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Pharmacognostical studies on the leaves of *Pisonia grandis* R. Br

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ABSTRACT

Pisonia grandis R.Br belonging to the family Nyctaginaceae commonly known as Leechaikottai kerai in Tamil, Velati salet in Hindi, is a medium sized evergreen tree. It is distributed throughout India, cultivated in the garden. In the alternative system of medicine the leaves of the this plant are used as analgesic, anti inflammatory, diuretic and hypoglycemic agent. The paper deals with the microscopical studies and physio-chemical analysis on the leaves of *Pisonia grandis,* one of the parameters in identification of medicinal plant for standardization purpose. For detailed study of microscopical features of the plant, Nikon Labphot 2 microscopic unit was used.

Keywords: *Pisonia grandis,* Microscopy, Nikon Lab photo 2 microscopic unit

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INTRODUCTION

The plant *pisonia grandis* (synonym: *pisonia alba, pisonia morindifolia*) commonly known as Leechikottai kerai in Tamil, Velati salet in Hindi [1].The plant *Pisonia grandis* R.Br belonging to the family Nyctaginaceae, is an evergreen glaborous garden tree with young shoots are minutely puberulous. It is a native of Hawai island also naturalized throughout India. In the alternative system of medicine, *pisonia grandis* leaves are used as analgesic, anti-inflammatory, diuretic and hypoglycemic agent [2]. Leaf moistened with Eau-de-cologne was used to bring down the inflammation of filariod nature. Leaves also consumed as vegetable and salad, also fed to cattle [3]. The phytochemical study reveals that the presence of steroids like octocosanol, betositosterol, alphaspinosterol, dulcitol and flavonoids in the leaves of the plant[4]. Lack of proper standards of indigenous medicinal plants may result in the usuage of improper drugs which in turn will cause damage not only to the individual using it , but also to the respect gained by the well known ancient system of medicine. So the present investigation involves the microscopical study and physiochemical analysis to identify and maintain the quality of the plant in the traditional system of medicine.

MATERIALS AND METHODS

Fresh leaves of *Pisonia grandis* were collected from Kodambakkam ,Chennai, Tamilnadu, India. The plant was identified and authenticated by taxonomist voucher specimen (2009/ 357) was deposited in the herbarium in the department of Pharmacognosy, Vels University, Pallavaram, Chennai, Tamilnadu.

Macroscopical characters

The plant is an evergreen tree, all parts are glaborous and the young shoots are minutely puberulous. The leaves are ovate-oblong to oblong, 15-25 cm long ,5-7 cm wide usually unequal obtuse at the base and acute apex. It has a long petiole with prominent reticulate venation and minutely puberulous in the axils of the nerves. Flowers are small, greyish, funnel shaped, dioecious with perianth about 3 mm long. Each flower has 5 toothed with 6-10 stamens and filaments were connate below into a tube.

Histological characters

Preparation of specimen

The sample was fixed in FAE (Formalin - 5 ml+ Acetic acid – 5 ml + 70 % Ethyl alcohol – 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol[5]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 ^{o} c) until TBA solution attained super saturation. The specimens were cast into paraffin blocks [5].

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Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the section was 10-12 µm. De waxing of the sections was by customary procedure [6]. The sections were stained with toluidine blue, since toluidine blue is a polychromatic stain [7]. The staining results were remarkably good and some phytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary, sections were also stained with Safranin and fast green and IKI (for starch). Powdered materials were cleared with NaOH and mounted in glycerin medium after staining. Different cell component were studied and measured. For studying the stomatal morphology, venation pattern and trichome distribution, paradermal Section as well as clearing of leaf with 5% sodium hydroxide and epidermal peeling by partial Maceration employing Jaffrey' maceration were performed. Gycerine mounted temporary preparation was made for macerated material.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab photo 2 Microscopic Unit. For normal observation, bright field was used. For the study of crystals starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.

As a part of quantitative microscopy stomatal number, stomatal index and vein islet number were determined for the fresh leaves of the plant. The total ash, water soluble ash , acid insoluble ash and extractive values for various solvents and loss on drying were determined[8].The dried powdered material of the leaves were also subjected to various chemical test for identification of phytoconstituents [9].

RESULTS AND DISCUSSION

The leaf has thick midrib with fairly prominent lateral vein and thin bilateral lamina [Fig-1]. The midrib is broadly semicircular with flat adaxial side. It has thin epidermal layer of small papillate epidermal cells, three to four layers of small collenchyma cells and parenchymatous ground tissue. The vascular system of the midrib consists of a wide bowl shaped outline of about nine discrete vascular bundles and one adaxial large bundle. All the vascular bundles are collateral with inner xylem and outer phloem[Fig-1]. The xylem elements are wide, angular, thick walled and somewhat diffuse in distribution. Phloem is in thick band with several clusters of sieve elements [Fig-1].The lateral veins of the leaf are Plano convex with flat adaxial side and slightly convex abaxial side. The lateral vein has adaxial and abaxial narrow bands of sub

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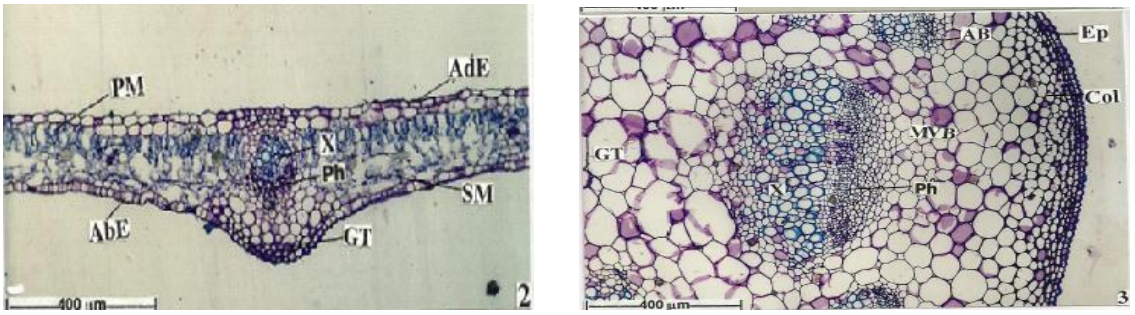
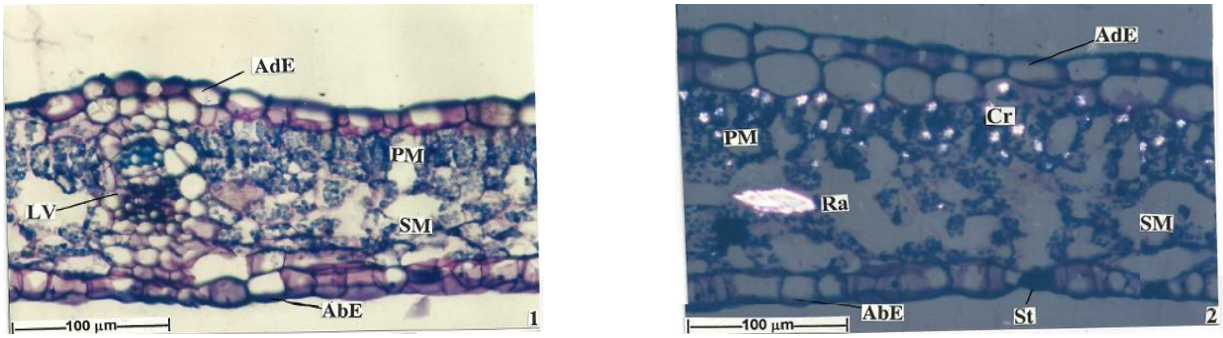
epidermal collenchyma cells and a central small collateral vascular bundle. The lateral vein is 280µm.Lamina is uniformly smooth and even on both surfaces. The lamina was 150 µm thick. It is hypostomatic on the abaxial epidermis and the adaxial epidermis was apostomatic. The adaxial and abaxial epidermal layers are equally thick with wide rectangular or tubular cells. The epidermal cells are 15 µm thick.



T.S of leaf through lateral vein with lamina Lateral vein with lamina enlarged

Figure -1 TRANSVERSE SECTION of *Pisonia grandis* LEAF

AB- Accessory bundle, AbE- Abaxial epidermis, AdE- Adaxial epidermis, AbS- Abaxial side, AdS- Adaxial side, Col- Collenchyma, EP- Ep, PM- Palisade mesophyll, SM- Spongy mesophyll, X- Xylem, Ph - Phloem , GT – Groundtissue.



T.S of lamina with lateral vein T.S of lamina under polarized light

Figure-2 ANATOMY OF THE LAMINA

AbE- Abaxial epidermis, AdE- Adaxial epidermis, AbS- Abaxial side, AdS- Adaxial side, Col- Collenchyma, PM- Palisade mesophyll, SM- Spongy mesophyll, Ra- Raphide, St- Stomata, Cr- Crystal & Lv- Lateral vein.

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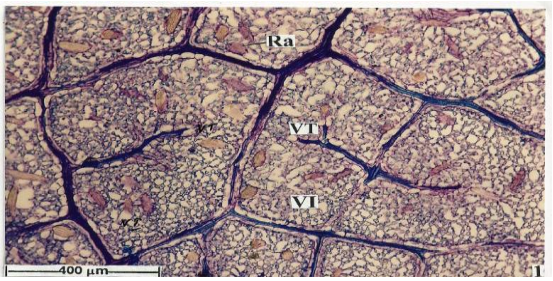
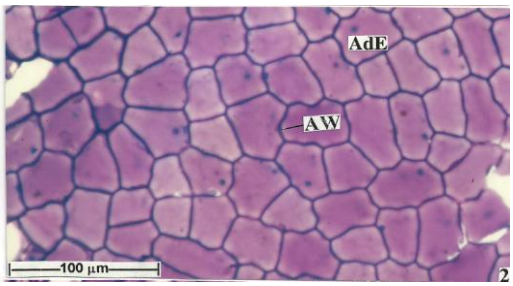
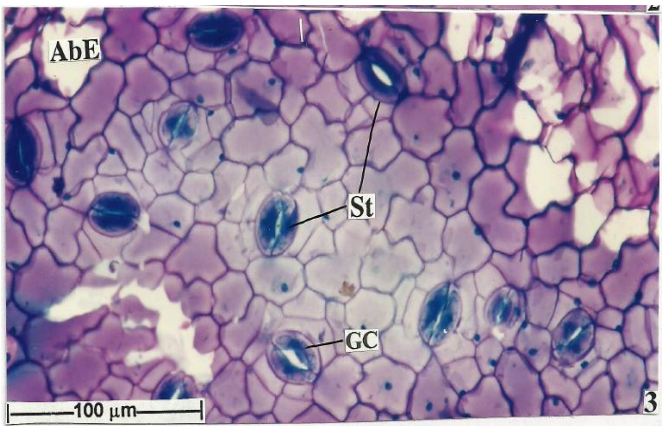


Paradermal section showing vein islets and vein termination



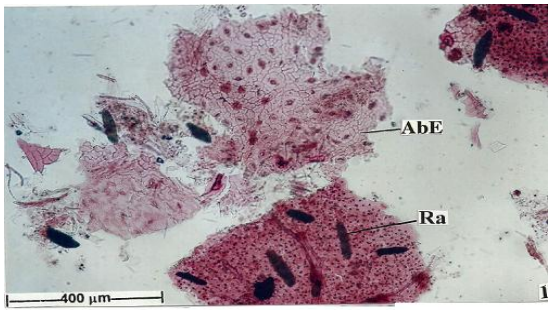
Adaxial epidermis



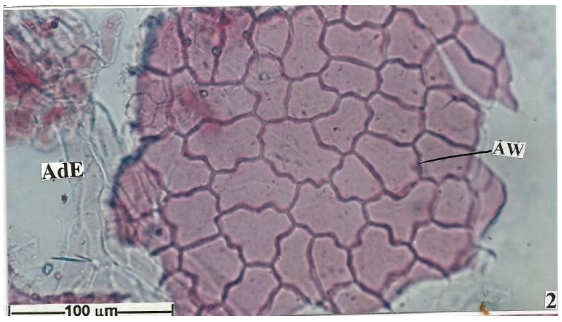
Abaxial epidermis with stomata

VI - Vein islets, VT- Vein termination , Ra – Raphides, AdX- Adaxial epidermis, AW – Anticlinalwall, S – Stomata, GC – Guardcell,

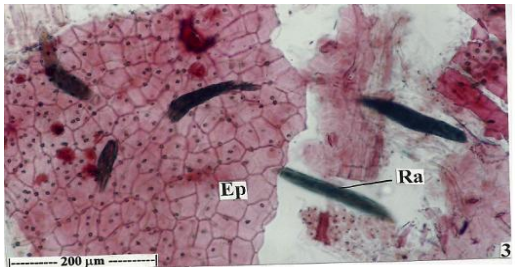
Figure-3 VENATION PATTERN AND EPIDERMAL MORPHLOGY



Fragment of abaxial epidermis with raphides



Adaxial epidermal cells



Adaxial epidermal cells with raphides enlarged

AbE- Abaxial epidermis, AdE- Adaxial epidermis, AW-Anticlinal wall, EP- Epidermis & Ra- Raphide

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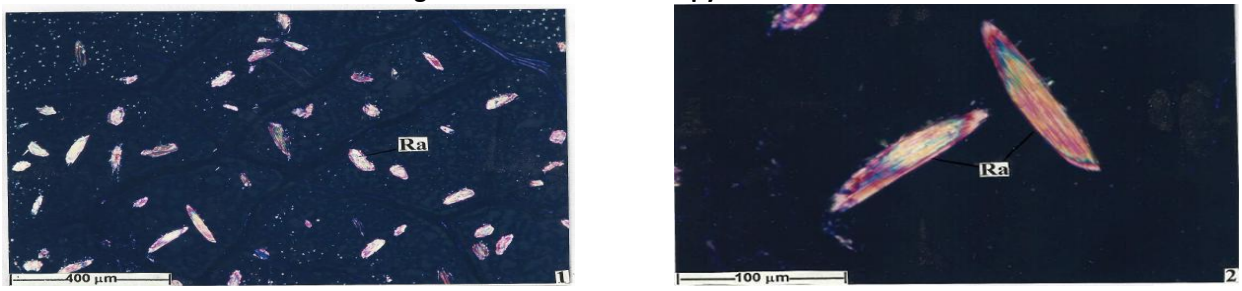
Figure-4 Powder Microscopy of the Leaf



Raphides in the mesophyll tissue

Two raphides enlarged

Ra - Raphides

Figure- 5 RAPHIDES DISTRIBUTION

The mesophyll tissues were differentiated into adaxial zone of two layers of short wide palisade cells and abaxial zone of cells, loosely arranged forming reticulate spongy tissue[Fig-2].The lateral veins are thick and straight. They form distinct vein islets and vein terminations. The vein islets are wide, rectangular and random in orientation. The vein terminations are long, thick and unbranched. They are straight or slightly bent [Fig-3].The adaxial epidermal cells as seen in surface are polygonal, random in orientation and lack stomata. Their anticlinal walls are slightly thick and straight [Fig-3]. The abaxial epidermal cells are amoeboid in outline due to wavy anticlinal walls. The walls are thin and smooth. The abaxial epidermis is stomatiferous. The stomata are broadly elliptical measuring 20x30 µm and anomocytic type. The powder microscopical studies revealed the presence of Fragmented abaxial epidermal peelings with dense stomata, which are anomocytic type (Rananculaceous) [Fig4].Adaxial epidermal peelings are wide polygonal cells with straight walls. Stomata was absent in this epidermis [Fig4]. Spindle shaped raphides are abundant in the powder which are diffuse in distribution and random in orientation. They are 120 µm long and 20 µm thick [Fig-4 & 5].Minute, star shaped calcium oxalate crystals are also seen in the leaf mesophyll.

Table-1 Analytical parameters *of Pisonia grandis* leaves

Parameters	Results(%w/w)
Stomatal number	18 - 23
Stomatal index	15.92%
Veinislet number	13 -16
Vein termination number	4 -7
Ash Values	
Total ash	5 (%w/w)
Water soluble ash	3 (%w/w)
Acid insoluble ash	1.02 (%w/w)
Extractive Values	
Water soluble extractive value	8 (%w/w)
Alcohol soluble extractive value	16 (%w/w)
Chloroform soluble extractive value	16 (%w/w)
Loss on drying	2.5 (%w/w)

Total ash value, water soluble ash ,acid insoluble ash and extractive values for various solvents and loss on drying of powdered leaves are given in the **Table -1.**The qualitative chemical test reveals the presence of favonoids, steroids and phenolic compounds in the leaf powder of *Pisonia grandis* R.Br.

CONCLUSION

The pharmacognostical investigation on the leaves of *Pisonia grandis* R.Br has been carried out for the first time. These parameters could serve in the identification and preparation of a monograph on this medicinal plant. This presented data also provides a set of anatomical features and physio-chemical parameters, which may helpful to those who involve in identifying this plant to maintain its quality control.

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