Iranian Journal of Pharmaceutical Sciences
2013:9 (3):45-54
www. ijps.ir

Original Article

Comprehensive Anatomical Investigation of Root of *Plumbago Roseus* Linn

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Abstract

Root of *Plumbago indica* Linn is used in ayurveda and siddha medicine for the treatment of colic inflammations bronchitis, helminthiasis, hemorrhoids, elephantiasis, hepatosplenomegaly, amenorrhea, odontalgia, piles, and diabetes. The present study aims to investigate the anatomical characters of thin and thick root and to confirm the physicochemical characters of the root. Anatomical studies and physiochemical evaluation of root were performed based on the customary protocols mentioned in WHO guidelines and Indian ayurvedic pharmacopoeia. Anatomical studies of root tissues were done as photographs with different magnifications by using Nikon lab photo 2 microscopic Unit. The elemental analysis was done by using perkin elmer 5000 atomic absorption spectrophotometer. We compared the anatomy of thin root to thick root; the periderm of the thin root was 70-80 µm thick, whereas in thick root the periderm was measured about 100 µm thick. In powder microscopy, two kinds of fibers were found; Wide fibers were 300-500 µm long and 30 µm wide, Narrow fibers were 340 µm long and 15 µm wide. Vessel elements are estimated about 140 µm long. Physio-chemical parameters such as total and acid-insoluble ash (8.33% w/w, 1.55% w/w, respectively), extractive values (aqueous 11.21% w/w and alcoholic 5.30% w/w) were performed. The study provides the quantitative data that was given assurance to identify and differentiate this species correctly for the purpose of traditional medicinal use.

Keywords: Fire plant, Helminthiasis, Iron tonic, *Plumbago indica*, *Plumbago roseus*, Plumbaginaceae.

1. Introduction

The genus *Plumbago* includes 3 species, namely *Plumbago indica* (*P. rosea*) *Plumbago capensis* and *Plumbago zeylanica*, which are disseminated in many parts of India [1]. Among these *Plumbago indica* Linn. Synonym. *Plumbago rosea*, Linn. Family Plumbaginaceae have been reported by several researchers about the categorization of numerous compounds namely plumbagin, sitosterol, stigmosterol, campesterol, plumbagic acid lactone, cyanin and two aliphatics palmitic acid, myricyl palmitate, α-amyrin, α-amyrin acetate, β-sitosterol, n-octacosanol, β-sitosterol, myricetin, roseanoic acid, ampelopsin [2].

The roots are acrid astringent, thermogenic, anthelmintic, digestive, gastric, sudorific and also useful in fever, cough, worms, leucoderma, dyspepsia, skin diseases, scabies, vitiated conditions of

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Cite this article as: Muthuswamy R, R S. Comprehensive anatomical investigation of root of *Plumbago roseus* Linn. *Iranian Journal of Pharmaceutical Sciences* . 2013. 9 (3): 45-54.

vata, pitta, kapha, and anemia. It is narcotic carminative, antiperiodic, nervous stimulant and rejuvenating and is useful in colic inflammations bronchitis, helminthiasis, hemorrhoids, elephantiasis, hepatosplenomegaly, amenorrhea, odontalgia, piles, diabetes and diarrhea [3]. Its leaf and root juice mixed with oil is externally applied for rheumatism, paralysis and leprosy. The roots are used in dyspepsia, chronic intermittent fever, ringworm, anemia, skin diseases, diarrhea, and abortifacient [4]. Since root is extensively used in Siddha and Ayurveda medicine in India, therefore the current study has decided to make the comprehensive study of the root of this species.

2. Materials and Methods

2.1. Collection of Specimens

The fresh root was collected early morning during the summer season in the month of April 2012 from the fire plant in rayiarath garden, Pattikadu, Palakkad, Kerala, and South India. The plant material was taxonomically identified by Prof. P. Jayaraman, taxonomist, Plant anatomy research centre, Chennai. The voucher specimen (No.PARC/2010/2098) was deposited in the medicinal plant documentation unit in Pharmacognosy and Phytochemistry department, Nehru College of Pharmacy, Pampady, Thiruvilwamala-680597, Thrissur district, Kerala state, India. Fresh root used for microscopic characterization and root powder had been used to determine the physicochemical parameters such as ash values, extractive values, powder microscopic characters as well as qualitative and fluorescence analysis. Reagents, Chemicals of analytical grade, were used from Sigma Chemical, Bangalore, India as well as Fine Chemicals Ltd., Mumbai, India.

2.2. Microscopic Slide Preparation

A piece of root was fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Alcohol-90ml). Afterwards, microscopic slides were arranged as indicated by standard procedures [5-6]. The transverse cross sections of the root have been prepared by cutting the paraffin implanted specimen by Rotary Microtome. The thickness of the sections was made about 10-12 µm. The dewaxing of the root sections was done by customary method [6]. Later on the root sections were stained with toluidine blue [7]. Sections of the root were cleared in sodium hypochlorite solution at 30% and stained with safranin[6]. Microscopic characterization was done by Olympus optical microscope connected to a Sony digital camera. Powder microscopy was done by regular actions [8-9]. The micro chemical tests for anatomical regions were carried out based on the usual methods [6,10,12].

2.3. Behaviour of Powder, with Different Chemical Reagents

The root powder material has been treated with varies chemical reagents to observed colour changes under ordinary daylight and colour and consistency of extracts were also monitored by the customary techniques [13].

2.4. Fluorescence Analysis of Powder and Extracts

The root extracts were observed under daylight, short and long UV light for fluorescence analysis [14].

2.5. Estimation of Inorganic Constituents (Elemental Analysis)

To estimate the inorganic elements content, 1 g of the dried root powder was triturated in concentrated nitric acid and perchloric acid (3: 1) until a clear solution was obtained. Later on cooling, the solution was made up to a definite volume with de mineralized water and analyzed in Perkin

Table 1. Physicochemical values of root of *Plumbago roseus.*

Parameters	Results
1. Organoleptic characteristics	
Appearance	Coarse powder
Colour	Blackish grey
Odour	Characteristic
2. Loss on drying	7.66%w/v
3. pH value	
pH of 1% aqueous solution	6.3
4. Ash values (%)	
Total ash	8.33%
Acid insoluble ash	1.55%
Sulphated ash	2.31%
Water soluble matter (%)	11.21%
Alcohol soluble matter (%)	5.30%
5. Successive solvent extracts percentage yield and its appearance	
Hexane extract Blackish green solid mass	2.24%w/w
Chloroform extract Blackish brown mass	1.64% w/w
Ethyl acetate extract Blackish dull red, sticky mass	2.14% w/w
Ethanol extract Blackish brown mass	5.12% w/w
6.Foreign organic matter	0.72% w/w
7. Crude fiber content	10.52% w/w
7.Elemental analysis	
In organic Elements	Quantity of elements (µg/g) in dried powder
Zn	21.5
Mn	32.3
Cu	15.3
Cr	18.5
Pb	07.2

Elmer 5000 an atomic absorption spectrophotometer [15,16].

2.6. Physicochemical Parameters

Loss on drying, [16], pH of 1% water soluble solution of root was measured at 25C° [17]. Ash values and extractive values were performed according to the customary technique [9]. Thin Layer Chromatography (TLC) was performed by using the standard procedure [18].

3. Results and Discussion

The current study found that Mn, Zn were major contents in the root. The important physicochemical parameters and organoleptic characters determined was depicted in Table 1. The TLC Profile of successive solvent extracts is a very important module of the modern monograph given in Table 2. The behaviour of root powder with different chemical reagents is depicted in Table 3. The fluorescence analysis of root powder was made with different acids and alkalis (Table 4). The qualitative analysis report shows that the presence of steroids, triterpene, tannin and phenolic compounds as well as flavanoids were the secondary metabolites in the root.

3.1. Botanical Description

Fire plant is perennial shrub, 1 to 1.5 meters height, Stems are woody and straight with stretchy twigs; leaves are simple, alternate, extipulate, entire, membraneous, curvy, short, cuneate at the bottom passing in to a very short

Table 2.TLC finger printing of different extracts of root of *Plumbago roseus.*

Extract	Solvent system	Detection		Spots	hR_f. Values
Hexane	Toluene: methanol (10:80)	Anisaldehyde acid (65%)	Sulphuric	4	16;34;53;76
Chloroform	n-hexanae: Ethyl acetate (80:20)	Anisaldehyde acid (65%)	Sulphuric	2	14; 24
Ethyl acetate	Benzene:Methanol (40:60)	Anisaldehyde acid (65%)	Sulphuric	3	19;34; 68
Ethanol	n-Butanol: Acetone (35:65)	Anisaldehyde acid (65%)	Sulphuric	5	12;23;34;56;68

Table 3. Behavior of the root powder of *Plumbago roseus* with different chemical reagents.

Reagents	Observation	Chemical nature
Aqeous $Fecl_3$	No change	Starch absent
Dilute ammonia solution	Black brown	Anthraquinone present
5%Aqeous KOH	Black precipitate	Anthraquinone present
Aqeous Hgcl2	White precipitate	Alkaloids present
Picric acid	No change	Alkaloids absent
Aqeous AgNo3	No white precipitate	Protein absent
Conc H2So4	No change	Lipids absent

amplexicaul, auriculate, red petiole; roots elongated, cylindrical inflexible, bent or curved, corpulent,1-2 cm broad, 120-180 cm long, tangential branches not many, light yellowish brown on the exterior, reddish white inside; flowers scarlet or bright red coloured in lengthy terminal spikes, bracts and bracteoles ovate, almost equal in size, shorter than the calyx; calyx greenish red, sub–sessile, short cylindrical, acutely five toothed, five ribbed, enclosed with stipulate, bifarious and sub sessile glands; corolla scarlet, corolla tube slender, longer than the calyx, 5 toothed, stamens five, filaments unified at the bottom and made a nector disc around the ovary, anthers linear, two fissure at the bottom; ovary superior, ovate, lone cell ovule, style filiform, hairy with five stigmatic branches assembled to made line up of glands [3].

3.2. Anatomy of Roots

The anatomical descriptions were made with the help of standard book [19]. Both thin and thick roots were studied.

Table 4. Florescence analysis of root powder of *Plumbago roseus* with various acids and alkalis.

S.NO	Treated chemicals	Day light	UV long	UV Short
1	Powder as such	Blackish brown	Blackish brown	Blackish brown
2	Powder +1N HCL	Pale yellow	colorless	colorless
3	Powder +1N NAOH	yellow	Green	Green
4	Powder +1N NAOH in MEOH	Pale green	Green	green
5	Powder +50%HNO3	Green	Greenish yellow	Greenish yellow
6	Powder +50%H2SO4	Yellowish green	Green	Green
7	Powder +MEOH	Green	Green	Green

3.2.1. Thin Root (Young Root)

Thin root measures 2.4 mm in diameter. It consist uniformly thick periderm all around the root (Fig.1). The periderm is 70 or 80 µm thick. The periderm cells are squarish in shape and occur in compact radial files these are 5 or 6 cells in each row. The epidermis were broken and disintegrated. Inner to the periderm is homogeneous, parenchymatous cortex, which is 700 µm of radial width. The cortical cells are angular, thin walled and compact (Fig.1.1).

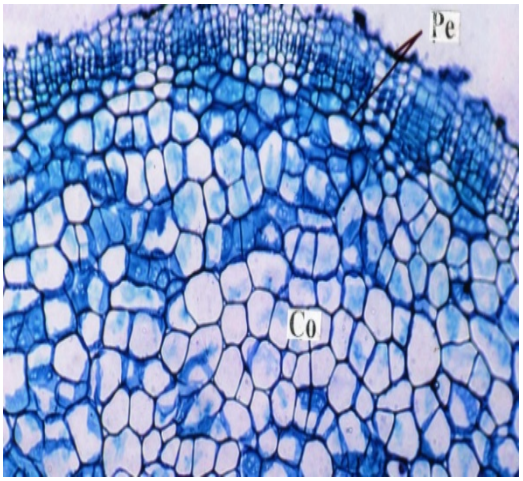

Figure 1.1. T.S. of thin root shows cortex area and periderm.

Co-Cortex, Pe-Periderm

The cortex is followed by secondary phloem there is no distinct border line between the phloem and cortex. The transition from the cortex with phloem is gradual. In the secondary phloem zone there are small groups of sieve elements which are narrow oblong large circular parenchyma.

The secondary xylem consists of wide compact cylinder which are diffusely merges with the outer phloem zone (Fig.1.1) there are many fairly wide circular thick walled vessels which are disused in bi or uniseriate radial multiplies. The radial lines of vessels are separated from each other by wide, thick walled lignified fibers. The vessels are 10 x15 µm wide.

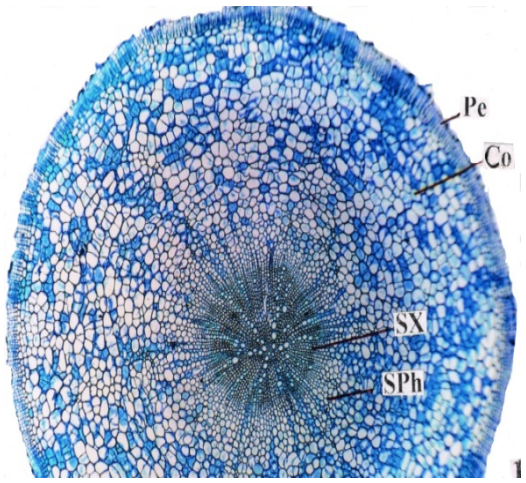

Figure1. T.S. of thin root entire view
CO- Cortex, PE- periderm, SX- secondary xylem, SPH- Secondary phloem.

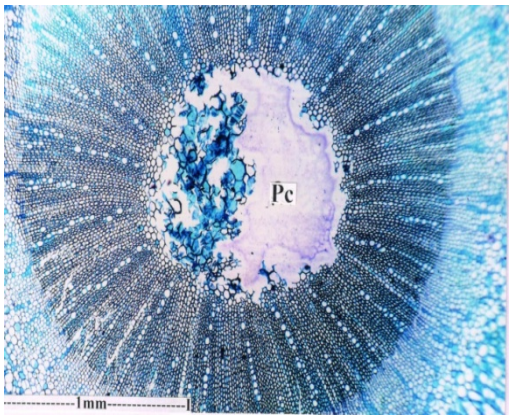

Figure 2. T.S. of thick root showing central empty pith canal and pith cavity, secondary phloem, secondary xylem.

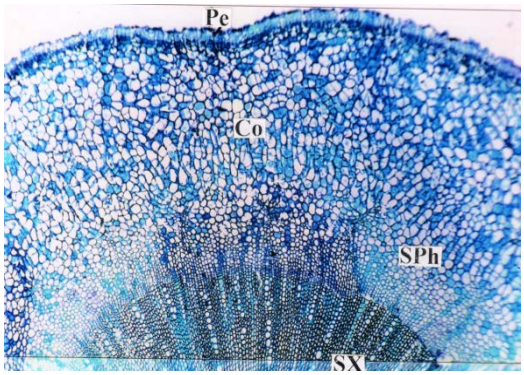

Figure 2.1. T.S. of thick root showing SPh-secondary phloem, SX-secondary xylem, Co-Cortex, Pe-Periderm.

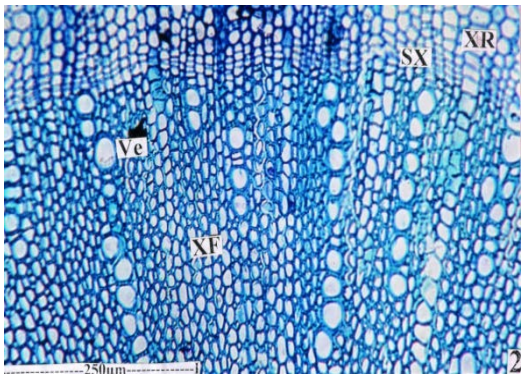

Figure 3. Thick root showing periderm and cortex
Thick root showing radial lines of phloem elements and secondary xylem with long radial multiplies of vessels and fibres.
Ve- vessel, XF- xylem fiber, SX- secondary xylem, XR- xylem ray.

3.2.2. Thick Root (Matured Root)

The thick root is about 5mm thick, it is similar to the thin root except that the vascular cylinder is thicker and wider and there is a wide central pith cavity. The root has thick, well developed periderm which is 100 µm thick. The cortex is wide and parenchymatous tissue. The periderm cells are tubular in shape homo cellular and thick walled. The phellogen layer is evident which occurs in the inner part of the periderm. (Fig. 2). The vascular cylinder consists of an outer circular zone of secondary phloem and inner thick hollow cylinder of secondary xylem, in the phloem

zone sieve elements occur in thin radial lines with wide parenchymatous gaps in between (Fig. 2.1).

The secondary xylem consists of mostly uniserriate, thin radial lines of vessels and xylem fibers. The vessels are circular narrow, thick walled and are in long radial multiples (Fig. 3). The vessels are 30 µm in diameter the xylem fibers are wide fairly thick walled and lignified. Xylem rays are thin, straight and the ray cells are rectangular in outline (Fig. 4).

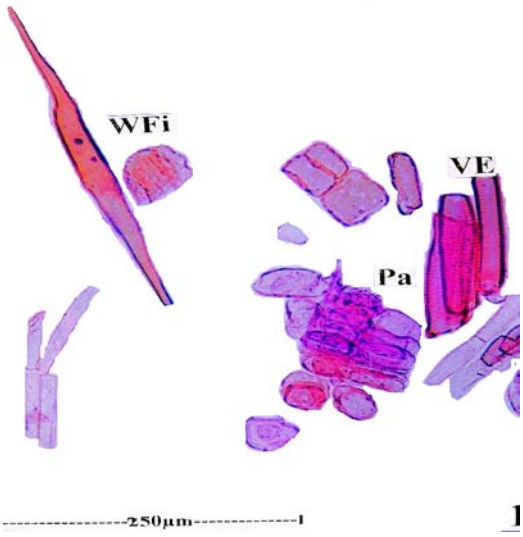

Figure 4. showing WFI-Wide fiber, narrow fiber and PA-parenchyma cells and VE-vessel elements.

3.3. Powder microscopy

The powder of the root material exhibits the following inclusions:

3.2.3. Fibers

There are two types of fibers in the powder. Some of the fibers are wide, thin walled and spindle shaped.The wide fibers are 300-500 µm long and 30 µm wide the second type of fiber is the narrow type which has got thick walls and narrow lumen. The narrow fibers are uniform in thickness and tapering at the ends. They are up to 340 µm long and 15 µm wide. (Fig.4.1)

3.2.4. Parenchyma Cells

Isolated parenchyma cells are frequently seen in the powder. The parenchyma cells may be short, thin walled and rectangular with thin walls and wide lumen (Fig. 4.2)

3.2.5. Vessel Elements

The vessel elements are narrow and cylindrical; the perforation is circular, wide and horizontal. The lateral walls have multiseriate, alternate, circular pits. The vessel elements are 140 µm long (Fig. 4.3)

The TS of the root is nearly cuticular in outline with 5-6 layered cork tissue, consisting of cubical to rectangular cells with their wall light yellow to yellowish brown content forms the major part of the root. The greater part of the cortex consists of thin walled rounded, polygonal tangentially elongate cells with well defined intracellular space. The cells are normally devoid of the starch grains. The innermost rows of cortical cells are nearly regular and rounded .There is no clear demarcation between cortex and bast. The phloem parenchyma cells are very small, thin walled and polygonal. Most of the cells have yellow contents. No mechanical elements are in the bast. The cambium consists of one or two layers. The xylem vessels are arranged mostly in the single file in radial rows and are surrounded by mechanical cells. The medullary rays are multiseriate. The root is triarch and protostelic [3].

Earlier studies on root shows the presence of elements were Fe (6.47), Mg (0.92), Zn (0.34), Cu (0.22), Co (0.06), Ni (0.01), Na (82), K (113), Ca (18), As (0.0001), Cd (0.007), Pd (0.3116), Hg (0.0053) and the absence of Cr [20]. The current study found that the presence of 18.5µg/g chromium in dried root powder. Plumbago rosea root has a major amount of iron. Therefore, it seems to be dark in color similar to dates fruits. Iron is diuretic, demulcent and used to treat diarrhea and croup. Copper deficiency leads to chronic or repeated diarrhea, low resistance to infection and anemia. Manganese is needed for hemoglobin development, reproduction and skeleton growth in humans. The highest content of Manganese was reported in all three species of *Plumbago zeylanica, Plumbago rosea* and *Plumbago capsensis* has been given to cure skin diseases, scabies, piles, and rheumatism. Zinc is constituent of many metalloenzymes and also membrane stabilizer and stimulator of the immune response. Its deficiency develops loss of appetite and impaired immune function. In case of vigorous zinc deficiency causes hair loss, delayed sexual maturation, impotence hypogonadism in males, the eye and skin lesions. The zinc was higher content in *Plumbago zeylanica,* whereas the amount is equal in *Plumbago capsensis, Plumbago rosea* which can be cure skin diseases and rheumatism. Chromium plays a major role in carbohydrate metabolism and its deficiency leads to Diabetes in humans. Chromium supports transmission in the neuromuscular system. Sodium is influenced in intracellular and extracellular fluid balance and safeguarding of viscosity of blood. Potassium is diuretic, both sodium and potassium facilitated the ionic balance of the human body and sustains tissue excitability. Calcium is essential in the

nerve impulse conduction and the mechanism of the neuromuscular system.

The Plumbagin content is high in *Plumbago rosea* 0.17% compared to other species of *Plumbago zeylanica,* 0.01% and *Plumbago capsensis* 0.04%. Crude fiber content largely depends on cellulose and lignin plus some mineral matter. Pesticide residue and Aflatoxin $B_1B_2G_1G_2$ were not detectable range in root powder [20]. Acid insoluble ash specified the occurrence of more siliceous matter in the drug. It was found to be 1.55%. The alcohol soluble extractive values reveal the contents of polar phytochemicals such as flavanoids, anthraquinone, glycosides, alkaloids, steroids, triterpenoids presented in the plant materials. It was estimated about 5.30% in root. The water soluble extractive values reveal the presence of water soluble materials such as vitamins, amino acids, sugars, carbolic acids, and it was calculated to be 11.21%. As this root contains a rich quantity of iron, manganese and other essential minerals and flavanoids it would be considered as a general tonic in the future.

4. Conclusion

The Microscopic characterization of the root will be helped to differentiate other species of the genus. The current study differentiates the microscopic characterization thick root to thin root. In the view of developing customary monographs, this study was a significant milestone.

Acknowledgements

We are grateful Mr. Rayairath, Rayairath herbal garden, Pattikadu, Thrissur, Kerala, India to provide all the facilities to collect plant parts from his herb garden.

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