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Pharmacognostical Studies and Evaluation of Anti-inflammatory Activity of *Ficus bengalensis* Linn

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ABSTRACT

Pharmacognostical parameters for the leaves of *Ficus bengalensis* were studied with the aim of drawing the pharmacopoeial standards for this species: macroscopical and microscopical characters, physio-chemical constants, extractive values with different solvents, fluorescence analysis of dry powder, its reaction after treatment with chemical reagents under visible light, and UV light at 254 nm and 366 nm. Preliminary phytochemical studies on the *Ficus bengalensis* leaves were conducted. The determination of these characters will aid future investigators in their pharmacological analyses of this species. The anti-inflammatory effect of ethanolic and petroleum ether extracts of *ficus bengalensis* were evaluated in experimental animals. We have determined the anti-inflammatory activity of ethanolic and petroleum ether extracts of the bark of *Ficus bengalensis* by oral administration of doses of 300 and 600 mg/kg/day of body weight to healthy animals. The extracts were studied for their anti-inflammatory activity in carrageenan-induced hind paw edema in rats and the paw volume was measured plethysmometrically at 0 3h after injection. The ethanolic and petroleum ether extracts of *Ficus bengalensis*, significantly reduced (P<0.05) carrageenan-induced paw edema in rats. The ethanolic and petroleum ether extracts showed a greater anti-inflammatory effect compared with the standard drug Indomethacin. The present results indicated the ethanolic extract of *Ficus bengalensis* exhibited more significant activity than petroleum ether in the treatment of inflammation..

Key words: *Ficus bengalensis*, moraceae, vada, inflammation, pharmacognostical, indomethacin

DOI: 10.4103/0975-1483.51879

INTRODUCTION

Ficus bengalensis (Moraceae, Mulberry family) is commonly known as a Banyan tree or Vata or Vada tree in ayurveda. There are more than 800 species and 2000 varieties of *Ficus* species, most of which are native to the old world tropics. *Ficus bengalensis* is a remarkable tree from India that sends down its branches and great number of shoots, which take root and become new trunks. This tree is considered to be sacred in many places in India. Earlier, glucoside, 20-tetratriaconthene-2-one,6-heptatriacontene-10-one,pentatriacontan-5-one, beta sitosterol-alpha-D-glucose, and meso-inositol have been isolated from the bark of the *Ficus bengalensis*.[1,2]

The fruit extracts exhibited antitumor activity in the potato disc bioassay.[3] The leaves contain 9.63% crude protein, 26.84% crude fibres, 2.53% CaO, and 0.4% Phosphorous. It yields latex containing Caoytchoue (2.4%), Resin, Albumin, Cerin, sugar, and Malic acid. It is used in ayurveda for the treatment of diarrhea, dysentery, and piles[4,5] and as a hypoglycemic.[6,7] The extracts of *Ficus bengalensis* were also reported to inhibit insulinase activity from the liver and kidney.[8] It was also found to inhibit

the lipid peroxidation.[9] Various extracts of *Ficus bengalensis* were screened for its anti-allergic and anti-stress potential in asthma by milk-induced leucocytosis and milk-induced eosinophilia.[10] Other species of *Ficus* viz. *Ficus Racemosa*,[11] *Ficus inspida*,[12] *Ficus religiosa*,[12] *Ficus elastica*,[12] *Ficus Indica*,[12] and *Ficus carica*[13] were found to have anti-inflammatory activity. Based on this, an attempt has been made to evaluate the inflammation potency of *Ficus bengalensis*. But no pharmacognostical work has been done so far. Therefore, an attempt has been made to study the Pharmacognostic parameters on the leaves of *Ficus bengalensis* in both whole form and powdered form.

MATERIALS AND METHODS

Plant material

The plant material was collected from the foothills of the Satpuda ranges in the district of Jalgaon (MS) in the months of May and June 2008. The plant was identified and authenticated by the Joint Director of Botanical Survey of India, Pune dated 09/09/2008 and letter No. BSI/WC/Tech/2008/411. The leaves were separated, dried, coarsely powdered, passed through sieve no. 40, and stored in a closed container for further use. Around 5 kg of fresh bark was collected and cut into small pieces (2-3 cm), dried in shade under normal environmental temperature for 15-20 days, and homogenized to get a coarse powder. This powder was stored in an air tight container and used for further successive extraction.

Preparation of extracts

The powdered plant material (450 g) was repeatedly extracted in a 5000 ml round bottomed flask with 2000 ml solvents starting with petroleum ether and ethanol. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature and evaporated to dryness under reduced pressure in a rotary evaporator.[14]

Toxicity studies

The extracts were given at the doses of 300 and 600 mg/kg/day of body weight per day and were selected from a range of 1/6 to 1/15 of LD_{50} based on the preliminary study conducted at our laboratory.

Animals

Wistar albino rats (120-200 g) of either gender supplied from Yash Farms, Pune, India were used. The animals were housed under standard laboratory conditions maintained at 25 ± 1^0 C and under 12/12 h light /dark cycle and fed with a standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water ad libitum. The protocol was approved by the institutional Animal Ethical Committee for the purpose of control and supervision of experiments and animals (CPCSEA), constituted under the directives of the Ministry of Social Justice and Empowerment, Government of India.

Drugs and chemicals

Indomethacin (Micro labs, Bangalore), Carrageenan (Sigma Chemicals), Ethanol AR (Thomas Baker Chemical Pvt. Ltd.), and Petroleum ether AR (60-80^{0}C, MCC) were used during the experimental protocol.

Methods

The macroscopical characters (size, shape, color, odour, texture, venation margin, base, apex, and petiole) of the leaves were observed [Tables 1 and 2].[15] Then, the powder was identified by anatomical study with routine reagents to study the lignified cells, trichomes, stomata, fibres, etc. Quantitative microscopy was determined by methods prescribed by Trease and Evans.[16,17]

The ash values and extractive values with various reagents were determined as per the Indian Pharmacopoeia [Table 3]. Extractive values with various solvents like petroleum ether, chloroform, alcohol, and water were performed as per the standard procedure [Table 4].[18] Measurements of the vein islet number, vein termination number, stomatal number, stomatal index, and length of trichome were determined [Table 5].[19] The behavior of powdered leaves with various chemical reagents was studied. The fluorescence characters of the powder with various acids were observed under visible light and UV light as per the procedures.[20] Preliminary phytochemical tests of the powder/extracts were performed using specific reagents through standard procedures.[17-21]

Evaluation of anti-inflammatory activity

The albino rats of either gender were divided into six groups of six animals each. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally for 7 days as a control group, Group II received 300 mg/kg body weight of ethanolic extract of *Ficus bengalensis* (EEFB-I) orally for 7 days, Group III received 600 mg/kg body weight of ethanolic extract of *Ficus bengalensis* (EEFB-II) orally for 7 days, Group IV received 300 mg/kg body weight of Petroleum ether extract of *Ficus bengalensis* (PEEFB-I) orally for 7 days, Group V received 600 mg/kg body weight of Petroleum ether extract of *Ficus bengalensis* (PEEFB-

Table 1: Ethnomedical information of *Ficus bengalensis*

Parts	Uses
Root	Obstinate vomiting, leucorrhoea
Bark	Burning sensation, hemorrhages, diarrhea
Fruit	Refrigerant, tonic and are useful in vitiated condition of *pitta*
Buds	Diarrhea and dysentery
Leaves	Ulcers, leprosy, allergic conditions of skin
Latex	Rheumatism, neuralgia, lumbago, gonorrhea

Table 2: Macroscopy of *Ficus bengalensis*

Parts	Observation
Bark	Grey
Fruit	Red (sessile in pairs)
Leaves	Coraceous, ovate to elliptic
Appearance	Green
Shape	Lanceolate
Length/height	10-30 cm long and 7-20 cm wide
Apex	Obtuse
Petiole	2.5-5 cm long
Stipules	Stout
Arrangement	Opposite
Venation	Reticulately Pinnate

Table 3: Determination of Ash Values *Ficus bengalensis* Linn

Ash type	Percentage of Ash
Total ash	11.63% w/w
Acid insoluble ash	4.5% w/w
Water soluble ash	7.56% w/w

Table 4: Determination of Extractive Values *Ficus bengalensis* linn

Solvent	Percentage of extractive
Petroleum ether	1.8% w/w
Chlororform	1.2% w/w
Ethanol	4.8% w/w
Water	6.4% w/w

Table 5: Determination of phytoconstants *Ficus bengalensis* linn

Leaf constants	Report
Vein islet number	10.7/mm^2
Vein termination number	12.6/mm^2
Stomatal index (upper epidermis)	7.5/mm^2
Stomatal index (lower epidermis)	16/mm^2

II) orally for 7 days, and Group VI received 10 mg/kg of body weight of Indomethacin intraperitoneally for 7 days as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1%w/v carrageenan into the sub-plantar region of the right hind paw of the rats. On the 7th day, paw volume was measured 1h prior to carrageenan injection using a plethysmometer and at 0 and 3h after the carrageenan injection.[22] Mean increase in the paw volume was measured and percentage inhibition was calculated [Table 6].

Percentage of inhibition =100 (1-Vt-Vc)

Where, Vc= edema volume in control and Vt= edema volume in test /standard compound.

Statistical analysis

Results were expressed as Mean ± SEM; statistical significance was calculated by applying t-test. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Analysis and Discussion

The leaves were green and odourless with a slightly bitter taste. Leaves are 7.5–18 cm in length, lanceolate in shape, have a glabrous surface, acute, apex, equal base, entire margin, reticulate venation, and are petioled. The physical constants such as total ash value (10.63%), acid insoluble ash (2.65%), water soluble ash (8.69%), and extractive values are specific identification. The soluble extractive values with solvents such as petroleum ether, chloroform, ethylacetate, ethanol, and water were (1.4%, 1.2%, 2%, 2.8%, and 5.4%), respectively, which indicates the nature of constituents present. A quantitative microscopical study also gives valuable information regarding specific leaf constants such as vein islet (11.5/mm^2), vein termination number (13.8/mm^2), stomatal number (6.5/mm^2 and 14/mm^2), and upper and lower epidermis, respectively. The

Table 6: The effect of bark of *Ficus bengalensis* on carrageenan-induced paw edema

Treatment	Dose mg/kg	Mean paw volume in ml						Percent
		0 min	15 min	30 min	60 min	120 min	180 min	inhibition
Control	2% CMC	0.79±0.04	1.05±0.10	1.32±0.16	1.69±0.12	1.77±0.11	1.48±0.04	-
EEFB-I	300	0.78±0.11	1.17±0.18	1.41±0.10	1.35±0.10	0.83±0.14	[a]0.71±0.20	55.03
EEFB-II	600	0.73±0.12	0.83±0.15	0.91±0.12	0.82±0.16	0.67±0.17	[a]0.51±0.15	65.54
PEEFB-I	300	0.77±0.10	1.11±0.11	1.29±0.15	1.62±0.13	1.59±0.11	[a]1.41±0.19	04.73
PEEFB-II	600	0.74±0.15	1.15±0.18	1.41±0.10	1.58±0.10	1.46±0.14	[a]1.37±0.12	07.43
Indomethacin	10	0.72±0.08	0.79±0.10	0.85±0.10	0.74±0.10	0.55±0.17	[a]0.28±0.04	81.08

Table 7: The behavior of powdered leaves of *Ficus bengalensis* with different chemical reagents

Particulars	Under visible light	U.V. light	
		Short wavelength	Long wavelength
Powder as such	Dull green	Dark green	-----
Powdered drug + Conc. HCl	Dull green	-----	-----
Powdered drug + Conc. H_2SO_4	Dull green	-----	Green
Powdered drug + Conc. HNO_3	Brown	Dull green	-----
Powdered drug + Glacial Acetic acid	Dull brown	-----	-----
Powdered drug + Aqueous NaOH	Dull green	Dark green	-----
Powdered drug + alcoholic NaOH	Dark green	Dark green	Orange
Powdered drug + 10% Hcl	Dull green	Dark green	Yellow
Powdered drug + 10% H_2SO_4	Dull brown	Dark green	Dull Yellow
Powdered drug + 10% HNO_3	Dull green	Dark green	Dull yellow
Powdered drug + 10% Glacial Acetic acid	Dull green	Dark green	Dull yellow
Powdered drug + Water	Dull green	Dark green	-----

length of the trichome is 20.43µ-- 40.79µ--80.86µ [Tables 1-5]. The behavior of the leaf powder upon treatment with different chemical reagents was also observed and reported in Table 7. Fluorescence studies of various powders with various reagents revealed the presence of green and orange fluorescence with Conc. sulphuric acid and sodium hydroxide, respectively under UV light at 254 nm and 366 nm.

Powder analysis of *Ficus bengalensis* Linn

Ficus bengalensis linn is a pale green, fine, odorless powder with a slightly bitter taste. The powder microscopy revealed the presence of trichomes, fibres, epidermal cells with anticlinal walls, calcium oxalate crystals, and spiral thickenings. Similarly, the fluorescence characteristic of the powdered leaf when treated with various chemical reagents and its extracts has also been extensively studied. The extractive values of the powder with different solvents were determined and its result was reported in Table 7.

The various qualitative chemical tests of powder, ethanol extract, and aqueous extract [Table 8] indicates the presence of sterols, flavanoids, phenols, tannins, and saponins in large amounts whereas aromatic acids, carbohydrates, triterpenoids, gums, mucilage, and volatile oils were totally absent in the leaf extract of this plant.

In this study, a carrageenan-induced paw edema method shows the result given in Table 6. Ethanolic extract of *Ficus bengalensis* at 300 mg/kg body weight per day (EEFB-I) when given orally as a suspension the paw volume was reduced by 55.03%, whereas the case of the ethanolic extract of *Ficus bengalensis* at 600 mg/kg body weight per day (EEFBA-II) shows a 65.54% inhibition after 3h, which indicates that the effect of ethanolic extract of *Ficus bengalensis* is reflected in a dose-dependent manner. Both EEFB-I and EEFB-II showed an inhibitory effect on carrageenan-induced paw edema thus, exhibiting anti-inflammatory effect against acute inflammation.

Petroleum ether extract of *Ficus bengalensis* at 300 mg/kg body weight per day (PEEFB-I) reduced the paw volume by 04.73% and petroleum ether extract of *Ficus bengalensis* at 600 mg/kg body weight per day (PEEFB-II) exhibited a 07.43% reduction in paw volume after 3h, therefore, petroleum ether extract of *Ficus bengalensis* does not possess significant anti-inflammatory activity when compared with control and Indomethacin-treated animals [Table 6]. It may be due to the absence of flavonoid in the petroleum ether extract.

Table 8: Preliminary phytochemical screening of *Ficus bengalensis* Linn

Tests	Pet ether extract	Ethanol extract	Water extract
Alkaloids:			
Dragendroff's test	-	+ ve	+ ve
Mayer's test	-	+ ve	+ ve
Hager's test	-	+ ve	+ ve
Wagner's test	+ ve	+ ve	+ ve
Carbohydrates:			
Fehling's test	+ ve	+ ve	+ ve
Molish test	+ ve	+ ve	+ ve
Gums:			
Rheuthenium red + HCl	+ve	- ve	- ve
		- ve	- ve
Tannins:			
Aq. FeC_{l3} Test	-	+ ve	+ ve
Alc. FeC_{l3} Test	-	+ ve	+ ve
Flavonoids:			
Lead acetate test	-	+ ve	+ ve
Shinoda test	-	+ ve	+ ve
Alkaline test	-	+ ve	+ ve
Sterols:			
Salfowaski test	+ ve	+ ve	+ ve
Liberman Burchad test	+ ve	+ ve	+ ve
Saponins:			
Foam test	+ ve	+ ve	+ ve
Lead acetate test	+ ve	+ ve	+ ve
Glycosides:			
Baljet test	+ve		
Legal's test	-ve		
Killer lillani test	+ve		
Bromine water test	+ve		

Inflammation has different phases. The first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes, and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan induced inflammation, which is one of the most feasible methods of screening anti-inflammatory agents. The development of carrageenan-induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin, and kinins and the second phase is related to the release of prostaglandins and bradykinins.[23-27] We observed that EEFB-I and EEFB-II showed significant inhibition against carrageenan-induced paw edema in the dose-dependent manner but in the case of PEEFB-I and PEEFB-II, the failure to possess the anti-inflammatory effect may be due to the absence of flavonoid in the petroleum ether extract.[28] This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the ethanolic extract. This anti-inflammatory effect of EEFB-I and EEFB-II may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory activity.[29] The presence of flavonoid identified might be responsible for the anti-inflammatory activity in ethanolic extract. Thus, it is concluded that the ethanolic extract of the bark of *Ficus bengalensis* produces significant anti-inflammatory activity in a dose-dependent manner.

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Source of Support: Nil, **Conflict of Interest:** None declared.