 182-203.

[8]Chase, C. R. and Pratt, R. 1949. Fluorescence of Powdered Vegetable Drugs with Particular Reference to Development of a System of Identification. *J. Amer. Pharm. Assoc. (Sci. ed.) 38***:** 324-331.

[9] Rayner, R.W. 1970, A Mycological Colour Chart. Commonwealth Mycological Institute, Kew Survey.

[10] Anonymous 1998. Quality Control Methods for Medicinal Plant Materials, World Health Organization, Geneva, pp 25-28.

[11] Harborne JB 1973. Phytochemical Methods. Jackman H. (Ed.), London, pp. 70.

[12] Stahl, E. 1969. Thin Layer Chromatography. Springer-Verlag, Berlin.

[13] Meenal A.K., Singh Bijendra, Kaur Ramanjeet,, Sachan Ayushy, Pal Bhavana Niranjan Uttam, Yadav A. K and Nagariya A.K. 2010. Pharmacognostic and Physicochemical Studies on *Plumbago zeylanica Linn*. Root. Drug Invention Today, Vol. 2, 217-219.