

ISSN 2278-4136

ISSN 2349-8234

JPP 2014; 2 (6): 134-139

Received: 14-01-2014

Accepted: 17-01-2014

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Pharmacognostic and Phytochemical evaluation of the root bark of *Plumeria acutifolia* Poir.

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ABSTRACT

The aim of the present study is to investigate the pharmacognostic and phytochemical investigation of the root bark of the plant *Plumeria acutifolia* belonging to the family Apocynaceae. In microscopic studies, transverse section (TS) of root bark and powder characters of the plant was studied and characteristic features were established. Physicochemical parameters such as total ash value, acid insoluble ash value and water soluble ash value were determined. The alcohol soluble extractive and water soluble extractive were also determined. Preliminary phytochemical analysis of ethanolic extract was carried out. The results of preliminary phytochemical screening were positive for alkaloids, tannins, glycosides, terpenoids and saponins. The results obtained from standardization of root bark of *Plumeria acutifolia* established the macro- and microscopical parameters, physicochemical parameters, TLC profiles that characterize the genuine plant drug. The present study provides pharmacognostical, physicochemical and phytochemical details of the root bark of *Plumeria acutifolia* which are useful in laying down standardization and pharmacopoeia parameters.

Keywords: *Plumeria acutifolia*, Root bark, Pharmacognostical, Phytochemical.

1. Introduction

The plant *Plumeria acutifolia* has been mentioned in ancient literature as anti-inflammatory, anti-allergic, diuretic, carminative, laxative, anti-ulcer, and useful in treating leprosy and ascites, also possess cytotoxic activity and anti-microbial activity ^[1,2]. *Plumeria* species are an ornamental plant that flowers throughout the year. It is the native of Mexico and widely seen in temples and Mohammedan burial grounds in India. It has spread to all tropical areas of the world and in Hawaii, it grows so abundantly ^[3]. The root bark is bitter, pungent, heating, carminative, laxative and useful in leprosy ^[4]. The plant is reported to contain alkaloid, saponin, terpenoids and steroids ^[5]. The literature survey revealed that the systemic evaluation, including pharmacognostical study of this plant is still lacking. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. The main aim of the present work is to study the macroscopic, microscopic, physico-chemical standards and phytochemical analysis of the root bark of the plant *Plumeria acutifolia*, which could be useful for the proper identification of this drug.

2. Materials and Methods**2.1. Plant material**

The Plant specimen for the proposed study was collected from Melmaruvathur Chennai, Tamil Nadu. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, (PARC) Tambaram, Chennai. A voucher specimen (accession No. 31238) was deposited in the Herbarium for future reference.

2.2 Microscopical characterization

The qualitative studies were performed. Free hand transverse section of root bark was, studied for different microscopic characters and photographs of different magnifications of the sections were taken with Nikon Lab Photo 2 (Two) Microscopic unit. For normal observations, bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed ^[6].

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2.3 Powder analysis

The shade dried root bark of the plant was powdered and powder was passed through 100 # sieve. A small amount of powder was taken onto a microscopic slide, cleared from chlorophyll by heating with chloral hydrate solution and was mounted in 50% v/v glycerol in water. This was then observed under microscope to study the characteristic features [7].

2.4 Physico-chemical evaluations

The ash values, extractive values and loss on drying were performed according to the official methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plant materials [8].

2.5 Extraction

The collected root bark of the plant was made thoroughly free from any foreign organic matter, dried under shade and powdered. The ethanol extract was prepared using ethanol by triple maceration process for 48 h each time. The extract was filtered and concentrated under vacuum. The concentrated extract was used for phytochemical screening and establishment of TLC profile.



Fig 1: Root bark of *Plumeria acutifolia*

3.2. Microscopical Study

3.2.1. Transverse Section of the root bark consists of three distinct zones viz., periderm, collapsed phloem and non-collapsed phloem. Periderm is outer most part and is superficial. It is 400 μm wide; outer, a wider zone of phellem bear the cells which are tabular surprised and occur in radial lines. Inner to the phellem a few rows of cells broken due to exfoliation. Inner part of the periderm is phelloderm; which consists of about five layers of tangentially stretched thin walled suppressed cells. Collapsed phloem is the widest zone, comprising of dilated phloem rays, parenchyma cells and sparsely distributed sclereid masses. There are also thin, darkly stained canal like structures which represent "laticifers". The dilated rays are in wide parallel radial bands. They consist of long rows of rectangular cells, which possess wide circular simple pits. Non-collapsed phloem is very narrow and comprises the inner most part of the phloem. In this sclereids are absent and phloem elements are intact. The sieve elements are wide polygonal and thin walled. They are 20 μm wide. Shown in Fig 2.

3.2.2. In T.L.S. view of bark, the characters of phloem rays and sieve tubes are visible. The phloem rays and sieve tubes are either

2.6 Preliminary Phytochemical Screening

The ethanol extract was subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids [9].

2.7 Thin Layer Chromatography

The ethanol extract was subjected to thin layer chromatography. Number of solvent system was tried. The solvent system which shows good resolution was used. The visualization of the spot was done by exposing the plate to iodine vaporizing in the solvent system Toluene: Chloroform: Ethanol (2:2:6) [10].

3. Results and Discussion

3.1. Macroscopical Study

The root bark was light brown in color externally and pale yellowish internally with characteristic odour, slightly bitter in taste. The root bark was soft in texture. (Fig. 1)

bi-seriate or multi-seriate; uniseriate rays are rare. The rays are non-steroidal. The sieve tubes are narrow with simple, oblique sieve plate. The cells of the ray are polygonal, wide and thin walled. They are 252 μm to 700 μm in height and 60 μm to 70 μm in wide. Phloem parenchyma cells occur in vertical rows. Shown in Fig 3.

3.2.3. In R.L.S. view of bark In RLS view, the bark exhibit lateral periderm measuring 200 μm in thickness, inner collapsed phloem. The phloem rays occur in a horizontal band with vertical, rectangular upright cells with simple pits. The sieve tubes are visible in dense vertical rows along the inner portion of the wall. The sieve-tubes are narrow and elongated with vertical rows of wide, circular sieve areas. Vertically elongated crystals are seen in RLS. Calcium oxalate prismatic crystals as well as druses seen near the sclereids. Shown in Fig 4.

3.2.4. Powder microscopy: Microscopic study of powder revealed the presence of thick masses of the periderm cells. Prismatic crystals are seen in vertical rows forming crystal strands. Parenchyma cells are scattered, large, thin walled, polygonal cells,

which are abundantly seen in the bark powder. These cells contain granular bodies. The starch grains are circular, slightly oblong. They are 10-20 μm in diameter.

Long, narrow, fibre-like cells with tapering ends called fibre sclereids are quite frequent in the powder. They have thick lignified wall and narrow lumen. Brachysclereids are polygonal, wide with

thick walled and wide lumen.

Latex containing tubes called laticifers are frequently seen in the powder. The laticifers are narrow and canal like septate and branched. They contain dense granular bodies which represent the latex content. Shown in Fig 5.

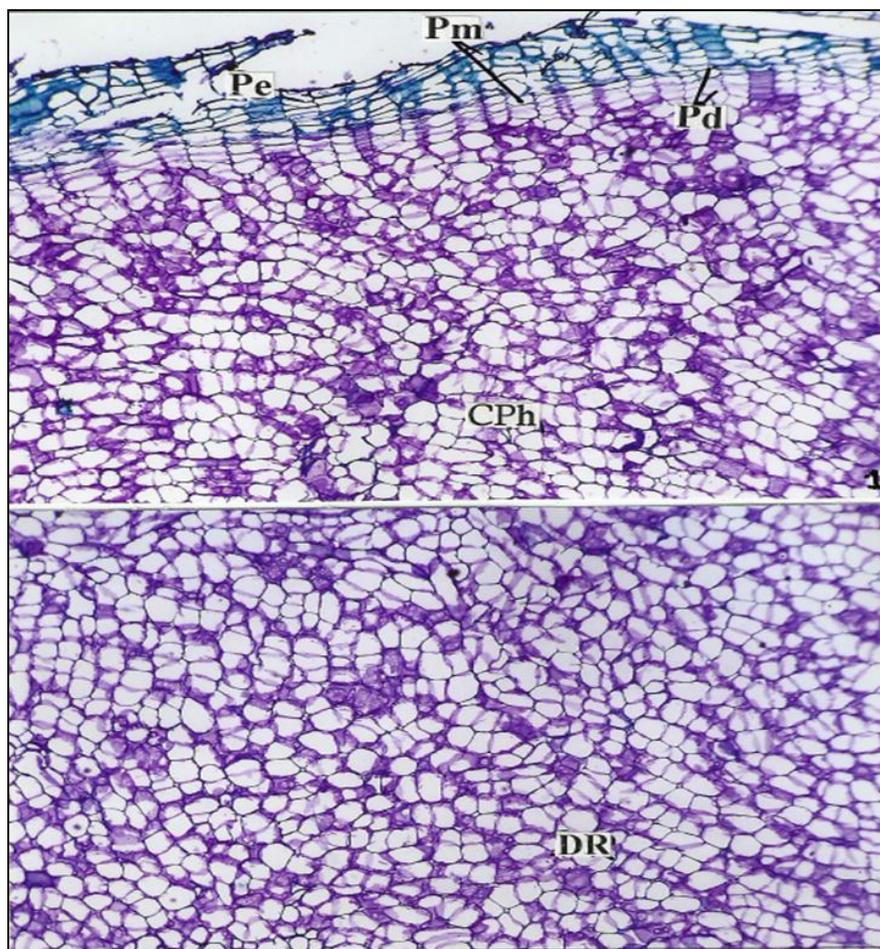


Fig 2: T.S of the bark- outer periderm and collapsed phloem, **CPh**- collapsed phloem; **DR**- dilated ray; **Pe**- periderm; **Pm**-phellem; **Pd**- Phelloderm

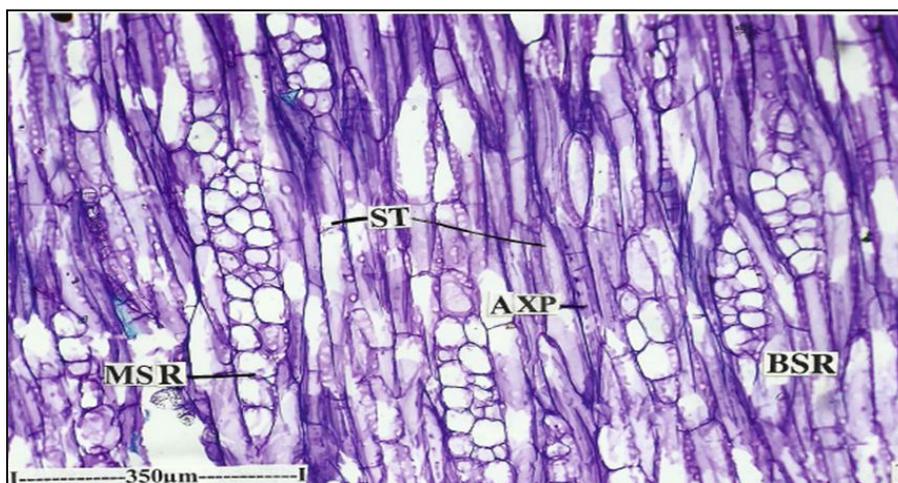


Fig 3: TLS of root bark, **AXP**- Axial parenchyma; **BSR**- Bi-seriate ray; **MSR**- multi-seriate ray; **ST**- sieve tube

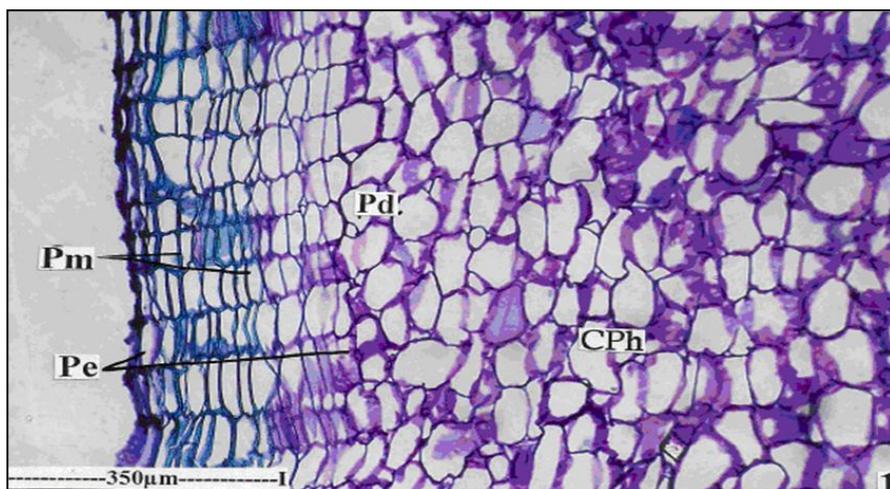


Fig 4: RLS of phloem showing periderm and collapsed phloem, **CPh-** collapsed phloem; **Pd-** Phelloderm; **Pe-** periderm; **Pm-** Phellem

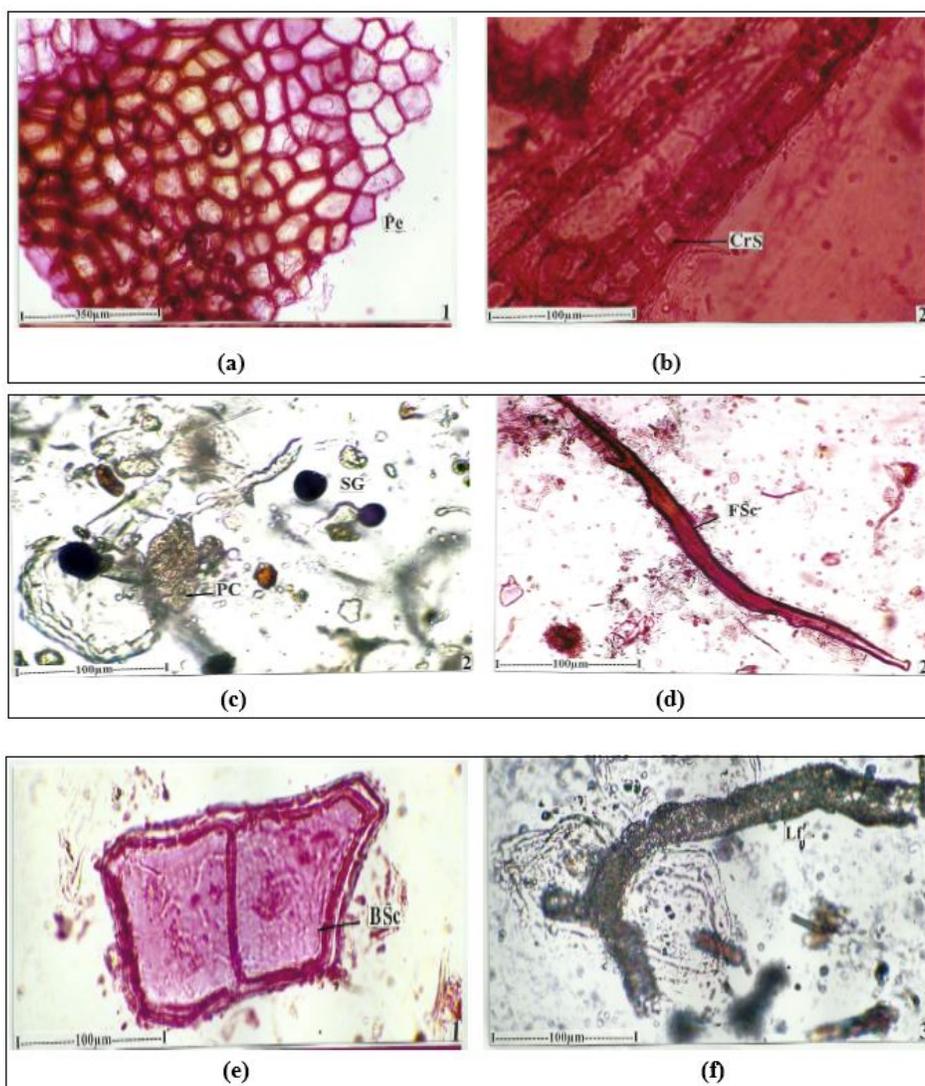


Fig 5: Powder microscopy: (a) periderm cells; (b) Crystal strand; (c) Starch grains; (d) Fibre sclereid; (e) Brachy sclereid; (f) Laticifers cells



Fig 6: TLC of ethanolic extract of the root bark of *Plumeria acutifolia*

3.3. Physicochemical Parameters

Various physicochemical parameters *viz.*, ash, extractive values and loss on drying were determined. The results were summarized in Table 1. These data's were helpful for identifying and ascertaining the quality of the collected crude drug.

3.4. Preliminary Phytochemical Screening

The ethanol extract of plant root bark shows the presence of carbohydrates, alkaloids, glycosides, saponins, steroids, tannins, triterpenoids.

3.5. Thin Layer chromatography (TLC)

To support phytochemical screening, the ethanol extract was subjected to thin layer chromatography. Number of solvent system was tried, but good resolution was seen in Toluene: Chloroform: Ethanol (2:2:6). The ethanol extract showed four well defined spots with R_f values 0.875, 0.775, 0.625 and 0.176. The results were presented in Table 2 and Fig 6.

Table 1: Physico-chemical analysis of the root bark of *Plumeria acutifolia*

S. No	Parameters	Values (% w/w)
1.	Ash values	
	Total ash	10%
	Water soluble ash	2.25%
	Acid insoluble ash	1.95%
2.	Extractive values	
	Water soluble extractives	6.7%
	Alcohol soluble extractives	2%
3.	Loss on drying	13%

Table 2: Thin layer chromatography of ethanol extract of *Plumeria acutifolia* root bark

Test extract	Solvent system	Number of spots	R_f values	Detecting agent
Ethanol extract	Toluene: Chloroform: Ethanol (2:2:6)	4	0.875 0.775 0.625 0.176	Iodine vapour

4. Conclusion

In the present investigation, the pharmacognostical and physicochemical characteristics of *Plumeria acutifolia* Poir (root bark) were studied. Various parameters established in the present study will help in controlling the standards and quality of the raw material of *Plumeria acutifolia*. Moreover, the plant has been traditionally used for its anti-inflammatory activity. The preliminary phytochemical analysis showed the presence of various phytoconstituents which may contribute to the anti-inflammatory activity of this plant. All the pharmacognostical characters and physico-chemical parameters have been reported for the first time. The present investigation adds to the existing knowledge of *Plumeria acutifolia* Poir. root bark and will be quite useful to pharmaceutical industries for quality control, ensuring batch to batch consistency of raw drug and in the field of medical, pharmacological evaluation and development of a formulation for treating various ailments.

5. Acknowledgement

Authors are extremely grateful to the management for the facilities provided to complete this work successfully.

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