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Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON LEAVES OF *FICUS MOLLIS* VAHL

RAVICHANDRA V D[1], PADMAA M PAARAKH[2,*]

[1]East West College of Pharmacy, Bangalore-91, [2,*] Department of Pharmacognosy, The Oxford College of Pharmacy, Bangalore 68. Email: padmaparas@hotmail.com.

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ABSTRACT

Ficus mollis Vahl, (Family: Moraceae) grows in Tropical and Subtropical regions of India, used for variety of purpose in traditional medicine. The usefulness of this plant is described in many folk books including Ayurveda, but no reports are available on morph anatomy, and phytochemical studies, hence present attempt was undertaken to investigate the microscopically and preliminary phytochemical and physico-chemical studies on the leaves of *Ficus mollis*. The study reveals the leaves are 6-15cm long, 3-9cm wide with a 5cm long stalk, arrangement is alternate distichous, sub-opposite, type is simple, elliptic, shape is ovate to fiddle, apex is broadly acute, base is sub-cordate and margin is entire. The transverse section of the leaves shows presence of epidermis, spongy parenchyma, bicollateral vascular bundles, nonglandular, glandular trichome and spiral vessels. The powder microscopy revealed the presence of anomocytic stomata, glandular trichome, covering trichome and prismatic calcium oxalate crystals. Physicochemical parameters like ash value, extractive value and phytochemical screening with different reagents showed the presence of fluorescence compounds, steroids, triterpenoids, phenols, tannins and flavonoids.

Keywords*: Ficus mollis,* Pharmacognostical studies, Phytochemical studies, Physico-chemical parameters, Fluorescence analysis.

INTRODUCTION

The family moraceae[1] comprises of the trees, shrubs, vines, or rarely herbs, frequently with milky or watery latex, sometimes spiny. Stipules present, frequently caducous. Leaves alternate, rarely opposite; petiole often present and well-defined; leaf blade simple , sometimes with cystoliths, margin entire or palmately lobed , venation pinnate or palmate. The genus *Ficus* comprises of trees, shrubs, or woody[2] vines, evergreen or deciduous, commonly epiphytic or scandent as seedlings with sap milky. Terminal buds surrounded by pair of stipules. Leaves alternate, monomorphic (dimorphic in *F. pumila*); stipules caducous, fused, enclosing naked buds. Leaf blade: margins entire (lobed in *F. carica*), rarely dentate; venation pinnate or nearly palmate. Figs occur in leaf axils, either in pairs or in clusters. They are stalkless, round up to 8mm across. Fig wall is somewhat fleshy, brownish, velvety.[3]The genus *Ficus* is made up of about 1,000 species from pantropical and subtropical origins[4]. Distribution is very common on rocks, foothills up to 900m in India and Sri Lanka.[5] Plants in the genus are all woody, ranging from trees and shrubs to climbers[6]. The anti bacterial[7], hepato protective [8], anti oxidant[9] and heavy metal analysis[10] of *Ficus mollis* has been reported. From the above literature, it is clear that no pharmacognostical work is carried out. The present study was therefore undertaken to investigate the pharmacognostical characters, fluorescence analysis and phytochemical analysis of the plant was carried out.

MATERIALS AND METHODS

Plant material collection

The plant material was collected from Sri Venkateshwara University, Tirupati, India. In October 2009. The plant was authenticated by Dr. Madhava Chetty, Deparment of Botany and voucher specimen of the plant were preserved at institute herbarium library. The leaves part were separated from other parts, washed, cleaned and dried for further use.

Reagents

All the reagents used were of analytical grade obtained from Science source, Bangalore, India.

Method

The external leaf morphology was observed and studied. Transverse free hand sections of fresh mature leaves were taken[11]. Whereas dried leaf powder material was used for the determination of ash values and extractive values[12, 13]. The phytochemical screening was done with the different extracts[11].The results were registered by botanical illustration and photos taken by means of the Motic digital microscope (Motic instrument Inc, Canada) fitted with 1/3" CCD camera imaging accessory with motic image 2000 image analysis software.

RESULTS AND DISCUSSION

Leaf morphoanatomy

Leaves are alternate or nearly opposite. 6-15cm long, 3-9cm wide with a 5cm long stalk, arrangement is alternate distichous, sub-opposite, leaf type is simple, elliptic, shape is ovate to fiddle, apex is broadly acute, base is sub-Cordate and margin is entire (Fig.1 a, b, c).

Fig. 1(a): *Ficus Mollis* Plant

Fig. 1(b): *Ficus Mollis* Dried Leaves

Fig. 1(c): *Ficus Mollis* Leaf Upper and Lower Surfaces

T S OF FICUS MOLLIS

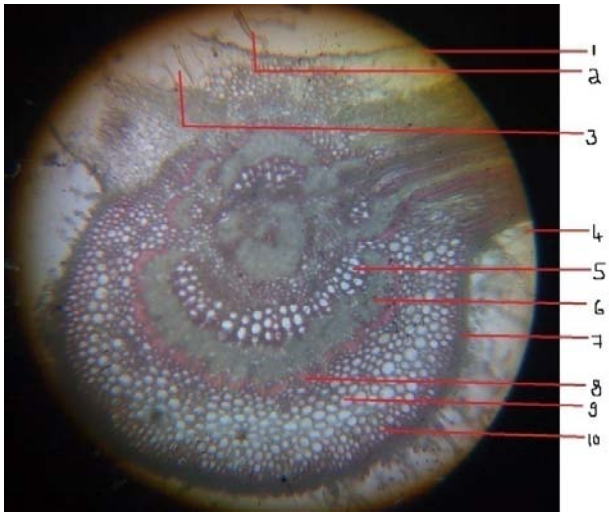


Fig. 2 (a): Lamina with midrib structure of *Ficus mollis*

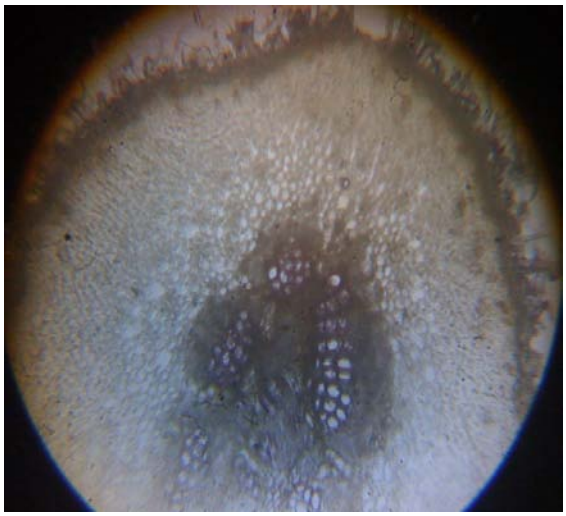


Fig. 3: T S of *Ficus Mollis* (Petiole)

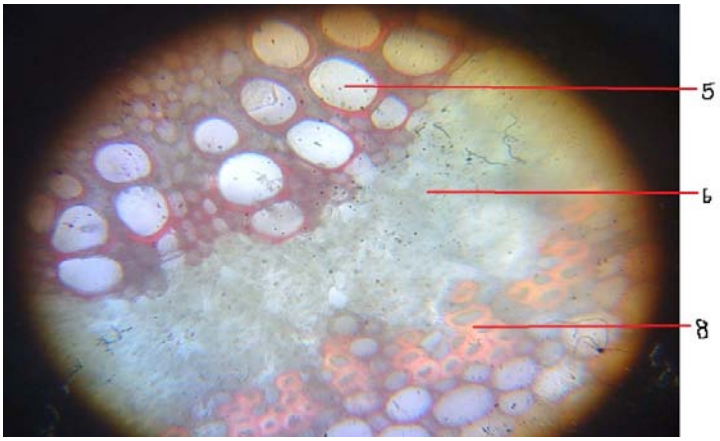
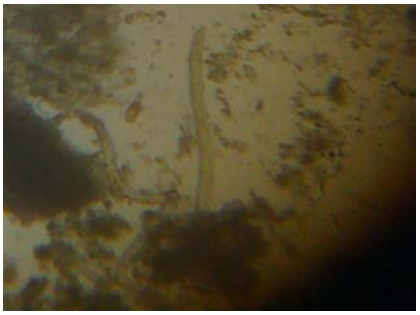


Fig. 2(b): Midrib [*Ficus Mollis*; 40X]

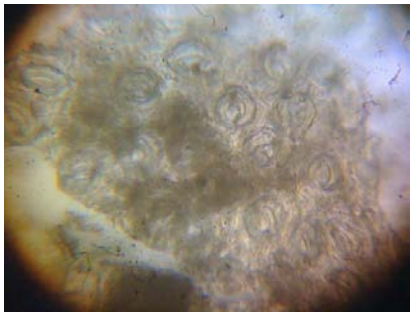
1. Upper epidermis
2. Trichome (unicellular)
3. Palisade cells
4. Glandular trichome
5. Xylem
6. Phloem cells
7. Lower epidermis
8. Pericyclic fibres
9. Parenchyma cells
10. Collenchyma cells

Powder analysis

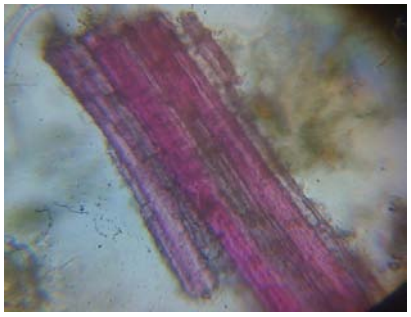
Powder Microscopy of *Ficus Mollis*



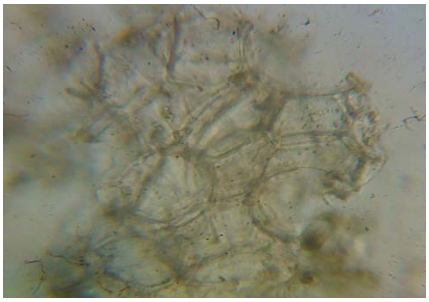
Unicellular trichome

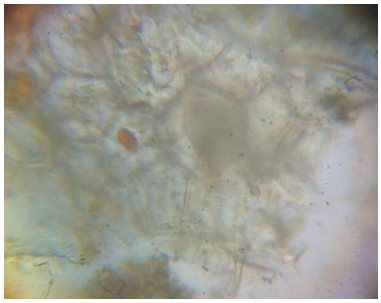


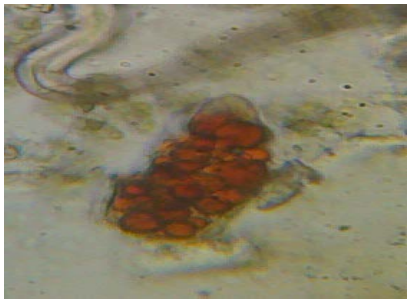
Stomata (anomocytic)



Phloem fibre

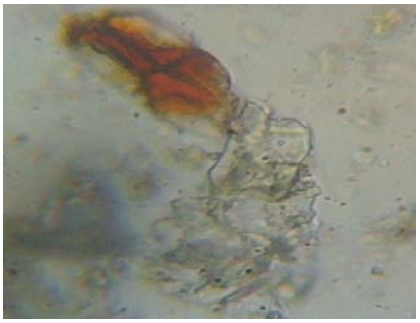






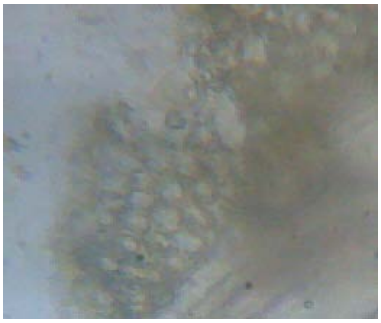
Parenchymatous layer with storage materials

Glandular trichome (multi cellular)



Epidermal cell with glandular trichome

Spiral vessel



Palisade cells

Fig. 4: Powder characters of leaf of *Ficus mollis*

Important Powder Microscopic Characters (Fig.4)

a) Unicellular, uniserrate covering trichomes are abundant, pointed toward the apex and broader at base, measure 240 - 460 microns in length.
b) Abundant multicellular glandular or non covering trichomes.
c) Paranchyamatous tissue with numerous storage materials like fixed oils and proteins.
d) Anomocytic stomata (the cells surrounding the stomatal pores are irregularly arranged).
e) Numerous xylem vessels of spiral type.
f) Palisade cells beneath epidermal layer.
g) Pericarp made up of pericyclic fibres between vascular bundles and endodermis an

Histochemical color reactions

The different histo-chemical color reactions were performed on the leaf transverse sections to differentiate the different cell compositions and identification[14] (Trease and Evans, 1986) and results were given in Table 1.

1. Behavior of leaf powder with different chemicals / reagents

Behavior of leaf powder with different chemical reagents was studied to detect the presence of phytoconstituents with color changes under daylight by reported method (Pratt and Chase, 1949) and the results were shown in Table 2.

2. Ash values

Total ash, acid-insoluble ash and water-soluble ash, values of the leaf powder were done as per the reported methods[11,12] (Anonymous, 1985) and the results are tabulated in Table 3.

3. Extractive values

Extracts were prepared with various solvents by reported method[16] (Kokashi *et al*;1958). Percentages of the extractive values were calculated with reference to air-dried drug (Table 5). Color and consistency of extracts (Pratt and Chase, 1949) are given in Table 4.

4. Fluorescence analysis of leaf powder

Fluorescence studies of various powders with various reagents revealed the presence of green and orange fluorescence with Conc. sulphuric acid and sodium hydroxide under day light and UV light by reported method (Kokashi *et al*; 1958). The observations are given in Table 5.

Phytochemical Screening

20 g of powdered dried leaf were extracted successively with petroleum ether, benzene, chloroform, acetone, methanol and distilled water. The extracts were concentrated, dried and phyotchemical screening was performed[11] and results are tabulated in the Table 6.

Table 1: Histochemical color reactions of *Ficus mollis* leaf powder

Reagents	Constituent	Color	Histological zone	Degree of intensity
Aniline So_4 + H_2SO_4	Lignin	Yellow	Xylem,	**
Phloroglucinol + Hcl	Lignin	Pink	Xylem, Sclerenchyma	***
Conc. H_2SO_4	Cellulose	Green	Mesophyll	**
Weak Iodine solution	Starch	--	--	--
Millon's reagent	Proteins	White	Spongy paranchyma	**
Dragendorff's reagent	Alkaloids	---	--	--
H_2So_4	Ca. Oxalate	Needles /prism	Mesophyll, and midrib paranchyma	*
$Sbcl_3$	Steroids/ Triterpenoids	Reddish pink	Mesophyll	***
5% Aq. KOH	Anthraquinone glycosides	--	--	--

***High, ** Moderate, *Slight, - Negative.

Table 2: Behavior of *Ficus mollis* leaf powder with different chemical reagents

Regents	Color/ppt	Constituents
Picric acid	Slight ppt.	Alkaloids present
Conc. H_2SO_4	Reddish brown	Steroids/triterpenoids present
Aq. $Fecl_3$	Bluish black ppt	Tannins present
Iodine solution	No change	Starch absent
Ammonia present	No change	Anthroquinone glycosides absent
5% Aq. KOH	No change	Anthroquinone glycosides absent
Mayer's reagent	Slight ppt	Alkaloids present
Spot test	Stains observed	Fixed oils present
Aq. $AgNo_3$	No ppt.	Proteins absent
Aq. NaOH	Yellow	Flavonoids present
Mg – Hcl	Magenta	Flavonoids present
Dragendorff's reagent	No ppt	Alkaloids absent
Aq. Lead acetate	White ppt	Tannins present
Liberman Bur chard's test	Reddish green	Steroids and tannins are present

Table 3: Ash values of *Ficus mollis* leaf

Types of ash value	% w/w
Total ash	6.66
Acid insoluble ash	1.39
Water soluble ash	2.11

Table 4: Extractive value of of *Ficus mollis* leaf

Type of solvent	%w/w
Petroleum ether 60-80^{0}C	2.41
Ethyl acetate	2.53
Alcohol	9.67
Water	18.88

Table 5: Fluorescence analysis of *Ficus mollis* leaf

Color reaction	Day light	UV light
Powder + NaOH	Light brown	Dark Green fluorescence***
Powder + NaOH in water	Dull brown	Dark green fluorescence **
Powder + NaOH in alcohol	Dull brown	Dark green fluorescence ***
Powder + Hcl	Dark green	Dark green fluorescence ***
Powder + H_2SO_4	Dark brown	Green fluorescence ***
Powder + HNO_3	Dull brown	Yellow fluorescence ***
Powder + 10 % Hcl	Light green	Yellow fluorescence ***
Powder + 10 % H_2SO_4	Dull brown	Clear yellow fluorescence ***
Powder + 10 % HNO_3	Dull Brown	Green fluorescence **
Powder + Glacial acetic acid	Light green	Light green fluorescence *
Powder + water	Dark brown	Dark green fluorescence **
Powder as such	Green	Dark green florescence ***

***High, ** Moderate, *Slight

Table 6: Phytochemical screening of *Ficus mollis* Extracts

Chemical Constituent	Tests	Pet ether	Benzene	Chloroform	Acetone	Methanol	Aqueous
Alkaloids	1. Mayer's test	-	-	+	-	-	-
	2. 2. Dragendorff's test	-	-	-	-	-	-
	3. Wagner's test	-	-	+	-	-	-
	4. Hager's test	-	-	+	-	-	-
Carbohydrates	1. Molisch's test	-	-	-	-	+	+
	2. Benedict's test	-	-	-	-	-	-
	3. Fehling's test	+	-	+	-	++	++
Phytosterols	1. Salkowski test	+	+	-	++	+++	-
	2.Libermann Burchard	-	-	-	+	++	-
Saponins	1. Foam test	-	-	-	-	-	-
Glycosides	1.Modified Borntrager's	-	-	-	-	-	-
	2. Legal test	-	-	-	-	-	-
Tannins	1. Alkaline Reagent	-	-	-	+	+++	+++
Phenols	1. Ferric Chloride test	-	-	-	-	++	++
Proteins	1. Xanthoprotein test	-	-	-	-	-	-
	2. Ninhydrin test	-	-	-	-	-	-
	3. Biuret test	-	-	-	-	-	-
Flavonoids	1. Gelatin test	-	-	-	-	-	-
	2. Lead acetate test	-	-	-	+	+++	+++
	3. Shinoda test	-	-	-	+	++	++

CONCLUSION

Microscopic analysis and quantitative parameters are carried out on plant samples in order to establish appropriate data that can be used in identifying crude drugs particularly those supplied in powder form. They are standard pharmacognostic parameters that can be used to differentiate closely related plant species or varieties with similar constituents or pharmacological activities. *Ficus mollis is* a greenish, fine, odorless powder with a slightly bitter taste. TS of the leaf lamina and midrib (Fig.2, 3) show the presence of bicollateral vascular bundles, collenchymas cells, spongy parenchyma cells. The powder microscopy (Fig.4) revealed the presence of glandular trichome, covering unicellular trichomes, fibres, epidermal cells and xylem vessels of spiral type.

The physical constants such as total ash value (Table 3) (6.66% w/w), acid insoluble ash (1.39% w/w), water soluble ash (2.11% w/w), and extractive values (Table 4) are specific identification. The soluble extractive values with solvents such as petroleum ether, ethyl acetate, ethanol, and water were (2.41% w/w, 2.53% w/w, 9.67% w/w and 18.88 % w/w), respectively, which indicates the nature of constituents present.

The behavior of the leaf powder upon treatment with different chemical reagents was also observed and reported in (Table 2). Fluorescence studies (Table 5) of powder with various reagents revealed the presence of green fluorescence with Conc. Hcl and sodium hydroxide, under UV light. The various qualitative chemical tests of petroleum ether , benzene, chloroform, acetone, methanol and aqueous extract (Table 6) indicates the presence of sterols, triterpenoids, flavonoids, phenols and tannins in large amounts whereas aromatic acids, carbohydrates, gums, mucilage, and volatile oils were totally absent in the leaf extract of this plant. As there is no pharmacognostical work on record of traditionally valued drug, the present work could be therefore be used as one of the tool for standardization of crude drug to identify and decide the authenticity of this drug in herbal industry/trade.

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