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PHARMACOGNOSTICAL PHYSICOCHEMICAL AND PHYTOCHEMICAL INVESTIGATION ON LEAVES AND STEM BARK OF *FICUS DALHOUSIAE* MIQ. (MORACEAE)

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ABSTRACT

Ficus dalhousiae Miq.(Family:Moraceae) known as Somalvalkam in Sanskrit is an endemic to peninsular India and very rare plant of Andhra Pradesh. It is found growing in rock crevices of dry deciduous forest. It is used in traditional system of Indian medicine in the treatment of liver disorders and cardiac problems. Ficus species are rich source of flavonoids and polyphenolic compounds that are responsible for the treatment of oxidative stress related diseases. As there are no reports available on the morphology and phytochemical studies of the leaves and stem bark of *Ficus dalhousiae,* the present investigation was carried out to lay down the standards, which could be very useful in the future experimental studies. The study includes macroscopy, microscopy, preliminary phytochemical screening, fluorescence analysis and physicochemical constants. The chief features of the transverse section of leaves are the presence of single layered palisade layer, cyclic stomata, druses of calcium oxalate and vascular bundles. Stem bark shows prismatic calcium oxalate, high tannin content in periderm, abundant starch. The main powder characters are calcium oxalate, unicellular covering trichomes, isolated cystoliths, laticifers. The preliminary phytochemical screening shows the presence of steroids, cardiac glycosides, alkaloids, flavonoids, tannins and carbohydrates. This study along with the quantitative microscopic data can be useful in the detection and evaluation of the leaf and stem bark material in any form or formulations of this rare and endemic species.

KEYWORDS: ***Ficus dalhousiae*, Cyclocytic stomata, Flavonoids, Chemomicroscopical study, Ash values**

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INTRODUCTION

Plants with healing properties have been known for thousands of years before and have been used as traditional medicines by the people to treat various diseases. Throughout history and prehistory, plant derived medicines have been played a chief role in treating human society[1]. The WHO estimates about 80% of the world population still rely on traditional system of medicine, for their primary health care, because drugs of synthetic origin have more side effects, and of high cost. In spite of the great advances of modern scientific medicine, traditional medicine is still the primary form of treating diseases of majority of people in developing countries including India. The number of people following one form or another of complementary or alternative system of medicine has been rapidly increasing worldwide. In such situation, proper identification and quality assurance of the starting materials is an important prerequisite to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy[2]. Pharmacognostical study is the preliminary step in the standardization of crude drugs, which is a simple and reliable method. In general medicinal plants are sold as fragments or powders; hence a detailed pharmacognostical evaluation gives valuable information regarding the morphology, microscopic and physical characters of the crude drug[3]. *Ficus dalhousiae* Miq. belongs to the family Moraceae. It is endemic, rare plant of peninsular India. It is found in Nilgiri mountains Tamil Nadu, Kerala and AndhraPradesh[4,5,6,7]. In Sanskrit it is known as Somavalkhom, Pei aal or Kal aal in Tamil and Peddakulamarri in Telugu. This plant was named as *Urostigma dalhousiae* and then renamed as *Ficus dalhousiae* by Miquel. The leaves and the stem bark of the plant are used in liver and skin diseases[8]. Bark paste is used in the treatment of leprosy. Fruits are used as cardio tonic[9]. Considering the medicinal uses and lack of availability of data on the morphology and microscopy of this endemic and rare species of *Ficus dalhousiae,* the goal of the present work was to carry out the macroscopy, microscopic, physicochemical and phytochemical parameters of the leaves and stem bark of the species as a contribution to the pharmacognostical studies of the species of *Ficus.*

MATERIALS AND METHODS

Collection and authentification of leaves and stem bark

Leaves and stem bark of *Ficus dalhousiae* were collected from Tirupathi hills, AndhraPradesh, India, during the month of May 2013. The plant was authenticated by Dr.P.Jayaram at the Plant Anatomical Research Centre (PARC), Tambaram, Tamil Nadu. Herbarium and voucher specimen were prepared and deposited in the Department of Pharmacognosy, Ratnam Institute of Pharmacy, Nellore, Andhra Pradesh.

Pharmacognostical studies

Morphology of the leaves and stem bark of *Ficus dalhousiae* was studied. Photomicrography of the stained transverse section of the fresh leaves and stem bark was done as per the standard procedures[10,11,12]. Leaf constants were studied with the help of camera lucida, length and width of phloem fibres and trichomes were measured[13]. The leaves and stem bark were dried under shade and coarsely powdered, sieved in no.60 sieve and stored in airtight containers and used for powder microscopy, physicochemical studies and for extraction.

Chemomicroscopical examination

The transverse section of the leaf and stem bark were treated with various chemical reagents to detect the presence of chemical constituents such as lignin, starch, tannins, calcium oxalate and oil globules[14,15].

Physicochemical analysis

Percentage of ash values, extractive values, crude fibre content and loss on drying were performed according to the official methods prescribed in Indian Pharmacopoeia,1966[16,17]. Average of three determinations for each procedure was calculated. Fluorescence Analysis was studied at day light and UV light as per the standard procedure[18,19].

Preliminary phytochemical screening

The powdered plant material was subjected to successive solvent extraction using Petroleum ether, Chloroform, Ethyl acetate and Ethanol. The preliminary phytochemical screening of different extracts was carried out using various reagents as per the standard procedure[20].

RESULTS AND DISCUSSION

Botanical description

Taxonomy

Taxonomy of the plant is given in Table-1.

Table 1
Taxonomy of Ficus dalhousiae Miq.

Domain	**Eukaryota**
Kingdom	Plantae
Subkingdom	Viridaeplantae
Phyllum	Magnoliophyta
Subphyllum	Euphyllophytina
Class	Equisetopsida
Subclass	Rosidae
Order	Rosales
Family	Moraceae
Tribe	Ficeae
Genus	*Ficus*
Specific epithet	*Dalhousiae*-Miq.
Botanical Name	*Ficus dalhousiae*

Morphology

Leaves are simple(Fig.1), dorsiventral, 30X20cm, ovate- elliptic to broadly ovate, pubscent below, apex shortly acuminate, base more or less deep cordate, entire margin, reticulate venation, veins are prominent below, 10-14 pairs, petioles upto 10cm long. Receptacles are in pairs, pubescent, obovoid, shortly peduncled, with three apical scales and three bifid basal bracts. Stem bark (Fig.1) are flat or slightly curved, 6 to 8 mm thickness, outer surfaces ash or grey colour with a thin coat or membranous flakes, covered with lichen, brown or ash colour. Surface has irregular fissures and uneven. Inner surface is smooth and fibrous. (Table-2)

Figure 1
Leaves and Stem bark of Ficus dalhousiae Miq.

Table 2
Morphology of Leaves and Stem bark of Ficus dalhousiae Miq.

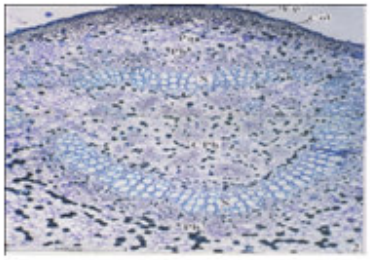
Characters	Leaves	Stem bark
Colour	Green	Outer –Greyish brown Inner – Light Brown
Odour	Slight Astringent	Woody, slight astringent taste
Taste	Characteristic astringent	Slight astringent
Size	30x20 cm	6 to 8 mm thickness
Shape	Ovate- elliptic to broadly ovate	Flat or slightly curved
Surface characters	Pubescent below	Cracked surface with irregular fissures

Microscopy

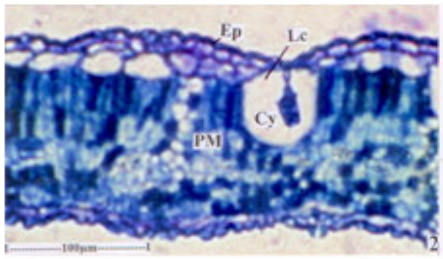
Epidermis of the leaf is composed of small squarish thick walled cells. It shows the presence of unicellular dagger shaped or straight covering trichomes. Beneath the epidermis single layer of wide elliptical hypodermal cells are present. Some of the cells get dilated, widened and circular forming lithocysts with cystolith. Palisade cells are single layered, cylindrical and compact. Mesophyll or spongy parenchyma beneath the palisade layer consist of circular, lobed, loosely arranged parenchyma cells. Lamina shows the presence of druses of calcium oxalate crystals. Midrib shows the presence of abundant covering trichomes. Collenchyma is well-developed and about ten layered. The ground tissue consists of small angular thin walled parenchymatous cells. Tannins are found in the cells of ground tissue. Vascular bundles are circular in shape. Stomata are cyclocytic type surrounded by four to eight rectangular subsidiary cells. Leaf powder contains unicellular covering trichomes, cyclocytic stomata, and isolated cystoliths. Laticifers are also found scattered in the powder. Bark shows several layers of thin walled squarish suberised phellem cells. Phelloderm is absent. Inner periderm shows dense tannin. Wide zone of collapsed phloem is observed. Dilated phloem rays, segments of phloem sclereids, inner phloem with intact sieve elements along with companion cells and parenchyma cells are seen. Spindle shaped, biseriate or multiseriate phloem fibres are found. Prismatic calcium oxalate crystals found in phloem ray cells and phloem sclerenchyma. Starch grains are abundant in phloem ray cells. Bark powder shows xylem and phloem fibres, sclereids, starch grains (Fig.2, 3, 4). In quantitative microscopy, Leaf constants such as Stomatal Number, Stomatal index, Vein islet number, Vein termination number, Palisade ratio, and length of trichome, length and width of phloem fibres were measured and reported in Table-3.

Table 3
Quantitative microscopy

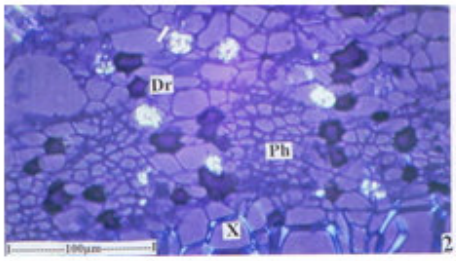
Leaf constant		Mean Value ± SD
Stomatal Number		
Upper Surface		3 ± 1
Lower Surface		7 ± 1
Stomatal index		
Upper Surface		11.11 ± 0.5
Lower Surface		16.27 ± 0.5
Vein islet number		10 ±1
Vein termination number		14±1
Length of trichome		145 µm - 190 µm - 270µm
Palisade ratio		3.5
Length and width of the phloem fibres (in µm)		
Leaf	Length	162 - 713.34 – 1350
	Width	40.5 - 77.22 – 162
Bark	Length	135 - 413.1 – 1080
	Width	27 - 43.2 - 94.5



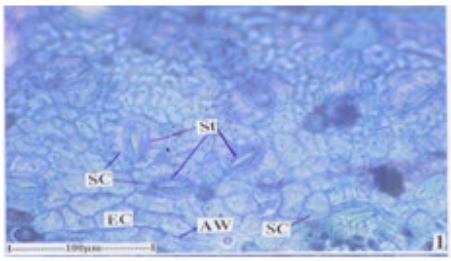
a) T.S. of leaf through midrib



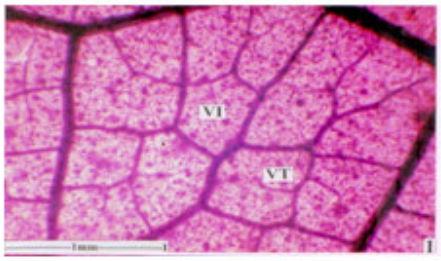
b) T.S. of Lamina showing lithocyst with cystolith



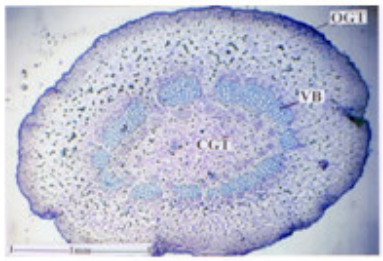
c) Calcium oxalate druses in the Phloem parenchyma



d) Cyclocytic stomata with a circle of subsidiary cells



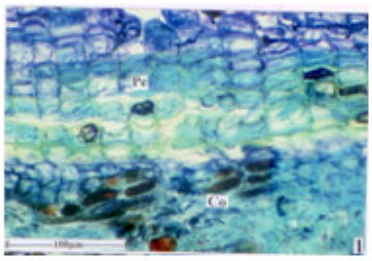
e) Venation pattern of the Lamina



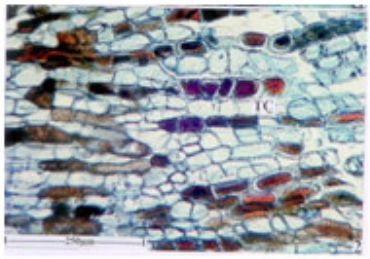
f) Cross sectional view of the petiole

Figure 2
(a-f) Microscopy of Ficus dalhousiae leaf

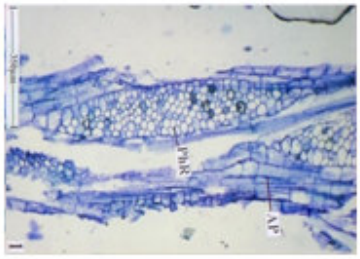
Ep – Epidermis; Col – Collenchyma; CPh – Collapsed Phloem; Pa: Parenchyma; Ph: Phloem; X – Xylem
Dr – Druses; Cy – Cystolith; PM – Palisade mesophyll; Lc – Lithocyst; St – Stomata; SC – Subsidiary cell;
EC – Epidermal cell; AW – Anticlinal wall; VI – Vein islet; VT – Vein termination;
OGT – Outer ground tissue; CGT – Central ground tissue; VB – Vascular bundle



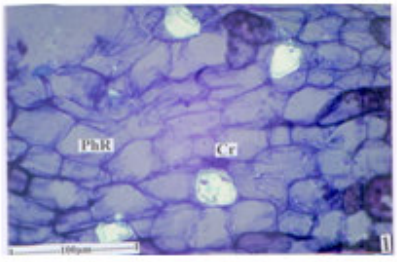
g) T.S. of the bark



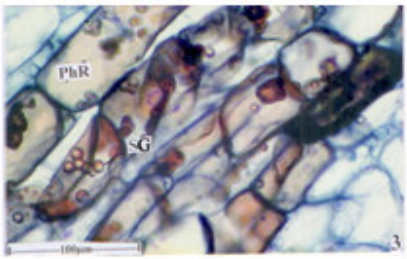
h) Phloem ray cells showing tanniferous cells



i) Multiseriate elongated phloem ray



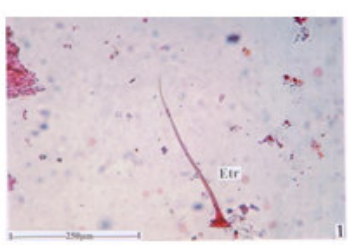
j) Calcium oxalate prismatic crystals in the phloem ray



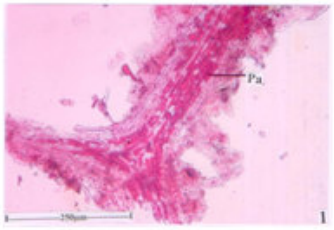
k) Starch grains found in Phloem rays

Figure 3
(g-k) Microscopy of Ficus dalhousiae bark

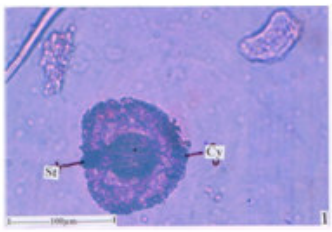
Pe – Periderm; Co – Cortex; TC – Tannin containing cell; PhR – Phloem ray; AP – Axile parenchyma; Cr – Crystals; SG – Starch grains



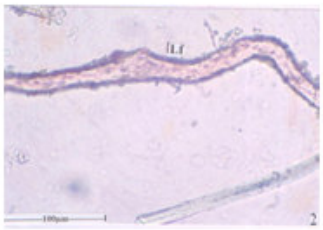
l) Epidermal trichomes



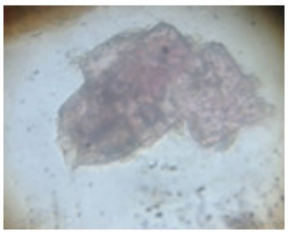
m) Parenchyma cells



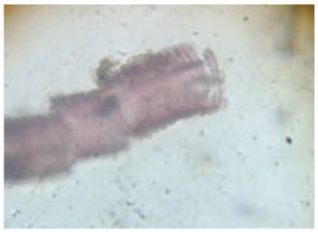
n) A cystolith



o) A laticifer



p) Sclereids



q) Xylem vessels

Figure 4
(l-q) Powder microscopy of leaf and stem bark

Physicochemical Constants

Percentage of ash values, extractive values, crude fibre content and loss on drying are given in Table-4. Alcohol soluble extractive value was found to be very high in stem bark and water soluble extractive value in leaf when compared to other extractable matter in the drug. Preliminary phytochemcial screening shows the presence of alkaloids, glycosides, coumarins, flavanoids, steroids, tannins are shown in Table-5. Presence of different organic compounds in the leaf and stem bark of the plant is confirmed by using various chemomicroscopical tests, which is tabulated in Table-6. Many herbs show fluorescence behaviour when the cut surface or powder is exposed to uv light and this can help in their identification and the results were shown in Table-7.

Table 4
Physico chemical parameters of Ficus dalhousiae Miq.

Parameter	Leaves (% w/w)	Stem Bark (% w/w)
Total ash value	12.78	12.86
Acid insoluble ash	10.20	8.36
Water soluble ash	12.92	8.16
Solvent Extractive Values		
Ethanol Soluble Extractive	11.80	20.95
Water Soluble Extractive	28.66	15.53
Ether Soluble Extractive		
a. Volatile	0.20	0.12
b. Non-Volatile	4.24	3.42
Loss on drying	2.23	2.13
Crude Fibre Content	16	30

Table 5
Preliminary phytochemical screening of leaves and stem bark of Ficus dalhousiae Miq.

Description	Leaves				Stem bark			
	PE	CHL	ETAC	ETH	PE	CHL	ETAC	ETH
Alkaloid	+	+	+	+	+	+	-	+
Glycosides	+	+	+	++	++	+	+	++
Cardiac	+	+	-	+	+	+	-	++
Carbohydrates	-	-	+	+	-	-	+	+
Reducing Sugars	-	-	+	+	-	-	+	+
Flavonoids	-	-	+	+	-	-	+	+
Steroids	++	++	-	++	++	++	-	++
Tannins	-	-	++	++	-	-	++	++
Phenols	-	-	+	+	-	-	+	+
Proteins and Amino acids	-	-	-	+	+	-	-	+
Saponins	-	-	-	-	-	-	+	+
Gums and Mucilage	-	-	-	-	-	-	-	-
Terpenoids	-	-	-	++	+	+	-	++
Resins	+	-	-	+	+	-	-	+
Chlorgenic Acid	+	-	-	-	-	-	-	-
Quinones	+	+	-	++	+	+	-	++
Anthocyanins	-	-	-	-	-	-	-	-
Diterpenes	-	-	+	+	-	-	+	+
Anthraquinone	-	-	-	-	-	-	-	-
Coumarins	+	+	-	++	+	+	-	++
Fats and Oils	+	+	-	-	+	+	-	-

Table 6
Chemomicroscopical test for Ficus dalhousiae Miq.

Test for	Reagents	Results	Colour
Starch	Iodine solution	Positive	Blue
Tannin	Aqueous ferric chloride solution	Positive	Black
Lignin	Dil. HCl and pinch of phloroglucinol	Positive	Effervescent reaction and magenta colour
Oil globules	Sudan III	Negative	No change

Table 6
Fluorescence analyses of powdered leaves and stem bark of Ficus dalhousiae Miq

Powdered crude drug + Reagent	Leaves			Stem bark		
	Day light	UV (Short) 254 nm	UV (Long) 366 nm	Day light	UV (Short) 254 nm	UV (Long) 366 nm
Powdered crude drug as such	Green	Dark Green	Dark Brown	Light Brown	Light Green	Light Brown
Drug + Water	Pale Yellow	Dark Green	Light Green	Dark Brown	Green	Dark Brown
Drug + 1M HCl	Pale Yellow	Pale Green	Pale Yellowish Fluorescence	Colourless	Pale Green	Colourless
Drug + 1M HNO_3	Light Brown	Dark Green	Yellow Fluorescence	Light Brown	Dark Green	Light Brown
Drug + 1M H_2SO_4	Light Yellow	Dark Green	Light Yellow	Light Brown	Light Green	Light Orange
Drug + 1M NaOH	Light Yellow	Dark Green	Yellow Fluorescence	Light Brown	Dark Green	Light Brown
Drug + Methanolic NaOH	Light Green	Dark Green	Orange Fluorescence	Light Brown	Dark Green	Light Yellow Fluorescence
Drug + Methanolic KOH	Olive Green	Dark Green	Cream Colour	Brown	Dark Green	Brown
Drug + dil. Ammonia	Pale Yellow	Light Yellowish Green	Light Green Fluorescence	Light Brown	Olive Green	Light Green
Drug + Acetic Acid	Light Brown	Dark Green	Dark Brown	Light Yellow	Light Green	Pale Yellow
Drug + 5% Iodine	Reddish Brown	Yellowish Green	Reddish Brown	Dark Brown	Greenish Brown	Dark Brown
Drug + 5% $FeCl_3$	Yellowish Green	Dark Green	Dark Green	Yellowish Brown	Dark Green	Dark Brown
Drug + Methanol	Light Green	Light Green	Orange Fluorescence	Dark Brown	Dark Green	Light Orange
Drug + KOH	Orange Yellow	Dark Green	Green Fluorescence	Dark Brown	Dark Green	Dark Brown
Drug + Conc. HNO_3	Light Brown	Greenish Yellow	Reddish Brown	Brown	Yellowish Green	Reddish Brown
Drug + $K_2Cr_2O_7$	Greenish Brown	Dark Green	Reddish Brown	Yellowish Brown	Greenish Brown	Reddish Brown

CONCLUSION

Standardization is important for herbal drugs to establish the identity, quality, purity and safety. The pharmacognostical standards for leaves and stem bark were laid down for the first time in this study. Morphology and anatomical studies of the leaves and stem bark will enable to identify the correct crude drug and also to find the closely related species. Ash values, extractive values, moisture content, crude fibre content are reliable and simple physical methods for detecting the adulteration. The preliminary phytochemical screening indicates the presence of active constituents that will be helpful in finding the genuinity of the drug. Further studies should be needed to carry out the isolation of the chemical constituents and detailed pharmacological activities in suitable scientific way.

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