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Pharmacognostic and Phytochemical Investigations of *Plumbago zeylanica* Linn. root

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ABSTRACT

Objective: To study detailed Pharmacognosy of the root of *Plumbago zeylanica* Linn (Plumbaginaceae), well known Ayurvedic drug (Chitrakah).

Method: the macroscopy, microscopy, physicochemical analysis, preliminary phytochemical testing of the root and other WHO recommended methods for the standardization was done.

Results: T.S. of the young and thick root confirmed the presence of periderm with tanniferous rectangular cells. Cortex wider comprises large, angular, thin walled compact parenchymatous cells and most of the cells have tannin. The vascular cylinder is circular, solid and dense with prominent two or three growth rings. Secondary phloem consists of small groups of sieve elements and parenchymatous cell. The secondary xylem elements include vessels and xylem fibers. Xylem vessels circular thick walled. The xylem fibers are heavily thick walled and lignified.

Keywords: *Plumbago zeylanica* L, Plumbaginaceae, Purgative.

1. Introduction

Plumbago zeylanica Linn. (Plumbaginaceae) is one of the well-known Ayurvedic drug. It is commonly known as chitramula and chitrack. This is perennial herb grow in shady places in the garden and found in Sri Lanka (Ceylon) and parts India, which include Bengal, Uttar Pradesh, and Southern India. Stems are 0.6 to 1.5 m long, somewhat woody, spreading, terete, striate and glabrous. Leaves are thin 3.8 – 7.5 by 2.2 – 3.8 cm. ovate, subacute, entire, glabrous, somewhat glaucous beneath, reticulately veined, shortly and abruptly attenuated into a short petiole. Petiole is narrow, amplexicaul at the base and they're often dilated into stipule like auricles. Flowers are elongate spikes, rhachis glandular, striate; bracteoles ovate acuminate, shorter than the calyx. Calyx is 1 – 1.3 cm long, narrowly tubular, persistent, densely covered with stalked glands; teeth small with membranous margins. Corolla white slender tube 2 – 2.5 cm long, lobe 8 mm long, obovate-oblong, acute, apiculate. Filaments as long as the corolla-tube; anthers exerted just beyond the throat. Capsule oblong, pointed; pericarp thin below, thick & hardened above [1, 2]. Leaves shows anti-inflammatory, antinociceptive [3], larvicidal activity [4]. Ethanolic extracts of *Plumbago zeylanica* stem show inhibition of immediate allergic reactions [5]. Plumbagin, naphthoquinone obtained from *Plumbago zeylanica* root inhibits cell growth and potentiates in human gastric cancer cell [6], inhibits ultraviolet radiation induced development of squamous cell carcinomas [7] and genotoxic damage induced by potassium canrenoate in culture human peripheral blood lymphocytes [8]. Root also shows anti-fertility activity [9]. Neo and 1-epineo-isoshinanolones isolated from *Plumbago zeylanica* root show antimicrobial activity [10]. For the standardization and quality assurance purpose, the following three attributes must be verified: authenticity, purity and assay. Hence the objective of the present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical studies of *Plumbago zeylanica* root.

2. Materials and Methods

2.1. Plant material

The root of *Plumbago zeylanica* was Purchased from Shri Shail Medicinal Plants Farms (Supplier), Nagpur (2013) and authenticated by Dr. A.S. Upadhye, Agarkar Research Institute, Pune, where a sample specimen (voucher number: R-155) has been deposited.

2.2. Macroscopic and Organoleptic studies

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug. The fresh root was subjected to macroscopic studies which comprised of organoleptic characters viz., color, odour, appearance, taste, texture etc.

2.3. Microscopic studies

2.3.1. Root Microscopy

For qualitative microscopic evaluation, the collected root was fixed in an FAA solution (Formalin-Aceto-Alcohol: Formalin, Acetic acid each 5 ml, in 90ml of 70% ethanol) for 24 hrs then dehydrated with a graded series of tertiary-butyl alcohol and castled in paraffin blocks. Later, the paraffin embedded specimens were subjected for sectioning with the help of rotary microtome and de-waxed the sections. These sections were stained with safranin and observed under a compound microscope at projection 10X and 40X [11].

2.3.2. Powder Microscopy

To study the presence or absence of various types of tissues or structures, the dried root is powdered using electric grinder, passed through sieve No. 60 and then subjected for microscopic studies. The powder microscopy was performed according to the methods of Kokate [12] and Khandelwal [13].

2.4. Physicochemical parameters

Physicochemical values such as the percentage of ash value and extractive values were determined according to the official methods [14, 15] and as per WHO guidelines on quality control methods for medicinal plant materials [16, 17].

2.4.1. Determination of ash values

For determining ash content of drug, about 3 gm of powder was spread in a pre-ignited and weighed silica crucible. Then the crucible was incinerated gradually to make the crucible free from carbon. After cooling, the crucible was weighed to get the total ash content and then the ash was subjected for determining the acid insoluble and water soluble ash. The percentage of total ash was calculated by taking the air dried sample as standard.

2.4.2. Determination of extractive values

Considering the diversity and chemical nature of the drug, five different solvents viz. petroleum ether, chloroform, alcohol and water were used for determination of extractive values. About 5 gm of powdered material was subjected continuous Soxhlet extraction with 100 ml of petroleum ether, chloroform, alcohol as solvents while using maceration process water extraction done.

Determination of extractive values of a crude drug is beneficial in its evaluation process wherever evaluation of chemical components is applicable. After extraction, the extracts are concentrated in rota vaporizer and dried in vacuum desiccator. Then the extractive values are calculated as percentage w/w of solvent soluble

extractive with reference to the air dried drug.

2.4.3. Determination of moisture content

The moisture content was determined by loss of weight on drying (LOD) method. For this 5 gm of drug (powdered root material) was taken and kept in an oven at 105 °C till a constant weight was obtained. Amount of moisture present in the sample was calculated as a reference to the air dried material.

2.4.4. Fluorescence analysis

Crude drugs show their own characteristic fluorescence when exposed to ultraviolet radiation and is dependent on its chemical constituents. This analysis is useful to identify adulterants during crude drug evaluation. In the present study, one gram of crude drug was taken in a watch glass and subjected to fluorescent analysis as such and after treatment with different reagents.

2.5. Preliminary phytochemical screening

Plants are considered as bioreactors or biosynthetic laboratories as they synthesize a wide range of characteristic therapeutically important molecules in the form of secondary metabolites. Thus, a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. For preliminary phytochemical extracts were subjected for preliminary screening using the standard procedure for identifying various phytoconstituents [12].

3. Results

A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. This detailed and systematic pharmacognostic study will give valuable information for future research work.

3.1. Macroscopic and Organoleptic Studies

Root is dark brown in color. The root surface is rough and firm due to scaling off of longitudinal striation. Inner side of root is creamy white, soft and collapsed and non-collapsed phloem zone distinctly visible (Fig. 1).



Fig 1: Morphology of *Plumbago zeylanica* root.

3.2. Microscopic Studies

3.2.1. Root Microscopy

Both young and fairly old root are studied. The young root is 3 to 3.5 mm in diameter while thicker root exhibits more distinct growth is around 2.85 to 3.5mm in diameter. In young root periderm and cortex is around 1.2 to 1.4 mm wide and central xylem and phloem region is 2.2 to 2.4 mm in diameter. In thick root periderm and cortex is around 0.51mm to 1.0 mm wide and central xylem and phloem region is 2.7 to 2.9 mm in diameter. Each growth in thicker root is distinguished by thick walled fibers and semi-ring porous vessels (Fig. 2 a, b & c).

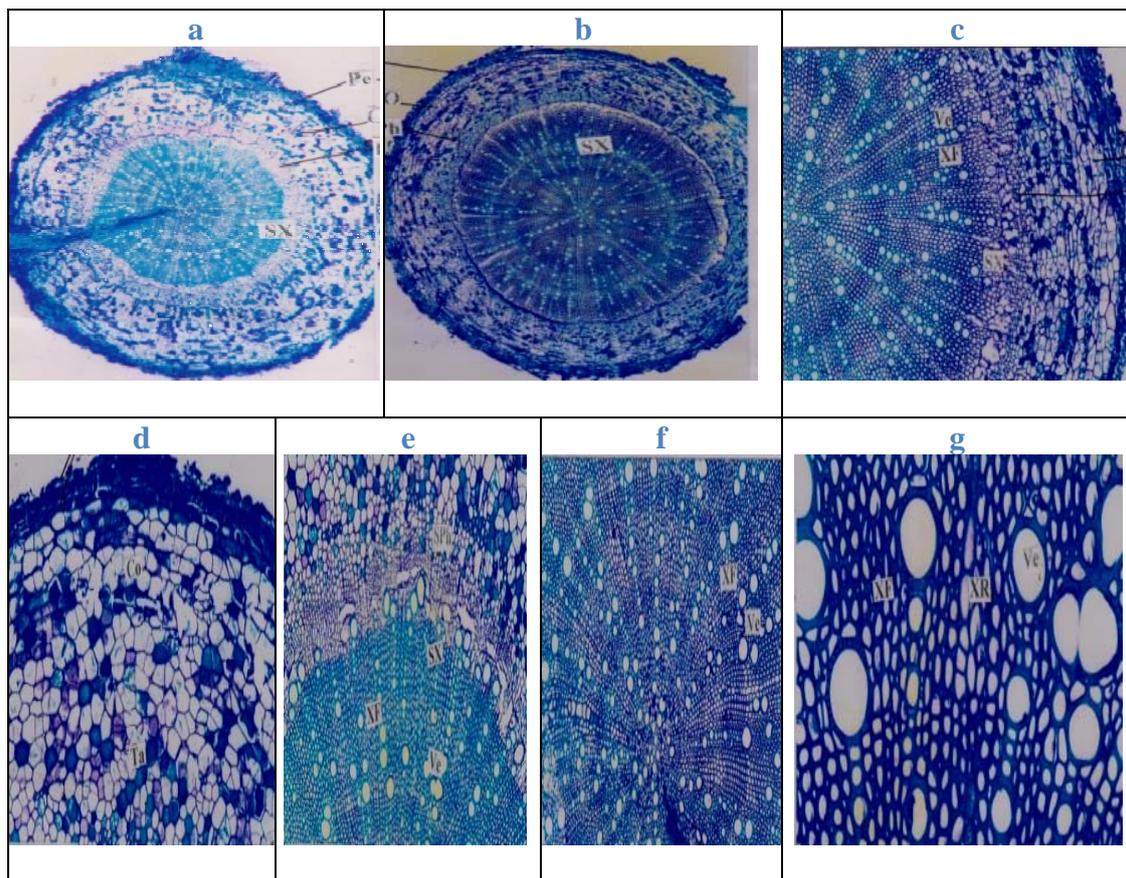


Fig 2: Microscopic study of *Plumbago zeylanica* root:

(a) T.S of thin root entire view, (b) T.S. of thick root entire view, (c) T.S of thick root (enlarged section view), (d) T.S of root showing periderm and cortex, (e) T.S. showing secondary phloem and secondary xylem, (f) T.S of the central portion of the root showing the distribution pattern of vessels, (g) T.S of root showing xylem vessel and xylem rays

Where, Co – cortex, Pe- periderm, Ph – phloem, SX – Secondary Xylem, SPh – Secondary Phloem, Ve – Vessels, XF – Xylem Fibers, XR – Xylem Ray, Ta – tannin.

a. Periderm

It consists of thin continuous periderm which includes four or five layers of aromatic content rich cells. The epidermis are broken and obliterated. While in thicker root periderm is slightly distinct with tanniferous cells (45 μm in diameter). The periderm in thick root is around 100 μm in wide (Fig. - 2 d).

b. Cortex

The cortex is wide and homogeneous. The cortical cells are tangentially oblong elliptical parenchyma cells. Some of the cortical cells have tannin content. While in thicker root the cortex wider comprises large, angular, thin walled compact parenchymatous cells. Most of the cells have tannin and mucilage (Fig. - 2 d).

c. Secondary Phloem and Xylem

The vascular cylinder is circular, solid and dense. It exhibits less prominent two or three growth rings. Secondary phloem is a thin continuous cylinder comprising small groups of sieve elements. Secondary xylem has several long uniseriate radial multiple vessels with wide gaps in between the vessel lines. Secondary phloem includes small radially arranged cells. Phloem consists of both

sieve elements and parenchyma cells. The sieve elements are in circular cluster. The secondary xylem elements include vessels and xylem fibers. Xylem parenchyma cells are not evident. The vessels are variable in diameter, all vessels are circular thick walled and are in radial multiples, radial chain or solitary. The xylem fibers are heavily thick walled and lignified and 4 μm to 9 μm . They have fairly wide lumen. The vessels are 19 μm – 42 μm in diameter. The ground tissue of the secondary xylem is thin walled narrow fibers with lignified parenchymatous cells (Fig. 2 e, f & g).

3.2.2. Powder Microscopy

Powder microscopy mainly helps in detection of adulterated substances and also in the confirmation of purity of crude drugs. Powder microscopy of *Plumbago zeylanica* root shows the presence of thick dark color periderm structure. Numerous tanniferous cell observed filled with bluish color with the periderm structure and the secondary xylem elements include vessels and xylem fibers. Xylem parenchyma cells are not evident. The xylem fibers are heavily thick walled and lignified (Fig. 3 a, b & c)

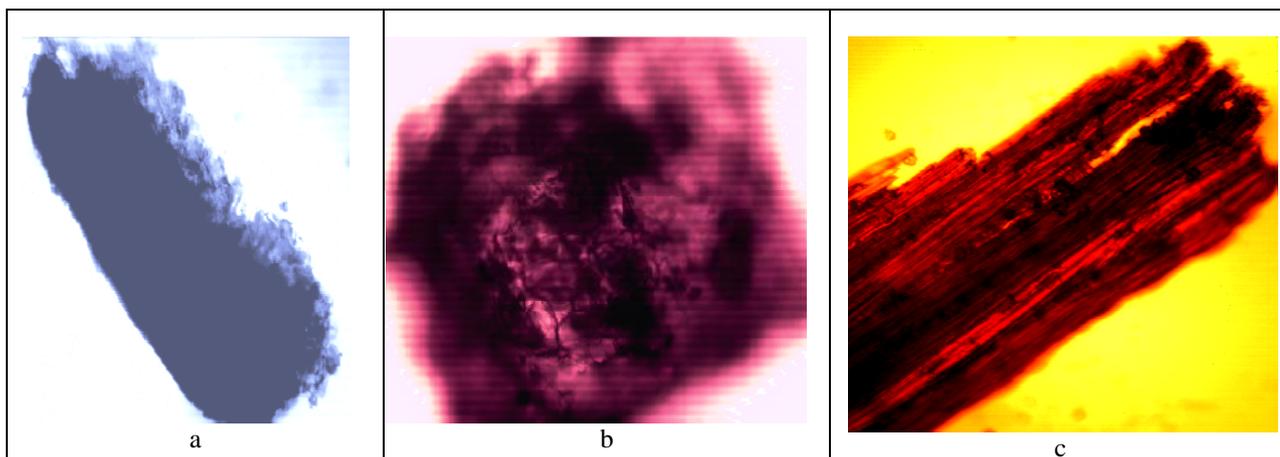


Fig 3: Powder microscopy of *P. zeylanica* root: a) Dark colour periderm; b) Tanniferous cell; c) Secondary xylem elements include vessels and xylem fibers.

3.3 Physicochemical constants

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physicochemical parameters like ash values, extractive values, moisture content and fluorescence on reaction with various

chemical reagents were investigated and the results are presented (Tables.1-3). Ash values of the drug give an idea about the earthy matter or inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of the exhausted or adulterated drug.

Table 1: Physicochemical parameters of *P. zeylanica* root powder.

Physicochemical parameter values (% w/w)		
1	Total ash	3 % w/w
2	Water soluble ash	2.09 % w/w
3	Acid Insoluble ash	0.86 % w/w
4	Moisture content	2 % w/w
5	Foreign organic matter determination	1 % w/w
6	Crude fiber determination	2.1 % w/w
7	Swelling index determination	0
8	Foam index determination	0

Table 2: Solvent extractive values (%w/w) of *P. zeylanica* root powder

No	Name of extract	Color	Extractive value
1	Petroleum ether Extract	Yellowish brown	1.4 % w/w
2	Chloroform Extract	Brownish red	4.06 % w/w
3	Methanol Extract	Red (Dark)	13.76 % w/w
4	Water Extract	Dark brown	15.5 % w/w

Table 3: Fluorescence analysis of *P. zeylanica* root powder with various reagents

No	Reagent	UV short (254 nm)	UV long (365 nm)	Visible
1	Methanol	Yellowish green	Green	Brown
2	1 N Methanolic NaOH	Green	Dark green	Brown
3	Ethanol	Florescent green	Yellow green	Brown
4	1 N Ethanolic NaOH	Dark green	Yellow green	Brown
5	1N HCL	Black	Faint green	Dark Brown
6	1N NaOH	Green	Green	Dark Brown
7	50 % H2S04,	Brownish black	Dark brown	Brown
8	50 % HNO3	Faint brown	Faint green	Brown
9	5 % KOH	Dark yellow	Purple green	Brown
10	Acetone.	Faint brown	Faint green	Brown

3.4. Preliminary phytochemical screening

Extracts obtained by continuous soxhlet were subjected to standard qualitative phytochemical tests to identify the presence of chemical

constituents (*viz.*, alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Preliminary phytochemical screening mainly revealed the presence of steroids

and triterpenes in petroleum ether extract; steroids, triterpenes and glycosides in chloroform extract; carbohydrate, tannin and

glycoside in methanol extract & carbohydrate & tannin in aqueous extract (Table - 4).

Table 4: Phytochemical analysis of *P. zeylanica* root extracts.

Chemical constituent	Chemical test	Pet. Ether extract	Chloroform extract	Methanol extract	Water extract
Alkaloids	Dragendorff's test	-	-	-	-
	Mayers test	-	-	-	-
Steroids	Salkowaski test	+++	-	-	-
	Liebermann-burchard test	+++	-	-	-
Triterpenes	Vanillin-sulphuric acid test	+++	++	-	-
Tannin	Ferric chloride test	-	-	++	++
Glycoside	Keller-killani test	-	++	++	+++
Carbohydrate	Molish test	-	-	+++	-
	Fehling's test	-	-	-	-
Flavonoids	Shinoda Test	-	-	-	-
Saponins	Lead acetate test	-	-	-	-
Proteins	Biuret test	-	-	-	-

+: Weak positive test; ++: Low positive test; +++: Strong positive test; -: negative test.

4. Discussion

Total ash values and extractive values are useful in identification and authentication of the plant material. Extractive values are useful to evaluate the chemical constituents of crude drugs. Preliminary phytochemical screening mainly revealed the presence of steroids and triterpenes in petroleum ether extract; steroids, triterpenes and glycosides in chloroform extract; carbohydrate, tannin and glycoside in methanol extract and carbohydrate and tannin in aqueous extract T.S. of the young and thick root confirmed the presence of periderm with tanniniferous rectangular cells, cortex, and Secondary phloem comprising small groups of sieve elements. Secondary xylem has several long uniseriate radial multiple vessels. The secondary xylem elements include vessels and xylem fibers. Xylem vessels circular thick walled. The xylem fibers are heavily thick walled and lignified.

5. Conclusion

In the present investigation, a set of pharmacognostical standardization parameter studies were conducted on *P. zeylanica* root as per pharmacopoeia and WHO guidelines. These studies revealed the presence of various important bioactive compounds and proved that the plant roots are also medicinally important. These results may help in standardization, identification and in carrying out further research in *P. zeylanica* root based drugs which are used in Ayurveda and modern pharmacopoeia.

6. Conflicts of interest statement

We herewith declare that we have no conflict of interest.

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