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Morpho - Playnological study on *Ficus religiosa* L. Leaves

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Abstract

Ficus is the Latin word for 'Fig', the fruit of the tree. '*religiosa*' refers to 'religion'. *Ficus religiosa* is large glabrous tree, belonging to Moraceae family, it is well known for curing a variety of ailments such as diarrhea, dysentery, vaginal disorder, eczema, leprosy, rheumatism etc. The present study was undertaken to investigate the Pharmacognostical parameters of *Ficus religiosa*. Linn leaves, macroscopical and microscopical characters were evaluated as per standard method. Morphological features of *Ficus religiosa* leaves, shape is ovate rotund, apex is caudate – acuminate, surface is glabrous shiny and coriaceous, stipules are small, sheathing, ovate – lanceolate, acute and caducous, venation is pinnate reticulate. Microscopical characters showed the presence epidermis, collenchyma, parenchyma, cystolith, prismatic crystals, stomata, spirals vessels etc. In this research article cystolith is investigated for the first time in *Ficus religiosa* L. Morphology, anatomy and other pharmacognostical studies serves as good taxonomical tools for delimiting taxa.

Keywords: *Ficus religiosa* leaves, moraceae, cystolith, prismatic crystals, stomata

Introduction

An herbal drug is defined as a plant-derived product used for medicinal and health Purposes. Traditionally used herbal drug preparations include a wide spectrum of natural herbal remedial products ranging from herbal tea to medicinal products, including cosmetics that are approved by national regulatory bodies [1]. World Health Organization recommended, over 80 percent of the world population relies on the traditional system of medicine, largely plant based, to meet their primary health care [2].

Ficus is the Latin word for 'Fig', the fruit of the tree. '*religiosa*' refers to 'religion'. *Ficus religiosa* is large glabrous tree, belonging to Moraceae family [3]. In the United States, it is grown in southern California, Florida, and Hawaii. *Ficus religiosa* generally used for the treatment of asthma, cough, sexual disorders, diarrhoea, haematuria, toothache migraine, gastric problems etc. [3] The leaf of *Ficus religiosa* may can be easily adulterated with inferior materias. This adulteration can be prevented by means of various evaluation parameters like macroscopic study. Microscopy is an important tool for authentication of crude drugs and study of powdered drugs. The present study of this plant was carried out the pharmacognostical parameters to identify the identical characteristics were mentioned in this article.

Taxonomy

- Domain : Eukaryota
- Kingdom : Plantae
- Subkingdom : Viridiaeplantae
- Phylum : Tracheophyta
- Subphylum : Euphyllophytina
- Class : Magnoliopsida
- Subclass : Dilleniidae
- Order : Urticales
- Family : Moraceae
- Tribe : Ficeae
- Genus : *Ficus*
- Specific epithet : *Religiosa* Linnaeus
- Botanical name : *Ficus religiosa*

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Synonyms

- *Ficus caudate* Stokes
- *Ficus peepul* Griff
- *Ficus religiosa* var. *Cordata* Mig
- *Ficus religiosa* var. *Rhynchophylla* mig.
- *Ficus rhynchophylla* steud.
- *Ficus superstiosa* link
- *Urostigma affine* mq
- *Urostigma religiosum* (l). Gasp

Material methods

Authentication

Ficus religiosa leaves was authenticated by Dr. D. Stephen Ph. D, Department of Botany, The American College, Madurai.

Collection of plant

The leaves of *Ficus religiosa* belonging to the family Moraceae were collected from in and around Madurai. Then the collected leaves were dried under shade finely powdered and passed through the Sieve no: 40.

Chemical and instruments

Sample was preserved in fixative FAA for more than 48 hr. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained ⁽¹⁰⁾. Transverse sections were photographed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss Axio Cam Erc5s digital camera under bright field light. Magnifications were indicated by scale bar.

Macroscopical evaluation ^[4]

Leaves are studied separately for its morphological characters by organoleptic test like colour, odour, size, shape and taste and other foliar morphological characters. Morphological examination and characterization of medicinal plants have always been accorded due credentials in the pharmacognostical studies.

Microscopical evaluation ^[5, 11]

Quantitative Microscopy of *Ficus religiosa* Linn

The fresh *Ficus religiosa* leaf samples were boiled with 0.1% cholral hydrate solution and slides prepared. Vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio were determined.

Stomatal Index

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

$$\text{Stomatal index} = \frac{S}{S+E} \times 100$$

Where, S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Determination of Stomatal Index

The procedure adopted in the determinations of stomatal number was observed under high power (45 X). Then counted the epidermal cells and stomata. From these values the stomatal index was calculated using the above formula.

Determination of Vein Islet Number and Vein Termination Number

It is the number of vein islets per sq. mm of the *Ficus religiosa* leaf surface midway between midrib and margin.

The vein islet numbers are used to distinguish closely related plants. The number of vein islets mm⁻² calculated from four attached square millimetres in the central part of the lamina, midway between the midrib and margin is termed vein islet number, when determined on whole leaves the area examined should be from central part of lamina midway between margin and midrib. Vein termination number is defined as number of vein termination present in 1 sq mm area of photosynthetic tissue.

Powder Microscopy

A pinch of the powdered *Ficus religiosa* sample was mounted on a microscopic slide with a drop of 50% glycerol. Characters were observed using Nikon ECLIPSE E200 trinocular microscope attached with Zeiss ERC5s digital camera under bright field light. Photomicrographs of diagnostic characters were captured and documented.

Results and discussion

Morphological studies of leaves

A large glabrous, deciduous, usually epiphytic tree, without aerial roots; bark fairly smooth, pale grey. Leaves alternate, leathery, tip narrowed into a long tail and half the length of the blade, rounded at the base, blade 4 – 7 in long. Margins entire, undulate shining and not tubercled above, paler and minutely tubercled beneath when dry; young foliage flushed with pink ^[6] (Fig 1).

The macroscopical description of the plant parts which was carried out by a naked eye placing the plant material on a white paper surface. Organoleptic features such as shape, size, colour, odour, taste of *Ficus religiosa* L. leaves were evaluated.

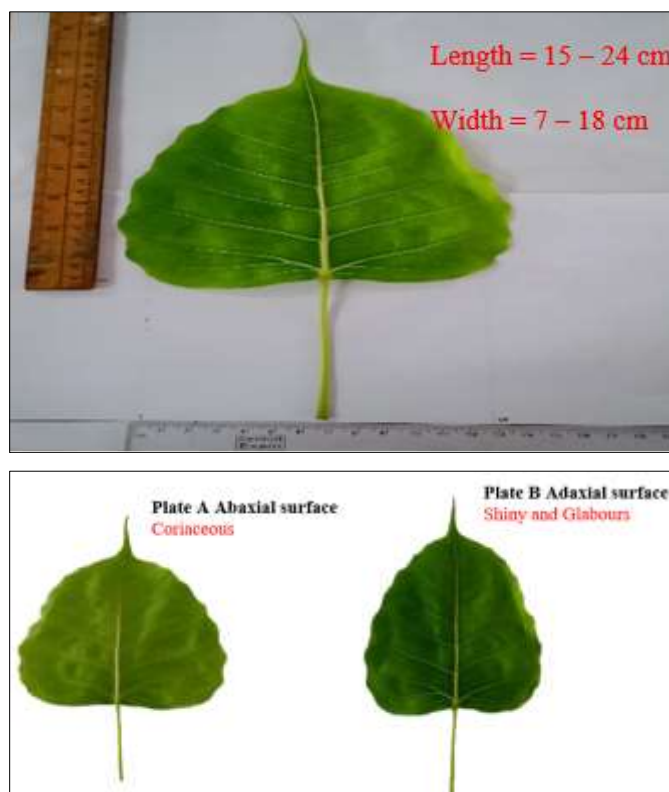


Fig 1: Leaf Size of *Ficus religiosa*

Result

The research of our study many interesting and morphological features of *Ficus religiosa* leaves, shape is ovate rotund, apex is caudate – acuminate, surface is glabrous shiny and coriaceous, stipules are small, sheathing, ovate –

lanceolate, acute and caducous, venation is pinnate reticulate. Critical examination and observation of study differentiated

religiosa species from other species of genus *Ficus* (Tab 1).

Table 1: Macroscopical characters of *Ficus religiosa* LINN Leaf

S. No	Morphological characters	Obesrvation
1.	Colour	Mature Leaf – Deep Green Tender Leaf – Rose To Light Red
2.	Duration	Evergreen
3.	Odour	Odourless
4.	Shape	Ovate-rotund
5.	Petiole colour size	Brownish yellow(dried) 7 – 12cm
6.	Taste	Not significant
7.	Apex	Caudate-acuminate
8.	Base	Cordate
9.	Composition	Simple
10.	Leaf arrangement	Alternate, Narrow upward
11.	Venation	Pinnate – Reticulate
12.	Lamina (colour)	Deltoid (dark green in fresh, dark brown in dried)
13.	Size	Length – 15 - 24cm Width – 7 – 18 cm Petiole – 2 – 5 inches
14.	Margin	Entire and slightly undulate (wavy)
15.	Texture	Thin, papery, smooth
16.	Petiole	Petioloated
17.	Surface	Adaxial – glabrous, shiny Abaxial – coriaceous
18.	Stipules	Small, sheathing, ovate – lanceolate, acute and caduceus

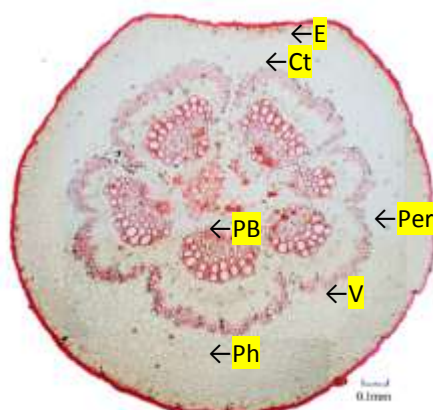
Microscopical study ^[7]

Foliar anatomical studies have shown that foliar characters too are strictly comparable over a wide taxonomic range, to those of foliar organs and so quite reliable. It plays an important role in identification of incomplete plants. Foliar anatomical study of *Ficus religiosa* leaves will reveal many important anatomical characters of petiole and lamina were listed below.

TS of Petiole

TS of petiole shows flat or slightly concave on the adaxial side and rounded convexity on the abaxial side (Fig 2). Epidermis (E) is single layered and covered by cuticle, it is Squarish shape. Walls of petiole in inner side is straight and outer side is arched. Hypodermal layers on adaxial surface is 2-3, abaxial surface is 2-3 (Fig 2a). Cork arising near the outermost layer of the cortex. Primary cortex becoming extensively sclerosed, containing a ring of stone cells. Cortex

(Ct) consists of 6 to 7 layers of loosely arranged collenchyma cells followed by 5-6 layers of thin walled parenchyma cells (Fig 2b). Pericycle is usually marked by separate strands of fibres which are frequently unligified. Pericycle (Per) including isolated strands of fibres, individual elements often being broad and flattened as seen in transverse sections. (Fig 2c). Vascular bundle is complete closed cylinder. Almost closed cylinder of xylem accompanied externally by numerous phloem groups and, in abaxial part of the pith, by others small phloem strands, a few of the latter being accompanied by a small amount of xylem. Veins (Ve) very diverse in structure. Solereder records a circle of bundles in the petiole. The inner region contains a cylinder of separate to partially merged vascular bundles, each with a fibre cap. Small vascular strands are present at the centre (Fig 2d). Pith (PB) commonly rather wide, usually composed of thin walled cells, but peripheral part sometimes consisting of cells with thicker walls than those at the centre (Fig 2 f) (Tab 2).



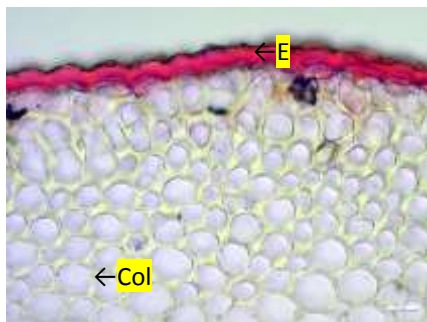


Fig 2 a: Adaxial portion

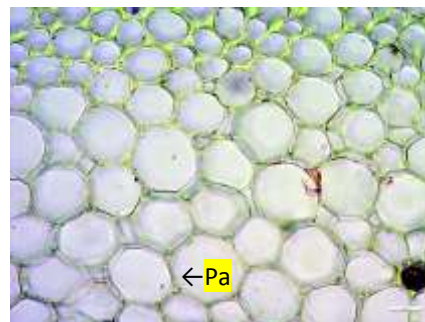


Fig 2 b: Cortex

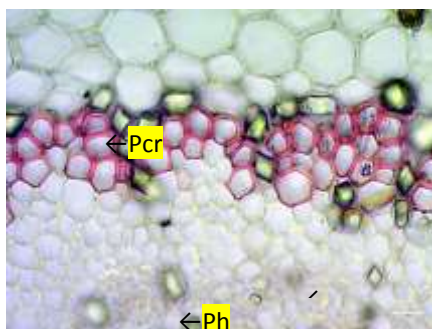


Fig 2 c: Pericycle and phloem

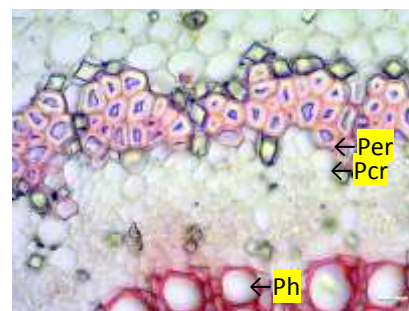


Fig 2d: Vascular bundle

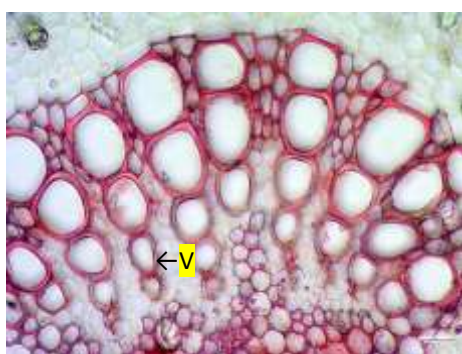


Fig 2 e: Vessels

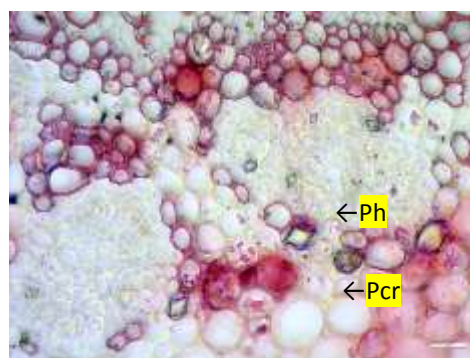


Fig 2f: Pith Bundle

E - Epidermis; **Col** - Collenchyma; **Ct** - Cortex; **Pa** - Parenchyma; **PB** - Pith bundle; **Pcr** - Prismatic crystals; **Per** - Pericycle; **Ph** - Phloem; **V** - Vessel; **Xy** - Xylem.

Fig 2: TS of petiole of *Ficus religiosa*

Foliar anatomy of lamina through midrib

Epidermis

Transverse section of *Ficus religiosa* is dorsiventral surface view of leaf, is elongated jar or eye drawn, anticlinal walls of adaxial epidermis (UE) and abaxial epidermis (LE) was characterized as straight to little sinuate. Epidermis (E) shape is polygonal. The upper or adaxial epidermis is formed of tangentially elongated multiseriate tubular cells covered by a thin cuticle (Cu) layer. Adaxial layer epidermis is 37.5 micrometer. Abaxial epidermis is uniseriate and the abaxial epidermis the length measurements reached to 44.5 micrometer as the highest range in *Ficus religiosa* and width measurements reached to 43 micrometer.

Adaxial Region of Midrib

At the midrib region 5 – 6 layer of loosely arranged collenchyma (Col) cells followed by 3 – 4 later of parenchyma cells at present below the epidermis pericycle (Per) are found below the parenchymatous cells (Fig 3a).

Abaxial Region of Midrib

In lower region, ground tissue is occupied by partially merged two opposed arcs of vascular bundles covered by a continuous sclerenchymatous bundle sheath. Prismatic crystals (**Pcr**) are found distributed in the periphery of bundle sheath. Pericycle is usually marked by separate strands of fibres which are frequently unligified. Pericycle including isolated strands of fibres, individual elements often being broad and flattened as seen in transverse sections (Fig 3e).

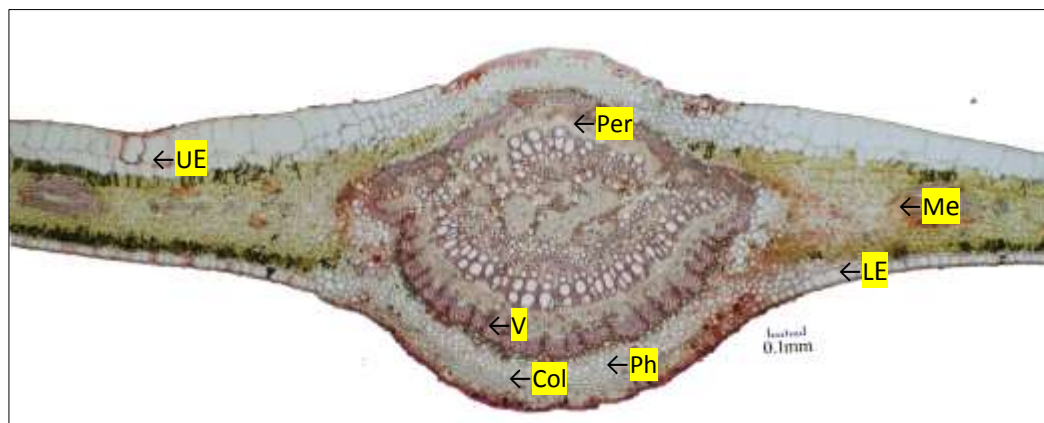
Vascular Bundle

Several layers of continuous sclerenchymatous bundle sheath around the vascular bundles. Prismatic crystals are found distributed in the periphery of bundle sheath, (Fig 3c) parenchyma (Pa) of midrib and in phloem (Ph) parenchyma, vascular bundles are collateral and closed with xylem (Xy) lying towards inner side and phloem towards outer side, central pith portion also contains phloem and xylem strands forming pith bundles. Outer part of the wall of the phloem fibres appearing as an envelope around the inner layers (Fig 3b).

Lamina

Transverse section of lamina shows well differentiated mesophyll tissue 2 – 4 layers of columnar cells with abundant chloroplast forms the palisade layer below upper epidermis. Mesophyll (Me) layer consists of palisade and spongy layer, the type of mesophyll (Me) in the ficus religiosa is isobilateral mesophyll i.e differentiated into upper and lower palisade (Pal) parenchyma with spongy parenchyma (sp) between them and less chloroplasts compared to palisade cells. The spongy layer were consisted 3 -5 rows of ordinary parenchyma cells, thickness of spongy parenchyma is 97.5 micrometer as

highest rate. Minor veins (Ve) are embedded in the mesophyll. Stomata (St) shape was anomocytic type uniformly hypostomatic and the stomata surrounded by six subsidiary cells and also the guard cells have reniform (Fig – 3h). Cystolith (Cys) is present spongy parenchyma cells (Fig – 3f). Solitary of epidermal cells, each containing a single clusters crystals, recorded, especially in the neighbourhood of the vein. Cystoliths present at abaxial surface of lamina, especially in the epidermis on or both surface of the leaf (Fig – 3f) (Tab. 2).



TS of Leaf

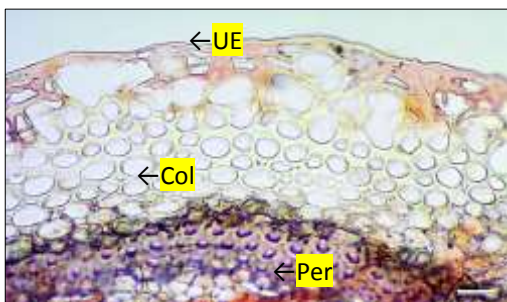


Fig 3 a: Adaxial region of midrib

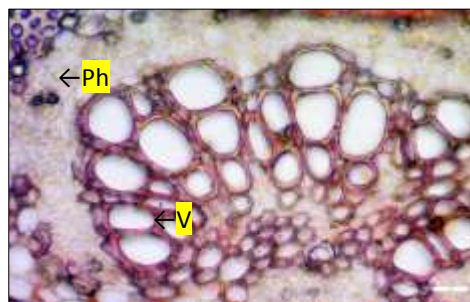


Fig 3 b: Vascular bundle

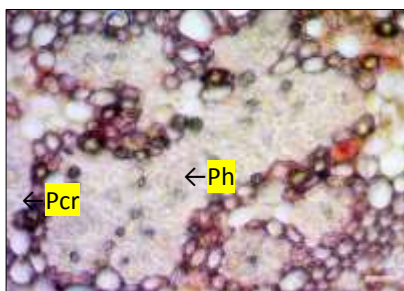


Fig 3 c: Phloem with prismatic crystals

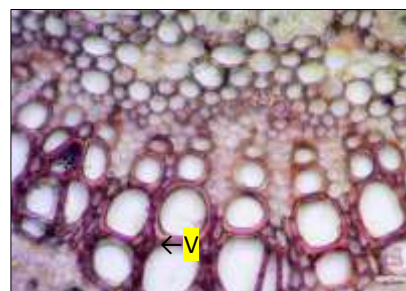
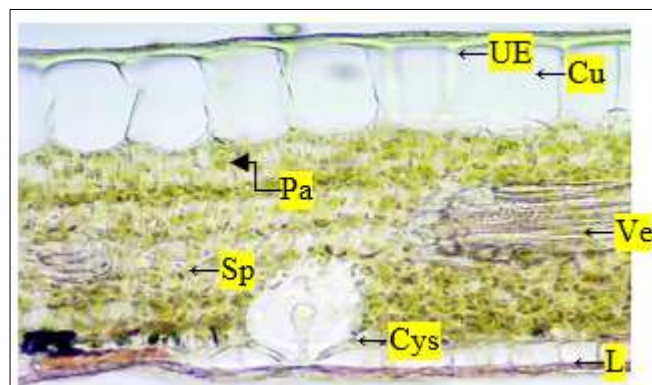


Fig 3 d: Vascular bundle at lower side of midrib

Fig 3: TS of lamina through midrib of *Ficus religiosa*



Col - Collenchyma; Cu – Cuticle; Cys - Cystolith; LE - Lower Epidermis; Me - Mesophyll Cells; Pa - Parenchyma; Pal - Palisade; Pcr - Prismatic Crystals; Per - Pericycle; Ph -Phloem; Sp - Spongy Parenchyma; St - Stomata; UE - Upper Epidermis; V- Vessel; Ve - Vein.

Fig 3: f - TS of Lamina

Table 2: Foliar anatomy of *Ficus religiosa* L

S. No	Microscopical characters	<i>Ficus religiosa</i> Linn
1.	Lamina	<ul style="list-style-type: none"> Length is 44.5 micrometer, it has highest range in <i>Ficus</i> genus <ul style="list-style-type: none"> Isobilateral mesophyll Hypostomatic, shape is anomocytic Cystolith is present at abaxial layer
2.	Petiole	<ul style="list-style-type: none"> Vascular bundle is complete closed cylinder Pericycle are makered by separate strands of fibre which is unligified. <ul style="list-style-type: none"> Epidermis is Squarish shape

Result

The study of foliar anatomy of *Ficus religiosa* leaves revealed a number of important anatomical characters and these character exhibit interesting interspecific variation that are of taxonomically significant. The adaxial epidermis length 44.5 micrometer, it has highest range in ficus genus. It has consisting 5 – 6 layer of loosely arrangd collenchyma cells followed by 3 – 4 later of parenchyma cells isobilateral mesophyll, prismatic crystals near bundle sheath, cystolith present at abaxial epidermis, hypostomatic anomocytic type.

Discussion

The study conferred to presence of cystoliths, lithocysts, hypostomatic anomocytic type at abaxial epidermis [7]. Trichomes are absent in *Ficus religiosa*. Laticifer, prismatic crystals are interspecific and identification for the

taxonomical classification and identification for the species *religiosa*. Cystoliths is special identical character in my study because in previous article [9] they reported only the *Ficus carica* and *Ficus elastic* have cystolith crystals in certain species of *Ficus* genus. Finally I conclude *Ficus religiosa* also having cystolith.

Quantitative microscopy [12]

The fresh leaf samples were boiled with 0.1% cholral hydrate solution and slides prepared. Vein islets, vein termination, epidermal number, stomatal number, stomatal index and palisade ratio were determined. The quantitative parameters obtained during microscopic observation of epidermal peelings of leaf were recorded in the table below. The leaf showed anomocytic stomata on abaxial sider with numerous of cystoliths.

Table 3: Quantitative Microscopy of *Ficus religiosa* L. Leaf

Parameters	Lower epidermis MEAN ± SEM (%W/W)	Upper epidermis MEAN ± SEM (%W/W)
Epidermal number	1062 ± 89.69	573 ± 6.67
Stomatal number	177.6 ± 5.92	-
Stomatal index	15 ± 0.57	-
Palisade ratio	66.33 ± 4.9	
Vein islets number	122.67 ± 2.60	
Vein termination number	153.33 ± 3.17	

Result

The results predict that the number of epidermal cells in the adaxial and abaxial layer are 573 ± 6.67 and 1062 ± 89.69. Stomatal number in the abaxial surface is 177.6±5.92, stomatal index of abaxial surface is 15±0.57. Palisade ratio present at lamina region is about 66.33±4.9. Vein islets number is 122.67±2.60 and vein termination number is 153.33±3.17 (Table 3).

Discussion

Based on study the *Ficus religiosa* is hypostomatic leaves the stomata is anomocytic stomata having a numerous number of cystoliths on abaxial surface. The presence of palisade parenchyma is restricted to the adaxial surface which is the evidence for bifacial mesophyll [9]. Based on the previous investigational reports and present observation, the quantitative constants were predicted significantly and they

are considered as valued taxonomical facts of *Ficus religiosa* leaves.

Powder microscopy

The powder shows characters like upper and lower epidermal cells in surface view, anomocytic stomata, palisade and

spongy cells, parenchyma and collenchyma cells, reticulate, pitted and spiral vessels, thick walled fibre, crystal fibre, prismatic crystals of calcium oxalate and cystolith. The powder is light green coloured with characteristic odour and taste (Fig 4).

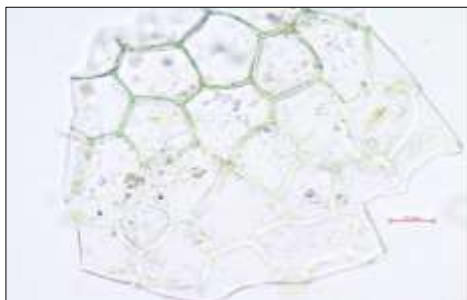


Fig 4 a: Surface view of adaxial epidermis



Fig 4 b: Surface view of Abaxial epidermis with anomocytic stomata



Fig 4 c: Reticulate and pitted vessels

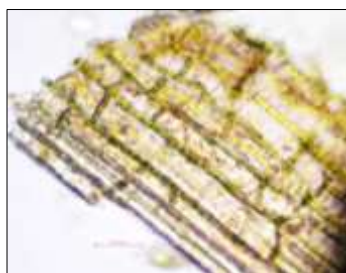


Fig 4 d: Pitted vessels



Fig 4 e: Spiral vessels



Fig 4 f: Crystal fibre



Fig 4 g: Fibre and pitted vessel

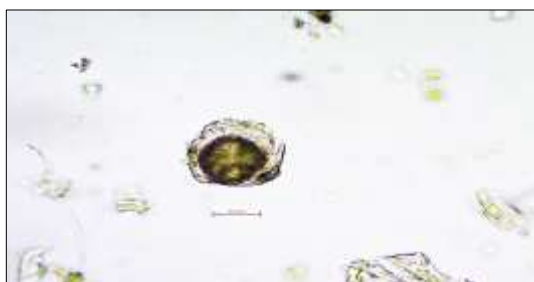


Fig 4 h: Cystolith



Fig 4 i: Prismatic crystals

Fig 4: Powder microscopy of *Ficus religiosa* Leaf

Result

The adaxial epidermis length 44.5 micrometer, it has highest range in *Ficus* genus. It has consisting 5 – 6 layer of loosely arranged collenchyma cells followed by 3 – 4 layer of parenchyma cells isobilateral mesophyll, prismatic crystals near bundle sheath, cystolith present at abaxial epidermis, hypostomatic, anomocytic type. Venation is pinnate reticulate.

Discussion

Critical analysis of *Ficus religiosa* powder reveals the following character. Cystolith is investigated for the first time in *Ficus religiosa*. For anatomy characters that might be useful for delimiting the taxa of this species at global level.

General Pharmacognostical Discussion

Pharmacognosy is an important monograph index of therapeutical important medicinal plants. For future reference researchers and pharmaceutical industries and also evidence based reference and guidance for the future scope of research, development and formulation for pharmaceutical industry. Moraceae family of dicotyledon plant containing 1500 species in 53 genera distributed mainly in warm regions of the world. This family includes a genus *Ficus*, represented about 700 species and one of largest genera of higher plants^[8]. Many *Ficus* species consists of numerous varieties of genetic diversity, outstanding therapeutical effect that are of remarkable commercial importance, it has been widely used to cure the disorders and diseases traditionally. Morphology, anatomy and other pharmacognostical studies serves as good taxonomical tools for delimiting taxa. The study explore the unique features of *Ficus religiosa* for identification plant from the numerous varieties of genus *Ficus*.

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