International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 2, 2011

Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON LEAVES OF *FICUS HISPIDA*

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Received: 15 Dec 2010, Revised and Accepted: 19 Jan 2011

ABSTRACT

Ficus hispida (Syn: *Ficus oppositifolia* Roxb; Family: Moraceae) commonly known as devil fig, hairy fig is 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 and is scientifically evidenced, and different biologically active phytoconstituents were isolated from plant. 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 hispida*. The study reveals the leaves are simple, opposite, decussate, caducous. The transverse section of the leaves shows presence of epidermis, sponge 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 hispida,* pharmacognostical studies, Phytochemical studies, Physico-chemical parameters, Fluorescence analysis.

INTRODUCTION

The genus *Ficus* is made up of about 1,000 species from pantropical and subtropical origins[1]. Plants in the genus are all woody, ranging from trees and shrubs to climbers[2]. *Ficus hispida* L.f is a shrubs to medium sized trees, up to 10 m tall with Bark brownish, lenticellate; blaze pink, Branchlets terete, with hollow internodes, densely hispid with brown or grey hairs, lenticellate. The plant is found to use traditionally for the prevention of disease. A mixture of honey and the juice of these fruit is a good antihemorrhagic[3] but the barks and leaves are used as an antidiarrhoeal[4], Antidiabetic[5] and as cardioprotective[6] activity.

A new norisoprenoid, ficustriol,phenanthroindolizidine alkaloid O-methyltylophorinidine[7], palmitic oil, 9,12-octadecadienoic acid, hexadecanoic acid ethyl ester, linalool, 1-hydroxylinalol,1-hydroxylinalool and benzyl alcohol were isolated so far from the plant[8]. 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, Department of Botany and specimen herbarium were preserved at institute herbarium library. The leaves part were separated form 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. Fresh mature leaves transverse free hand sections were taken[9]. Whereas dried leaf powder material was used for the determination of ash values and extractive values[10,11] The phytochemical screening was done with the different extracts[9].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 morph anatomy

Leaves simple, opposite, decussate; stipules to 2.5 x 1 cm, caducous, leaving annular scar; petiole 1-10 cm long, canaliculate, hispid; lamina 7-35 x3-16 (40 x 18 cm in saplings), elliptic-oblong, narrow ovate, narrow obovate, apex caudate-acuminate, base rounded subcordate or truncate-subcordate, margin entire or dentate sometimes irregularly toothed, scabrid on both surface, hispid beneath; midrib slightly raised above; 3-nerved at base; secondary nerves 4-9 pairs, often branched, ascending; tertiary nerves broadly reticulo-percurrent(Fig 1).

Fig. 1: Fresh leaf of *ficus hispida*

Transverse section of leaves of Ficus *microcarpa*

Lamina: It shows regular upper and lower epidermis with well developed thin cuticle and stomatal pores. Numerous unicellular, uniserrate covering trichomes are abundant, pointed toward the apex and broader at base measures about 260 - 450 microns in length.Parencymatous mesophyll is also present (Fig 3).

Midrib: Upper and lower epidermis layers continuous over the midrib, followed by a patch of collenchymas cells below the upper and above the lower epidermis. The epidermal cells show similar features as seen in the lamina region. Paranchyamatous tissue containing spiral vascular strands measuring 25 – 48 micron in diameter. Bicollateral vascular bundles are seen to the centre of the midrib. The rest of the midrib is occupied by the parenchyma cells (Fig 2).

Fig.2: Lamina with midrib structure of *Ficus hispida* L.

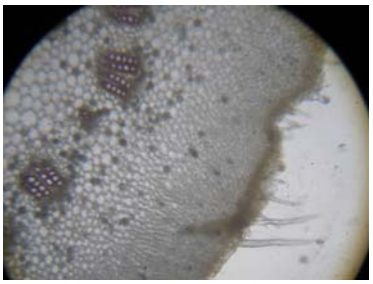

Fig. 3: TS of *Ficus hispida*

1. Upper epidermis
2. Trichome (unicellular)
3. Spongy parenchyma cells
4. Lower epidermis
5. Xylem
6. Phloem
7. Glandular trichome
8. Collenchyma

Powder analysis of leaves of Ficus *hispida*

Powder characters: The powder microscopy shows the fragments of unicellular covering and glandular trichomes, phloem fibres, parenchyma cells, numerous xylem vessels of spiral type and epidermal cells with anomocytic stomata (Fig 4).

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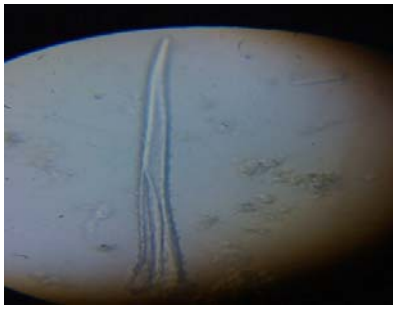
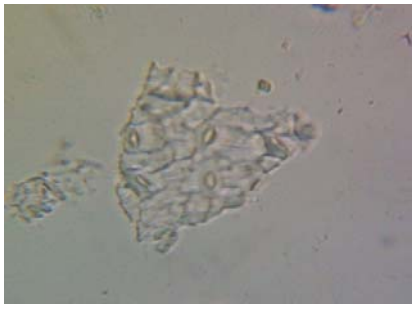
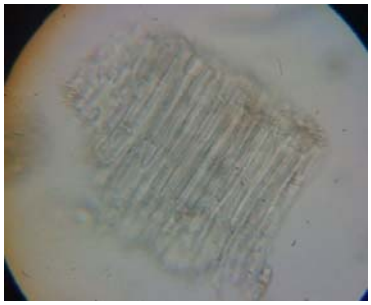


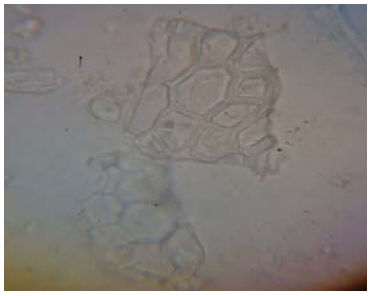
Unicellular trichome



Stomata (anomocytic)

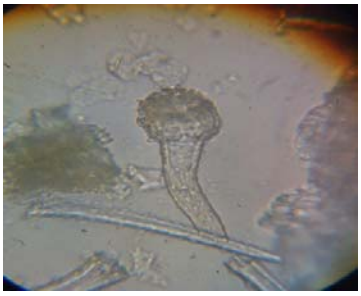


Spiral xylem



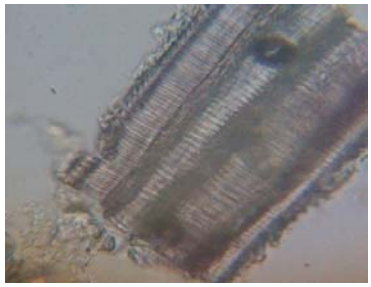
Parenchymatous layer

Palisade parenchyma

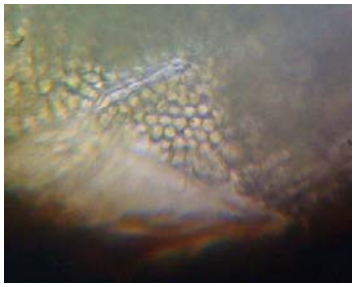


Glandular trichome

Xylem vessel (spiral)



Spiral vessels



Palisade cells

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

Histochemical color reactions

The different histo-chemical color reactions were performed on the leaf transverse sections to differentiate the different cell compositions and identification[12] and results were given in Table 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[13] and the results were shown in Table 2.

Table 1: Histochemical color reactions of *Ficus hispida* 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	--	--	--
Millons reagent	Proteins	White	Spongy paranchyma	**
Dragendroffs reagent	Alkaloids	---	--	--
H_2So_4	Ca. Oxalate	Needles /prismatic	Mesophyll, and midrib parenchyma	*
$SbCl_3$	Steroids/ Triterpenoids	Reddish pink	Mesophyll	***
5% Aq. KOH	Anthraquinone glycosides	--	--	--

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

Table 2: Behavior of *Ficus hispida* 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 precipitation	Proteins absent
Aq. NaoH	Yellow	Flavonoids present
Mg – Hcl	Magenta	Flavonoids present
Dragendroff's reagent	No ppt	Alkaloids absent
Aq. Lead acetate	White ppt	Tannins present
Liberman Burchardt's test	Reddish green	Steroids and tannins are present

Quantitative analysis

Ash values

Total ash, acid-insoluble ash and water-soluble ash, values of the leaf powder were done as per the reported methods[10,11] and the results are tabulated in Table 3.

Table 3: Ash values of *Ficus hispida* leaf

Types of ash value	% w/w
Total ash	7.18
Acid insoluble ash	1.66
Water soluble ash	1.48

Extractive values

Extracts were prepared with various solvents. Percentages of the extractive values were calculated with reference to air-dried drug[13] are given in Table 4.

Table 4: Extractive value of *Ficus hispida leaf*

Type of solvent	% w/w
Petroleum ether 60-80^0	2.26
Ethyl acetate	2.33
Alcohol	8.58
Water	14.91

Table 5: Fluorescence analysis of Ficus *hispida* leaf

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

***High, ** Moderate, *Slight

Fluorescence analysis of leaf powder

Fluorescence studies of various powders with various reagents revealed the presence of green fluorescence with Conc. Hydrochloric acid and sodium hydroxide under day light and UV light by reported method[14].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[9] and results are tabulated in the Table 6.

Table 6: Phytochemical screening of Ficus *hispida* extracts

Chemical Constituent	Tests	Pet ether (Green)	Benzene (Dark green)	Chloroform (Brown)	Acetone (Greenish brown	Methanol (Brown)	Aqueous (Brown)
Alkaloids	1. Mayer's test	-	-	+	-	-	-
	2. Dragendroff'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	-	-	-	-	-	-
Flavanoids	1. Gelatin test	-	-	-	-	-	-
	2. Lead acetate test	-	-	-	+	+++	+++
	3. Shinoda test	-	-	-	+	++	++

CONCLUSION

Microscopic analysis and qualitative 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 hispida is a pale green, fine, odorless powder with a slightly bitter taste. TS of the leaf lamina and midrib show the presence of bicollateral vascular bundles, collenchymas cells, and spongy parenchyma cells. The powder microscopy 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 (7.18 % w/w), acid insoluble ash (1.66 % w/w), water soluble ash (1.48 % w/w), and extractive values are specific identification. The soluble extractive values with solvents such as petroleum ether, ethyl acetate, ethanol, and water were (2.66 % w/w, 2.23 % w/w, 8.58 % w/w and 14.91 %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. Fluorescence studies 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 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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