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Pharmacognostical and Phytochemical Investigation on Leaves of *Ficus microcarpa* Linn.

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*

ABSTRACT
Ficus microcarpa Linn. (Syn: *Ficus nitidas*; 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 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 microcarpa*. The study reveals the leaves are variable, coriaceous, oblong, elliptic to broadly elliptic or obovate. 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 microcarpa,* 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] The species name, *microcarpa*, refers to the small size of the fruit. *Ficus microcarpa* with common names Chinese or Malayan banyan [1], Indian Laurel, Curtain fig [3] is a evergreen tree to 15 m (50 ft) or more in height, with a rounded dense crown, smooth gray bark, milky sap, and long, thin, dangling aerial roots. Leaves alternate, simple, leathery, deep glossy green, oval-elliptic to diamond-shaped, to 13 cm (5 in) long, with short pointed, ridged tips. Flowers tiny, unisexual, numerous, hidden within the "fig," a fleshy, specialized receptacle that develops into a multiple fruit (syconium), this green turning to yellow or dark red when ripe, sessile, in pairs at leaf axils, small, to 1 cm (0.5 in) in diameter. It is useful in conditions such as diabetes, ulcers, burning sensations, hemorrhages, leprosy, itching, liver disease and toothache. [4] The cytotoxic [5], antifungal [6] and hypoglycemic effect [7] of *Ficus microcarpa* Leaves has been reported.

Fig. 1: Vegetative apical branches of *Ficus microcarpa* L.

The phytoconstituents so far isolated from the plant are (2S,3S, 4R) -2-[(2'R) -2' - hydroxypentracosanoylamino]-heptadecane -1, 3, 4-triol, 12, 20 (30) -ursa-dien-3alpha-ol, epifriedelanol, alpha-amyrin acetate, beta-sitosterol, beta-daucosterol, hexacosanoic acid, heneicosanoic acid [8], ficuscarpanoside A, guaiacylglycerol 9-*O*-β-D-glucopyranoside, erythro-guaiacylglycerol 9-*O*-β-D-glucopyranoside, guaiacylglycerol, erythro-guaiacylglycerol, 4-methoxy guaiacylglycerol 7-*O*-β-D-glucopyranoside, and

***Corresponding author: Dr. Padmaa M Paarakh,** Principal and HOD, Department of Pharmacognosy, The Oxford College of Pharmacy, 6/9, I Cross, Begur Road, Hongasandra, Bangalore 560068; **Tel.:** +91-9880681532;
E-mail:padmaparas@hotmail.com,
rc.sunmoon2008@gmail.com

3-(4-hydroxy-3-methoxy phenyl) propan-1,2-diol [9], ficuscarpanoside B, (7*E*,9*Z*)-dihydrophaseic acid 3-*O*-β-d-glucopyranoside, ficuscarpanic acid, 2,2'-dihydroxyl ether, [(7*S*,8*R*)-syringoylglycerol, (7*S*,8*R*)-syringoylglycerol-7-*O*-β-d-glucopyranoside and icariside D2 [10] from the aerial roots.
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 speciman herbarium 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. Fresh mature leaves transverse free hand sections 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

Morph anatomy of leaf

Leaves variable, coriaceous, oblong, elliptic to broadly elliptic or obovate, usually 5-8 cm long, 3-5 cm wide, glabrous, margins entire, petioles 0.6-2 cm long. Syconia sessile, arising among or just below the leaves, depressed-globose, 6-10 mm in diameter, subtended by 3 broadly ovate, persistent bracts. Seeds minute, less than 1 mm (Fig. 1).

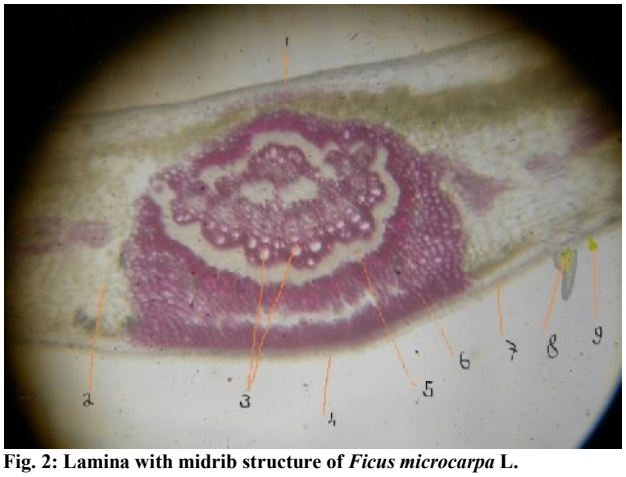

Fig. 2: Lamina with midrib structure of *Ficus microcarpa* L.

1. Upper epidermis
2. Spongy parenchyma
3. Xylem
4. Lower epidermis
5. Phloem
6. Collenchyma
7. Epidermal layer
8. Trichome (unicellular)
9. Trichome (glandular)

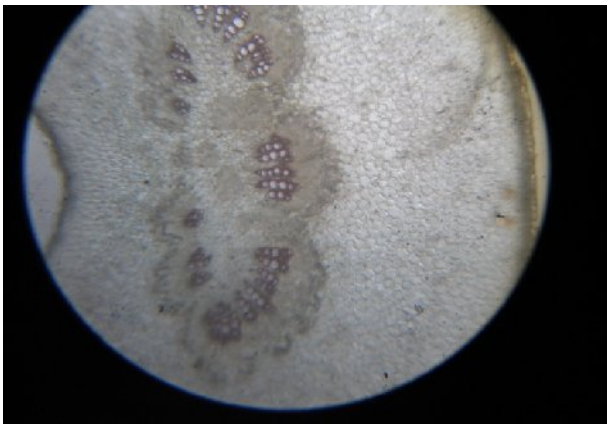

Fig. 3: TS of *F. microcarpa*

Transverse section of leaves of Ficus *microcarpa*

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).

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 230 - 425 microns in length.Parencymatous mesophyll is also present (Fig. 3).

Powder analysis of leaves of Ficus *microcarpa*

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).

Histochemical color reactions

The different histo-chemical color reactions were performed on the leaf transverse sections to differentiate different cell compositions and identification. [14] The 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 [15] and the results were shown in Table 2.

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 [12] and the results are tabulated in Table 3.

Extractive values

Extracts were prepared with various solvents. Percentages of the extractive values were calculated with reference to air-dried drug [15] and are given in Table 4.

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. [16] The observations are given in Table 5.

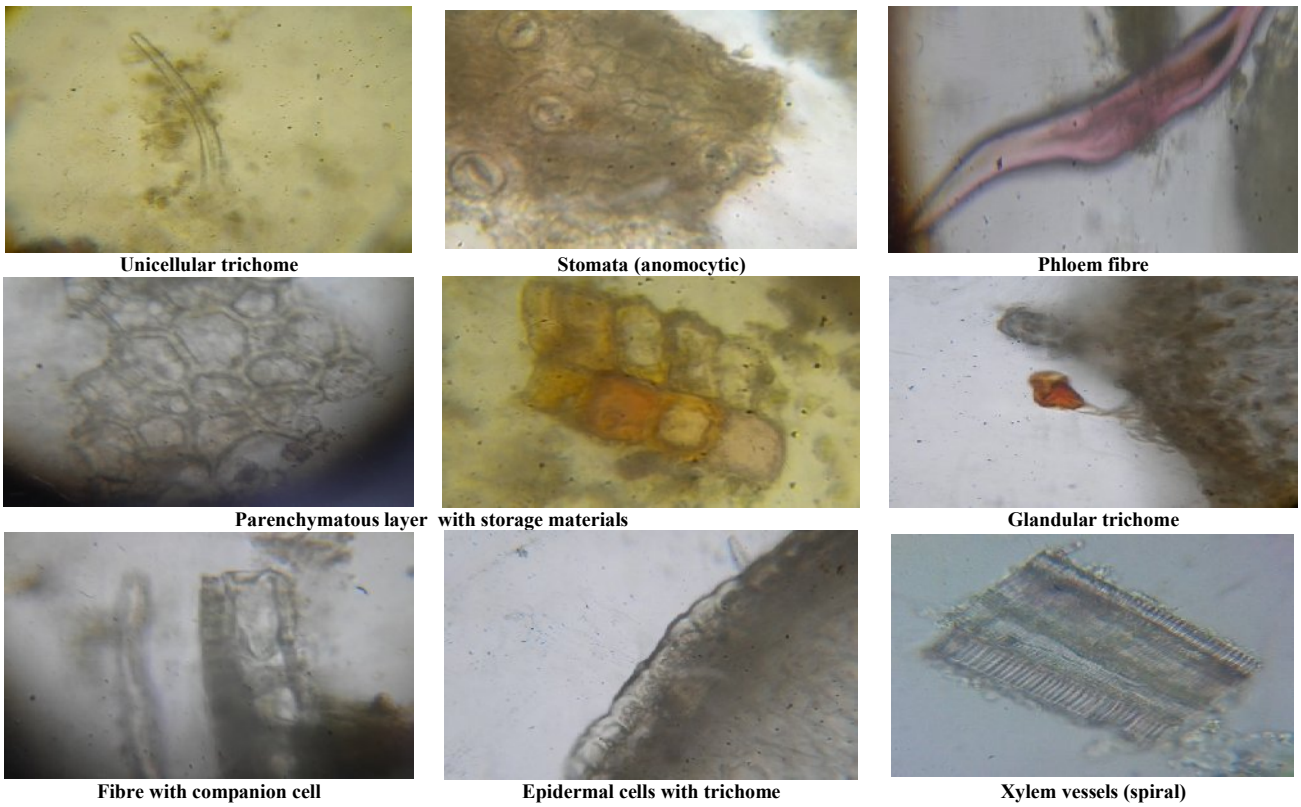

Fig 4: Powder characters of leaf of *Ficus microcarpa*

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

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

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.

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 microcarpa* is a pale greenish brown, 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.

Table 2: Behavior of *Ficus microcarpa* 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 Burchard's test	Reddish green	Steroids and tannins are present

Table 3: Ash values of *Ficus microcarpa* leaf.

Types of ash value	% w/w
Total ash	6.22
Acid insoluble ash	1.57
Water soluble ash	1.36

Table 4: Extractive value of Ficus *microcarpa* leaf.

Type of solvent	% w/w
Petroleum ether $60\text{-}80^0$	2.13
Ethyl acetate	2.46
Alcohol	8.0
Water	24.0

Table 5: Fluorescence analysis of Ficus *microcarpa* leaf.

Color reaction	Day light	UV light
Powder + NaOH	Dark brown	Dark Greenish yellow fluorescence***
Powder + NaOH in water	Dark brown	Dark green fluorescence ***
Powder + NaOH in alcohol	Dark brown	Dark green fluorescence ***
Powder + Hcl	Dull green	Dark green with faint green fluorescence **
Powder + H_2SO_4	Dull green	Dark brown
Powder + HNO_3	Brownish black	Brown
Powder + 10 % Hcl	Dull green	Dull green
Powder + 10 % H_2SO_4	Dull green	Dull green
Powder + 10 % HNO_3	Dull Brown	Dull Brown
Powder + Glacial acetic acid	Dull green	Dark green fluorescence **
Powder + water	Dull green	Dark green fluorescence *
Powder as such	Dull brownish green	Slight greenish florescence *

***High, ** Moderate, *Slight

The physical constants such as total ash value (Table 3) (6.22% w/w), acid insoluble ash (1.57% w/w), water soluble ash (1.36% 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 (2.13% w/w, 2.46% w/w, 8% w/w and 24% 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.

Table 6: Phytochemical screening of Ficus *microcarpa* Extracts

Chemical Constituent	Tests	Pet ether Extract (Green)	Benzene Extract (Dark green)	Chloroform extract (Brown)	Acetone Extract (Greenish brown)	Methanol extract (Brown)	Aqueous extract (Brown)
Alkaloids	1. Mayer's test	-	-	+	-	-	-
	2. 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 Burchardt's test	-	-	-	-	-	-
Saponins	1. Foam test	+	-	-	-	-	+
Glycosides	1.Modified Borntrager's test	-	-	-	-	-	-
	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	-	-	-	+	++	++

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