

## A Simple Anatomical Method for Identification and Authentication of Medicinally Important Herbal Drug 'Chitrak' (*Plumbago* species)

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### ABSTRACT

*Plumbago zeylanica* L. is used in Ayurveda to treat various diseases and is often called as Chitraka in Sanskrit. The root of this plant is used in Ayurvedic formulations that stimulates digestion and improves appetite. The raw materials or the plant parts are collected by untrained workers and supplied in dry condition to the drug manufacturing industries. Therefore, there is a chance of adulteration because of vested interest or ignorance as many plants are known by common names or in vernacular names. Three species of *Plumbago* viz. *Plumbago auriculata* Lam., *P. indica* L. syn. *P. rosea* L. and *P. zeylanica* L. are found in Odisha and amongst them *P. auriculata* Lam. is rare. All the species are known as Chitraka, but for commercial preparation of Ayurveda, Siddha and Unani (ASU) drugs in Indian System of Medicines, *P. zeylanica* L. is widely used because of its therapeutic activities. The present study deals on the anatomical characteristics of roots of these three *Plumbago* species for proper identification and authentication of the desired species in drug manufacturing industries before formulating the compound drugs. Root specimen of *Plumbago auriculata* Lam., was collected from the authentically identified plant specimens maintained in the nursery of Regional Plant Resource Center, Bhubaneswar and the roots of *P. indica* L. Syn. *P. rosea* L., and *P. zeylanica* L. were collected from authentically identified plant specimens maintained in the nursery of Silviculture office, Ghatikia, Bhubaneswar. Transverse sections were made manually for each specimen separately and were cleared by using 2% chloral hydrate. The sections were then subjected to staining with safranin and fast green solutions and then mounted over the glass slides using DPX (Qualigens, India) mountant and observed under the microscope. Results: Transverse section of the root of *P. auriculata* Lam. revealed the presence of a group of stone cells arranged in a ring occasionally interspersed with cortex surrounding the large stellar region. Transverse section of the root of *P. indica* L. revealed a wide cortical region comprising many layered rounded or oval shaped compactly arranged parenchymatous cells without any intercellular spaces. Stone cells are absent in the cortical region and also the cortical region do not show the presence of fibers. Microscopic observations of transverse section of the root of *P. zeylanica* L. showed small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring around the stellar region and also the cortical cells were found to be filled with starch grains of about 6 to 10µ in size. Conclusion: All the three species revealed distinct anatomical features. Microscopic observations of transverse section of the root of *P. zeylanica* L. showed small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring around the stellar region and also the cortical cells were found to be filled with starch grains of about 6 to 10µ in size which are the identifying features. This study will certainly help to authenticate the crude drug and ensure the quality.

**KEY WORDS:** ANATOMY, AUTHENTICATION, ASU FORMULATIONS, COMPOUND DRUGS, *PLUMBAGO ZEYLANICA* L.

### ARTICLE INFORMATION

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## INTRODUCTION

Plants are an important source of medicine and play a key role in world health. Medicinal herbs have been known to be an important potential source of therapeutics or curative aids. The use of medicinal plants has attained a commanding role in health systems all over the world because of their less toxicity. The genus *Plumbago* of family Plumbaginaceae comprises 10 species, extensively used for various purposes. *Plumbago zeylanica* L. is known as 'Chitraka' in Ayurveda has been used in alternative systems of medicine as component formulations for the treatment of multiple disorders such as arthritis, anemia, cardiovascular disorders, metabolic disorders, chronic rhinitis, sinusitis, dyspnea, anorexia and dyspepsia (Patel R.V. et al. 2011; Sharma, P.C.2001). The entire plant is used in traditional systems of medicine. This plant is used in treating leucoderma, piles, bronchitis, liver diseases and intestinal trouble.

The crude root paste is used by local people for the management of arthritis and other inflammatory disorders. A tincture of the root bark is used as anti-periodic. The root is used in Ayurvedic formulations that stimulates digestion and improves appetite (API, Part-II (Formulations), 2007). *Plumbago auriculata* Lam., *P. indica* L. Syn. *P. rosea* L., and *P. zeylanica* L. are the three species commonly found in India. *Plumbago auriculata* Lam. is known as blue *Plumbago*, Cape leadwort and Cape *Plumbago* and in Odia it is commonly known as 'Nila chita' and is native to South Africa (Botanica, 2004; Nico Vermeulen, 1998). *P. indica* L. Syn. *P. rosea* L. is known as Indian leadwort, scarlet leadwort, whorled plantain and in Odia it is called as 'Rakta chita' or 'Lal chita' and is native to Southeast Asia. *P. zeylanica* L. is known as white leadwort, Ceylone leadwort and in Odia it is commonly called as 'Agni' or 'Sweta chita' and is widely grown in India and has been used by rural and tribal communities for hundreds of years as traditional system of medicines. In Ayurveda, Siddha and Unani (ASU) drugs in Indian System of Medicines, *P. zeylanica* L. is used in treatment of intestinal troubles, leucoderma, dysentery, piles and bronchitis and roots are used to improve digestion by stimulating appetite.

All the plant parts are used in the Indian system of medicine. Plumbagin, a naphthoquinone well distributed among *Plumbago* species, specially found in their roots (Van der Vijver, 1972) with various pharmacological activities i.e., antimalarial (Likhitwitayawuid et al., 1998) antimicrobial (Didry et al., 1994), anticancer (Parimal and Sachadanandam, 1993), cardiogenic (Itoigawa et al., 1991) and antifertility action (Premkumari, P, 1977; Bhargava, 1984). Morphologically the roots of these three species look similar and difficult to distinguish (Figure 1 a-c). Also, most of the roots in dried form are procured for making formulations by the manufacturing industries and the procured roots look similar which leads to confusion and results in adulteration (intentional or un-intentional). In such circumstances, application of plant anatomy is an effective tool in identifying the plant samples. Although, a few reports on anatomy of

leaves of *P. zeylanica* L. reported earlier but report on root anatomy of *Plumbago* species is lacking. The purpose of the present study is to describe the anatomical features of the three species to distinguish from each other based on anatomical studies.

## MATERIAL AND METHODS

Root specimen of *Plumbago auriculata* Lam., was collected from the authentically identified plant specimens maintained in the nursery of Regional Plant Resource Center, Bhubaneswar. The roots of *P. indica* L. Syn. *P. rosea* L., and *P. zeylanica* L. were collected from authentically identified plant specimens maintained in the nursery of Silviculture office, Ghatikia, Bhubaneswar. Transverse sections were made manually for each specimen separately. The transverse section of the root was cleared by using 2% chloral hydrate for greater tissue transparency and light transmission. Chloral hydrate is known to preserve cellular features than many other clearing agents (Rost and Oldfield, 2000). The sections were then subjected to staining with safranin and fast green solutions (Johansen, 1940) and then permanently mounted over the glass slides using DPX (Qualigens, India) mountant and observed under the microscope.

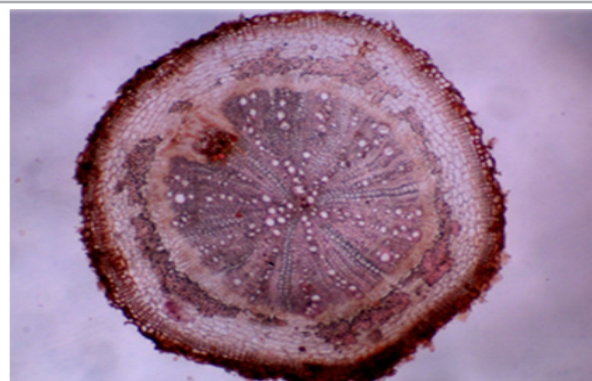


Fig. 1. T.S. of root of *P. auriculata* L. (Whole mount)

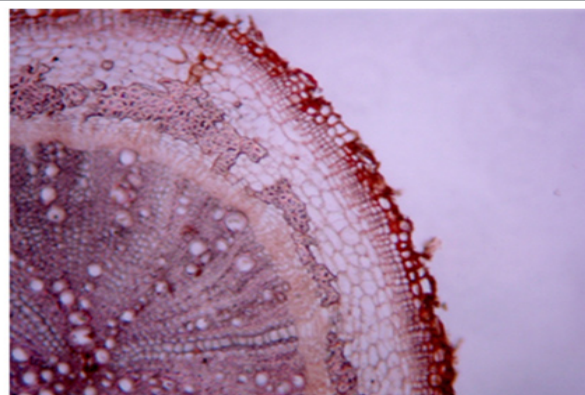


Fig. 2. T.S. of root of *P. auriculata* L. (A portion magnified showing group of stone cells arranged in a ring above the stellar region)

## RESULTS

Anatomical studies on the roots of *P. auriculata* Lam., *P. indica* L. Syn. *P. rosea* L. and *P. zeylanica* L. clearly showed distinguishing features and were described in the following heading for each species separately. *P. auriculata* Lam. Transverse section of the root of *P. auriculata* Lam. revealed 4–6 layered cork on the outer surface of the root consisting of flattened parenchymatous cells. Group of stone cells arranged in a ring occasionally interspersed with cortex surrounding the large stellar region. The innermost layer of cortex that surrounds the stele is called endodermis, which is very distinct. Cells of endodermis have special thickenings viz. casparian strips. Central stellar region occupies a larger area than the cortex. Xylem is exarch. Xylem vessels, medullary rays are prominent. Pith is absent (Fig. 1 & 2).

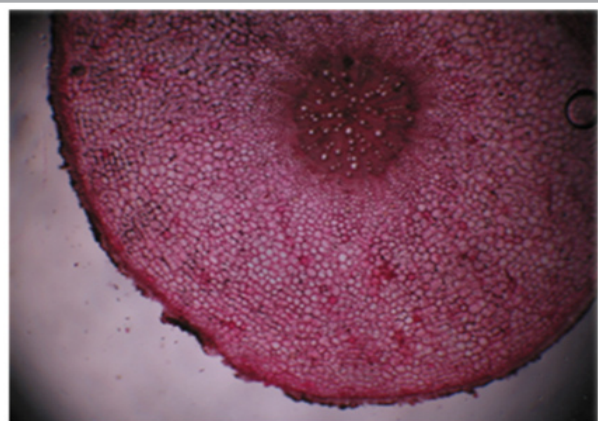


Fig. 3. T.S. of root of *P. indica* L.

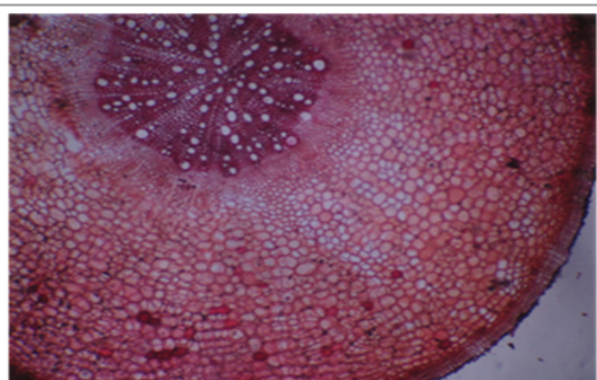


Fig. 4. T.S. of root of *P. indica* L. (A portion magnified)

*P. indica* L. Syn. *P. rosea* L. Transverse section of the root of *P. indica* L. revealed the presence of 4 or 5 layers of cork consisting rectangular cells followed by a layer of cork cambium; cortex showed wide cortical region comprising many layered rounded or oval shaped parenchymatous cells, compactly arranged without any intercellular spaces. Stone cells are absent in the cortical region, also the cortical region does not show the presence of fibers. The stellar region was found

to be narrow in comparison to *P. auriculata* Lam. (Fig. 3 & 4).

*P. zeylanica* L. Microscopic observations of the transverse section of the root of *P. zeylanica* L. showed the presence of cork consisting of 4 to 5 layers of rectangular parenchymatous cells with broad cortex made up of parenchymatous cells without any intercellular spaces. The cortical cells were found to be filled with starch grains of about 6 to 10  $\mu$  in size; small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring as in *P. auriculata* Lam.; endodermis present; pericycle a single layer of thin walled parenchymatous cells; usually polyarch xylem ring, surrounded by phloem (Fig. 5, 6 & 7). The cortex region of *P. indica* L. was broader than the other two species. *P. zeylanica* L. shows groups of stone cells haphazardly present in the cortical zone with large lumen, presence of starch grains in the cortical cells and narrow stellar region are the identifying characters from the above two species.

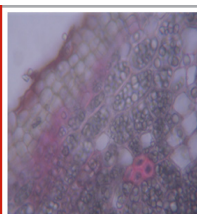


Fig. 5. T.S. of root of *P. zeylanica* L. showing starch filled cortical cells



Fig. 6. T.S. of root of *P. zeylanica* L. with the stellar region

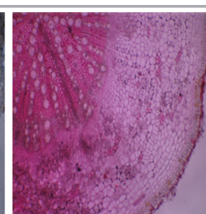


Fig. 7. T.S. of root of *P. zeylanica* L. showing cortical and stellar region after clearing with chloral hydrate solution

## DISCUSSION

Transverse section of the root of *P. auriculata* Lam. showed groups of stone cells arranged in a ring surrounding the centrally occupied larger stellar region which is larger than the cortex and occasionally found interspersed with cortex and is the unique identifying features of this species. The anatomical observations of the root of *P. indica* L. revealed a wide cortical region comprising many layered rounded or oval shaped compactly arranged parenchymatous cells without any intercellular spaces. Stone cells are absent in the cortical region and also the cortical region does not show the presence of fibers.

Microscopic observations of transverse section of the root of *P. zeylanica* L. showed small groups of stone cells with wide lumen present irregularly at cortical region which does not form a ring around the stellar region and also the cortical cells were found to be filled with starch grains of about 6 to 10  $\mu$  in size. Literature data revealed the identification of six *Ocimum* species namely *O. tenuiflorum* L. (both green and purple variety) *O. sanctum* L., *O. basilicum* L. (both green and purple variety), *O. canum* Sims. and *O. gratissimum* L. on the basis of their anatomical features (Parida et al, 2020).

Microscopic observations of roots of *P. auriculata* Lam. and *P. zeylanica* L. showed the presence of lignified pericyclic fibers whereas it is absent in *P. indica* L. (Galal et al., 2013) and the cortical region of *P. indica* L. is broader than the other two species.

## CONCLUSION

One of the major challenges that the pharmaceutical industries face is the genuinity of the raw materials due to its morphological resemblances. This ends in adulteration and substitution for genuine drugs. Such adulteration and substitution of genuine raw material is the main cause of degradation of the therapeutic effect of particular drugs used in Indian System of Medicine that lead to poor quality of herbal products. Developing suitable anatomical markers would help in clear identity of the raw materials. Present anatomical investigations on the roots of *Plumbago auriculata* Lam., *P. indica* L. Syn. *P. rosea* L. and *P. zeylanica* L. clearly showed distinguishing features among them and proved as an authentication tool to identify the crude drugs.

**Conflict of Interests:** There is no conflict of interest.

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