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Pharmacognostic, Phytochemical Investigation of *Ficus bengalensis* Linn.Bark

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Abstract : The bark of *Ficus bengalensis* Linn. belongs to Family Moraceae is very effective in diabetes, nervous disorders, ulcers and skin diseases and also important ingredient in various ayurvedic and traditional formulations. The bark of *Ficus bengalensis* Linn. is most of times adultrated with other species of *Ficus* because of less knowledge of identification. Hence the detailed pharmacognostic, phytochemical evaluation based on morphological, microscopical and standardization parameters like foreign organic matter, moisture content, total ash, water soluble ash, acid insoluble ash, foaming index, alcohol soluble extractive value and water soluble extractive value were carried out for the quality and identity of the drug. The preliminary phytochemical investigations of the Hydro-alcoholic extract of *Ficus bengalensis* Linn.bark shows the presence of major constituents like flavonoids, tannins, carbohydrates, saponinand steroids. The isolated saponin derivatives characterized by using UV and FTIR techniques.
Key words : Pharmacognostic, Phytochemical, *Ficus bengalensis* Linn.

Introduction:

Ficus bengalensis(Moraceae) is commonly known as a Banyantree or Vata or Vada tree in ayurveda. The tree sends downits branches and great number of shoots, which takeroot and become new trunks. The plant is a large evergreen tree distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India. It is a grown in gardens and road sides for shades.[1]

Leaves yield quercetin-3-galactoside, rutin, friedelin, taraxosterol, lupeol, β-amyrin along with psoralen, bergapten and β-sisterol. 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta-sitosterol-alpha-Dglucose and meso-inositol have been isolated from the bark of the *Ficus bengalensis*[2].

The uses reported are anti-inflammatory[3], antioxidant[4], antidiarrhoeal[5], antidiabetic activity[6] etc. Many plants of this genus are used in medicine for the treatment of skin diseases, enlargement of liver and

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spleen, dysentery, diarrhoea, diabetes, leprosy, lung complaints, leucorrhoea, heart diseases, cough, asthama, piles, ulcers, gonorrhoea, rheumatism and lumbago[7].

Pharmacognostic, phytochemical evaluation generally carried out for the quality and identity of the drug. In the present study, an attempt was made to study the pharmacognostic phytochemical investigation of the*Ficus bengalensis* Linn.bark.

Materials and Methods:

Plant material:

The bark of *Ficus bengalensis* Linn.were collected from local areas of Pune, Maharashtra and authenticated by Joint Director, at Botanical Survey of India (BSI), Govt. of India, Ministry of Environment and Forests, Pune, India.

Reagents:

All chemicals used in the present study were analytical grade and purchased from merck specialties pvt. Ltd. (Mumbai, India).

Morphological & Microscopical Study:

The bark of *Ficus bengalensis* Linn were investigated for its macroscopic characteristicsas per parameters of WHO guidelines1998.[8] The macroscopic characters such as color, odors, taste, shape, size and texture which were observed. For microscopical study the bark were boil in water over a Bunsen flame for a few minutes. Take sufficient number of sections, transfer the sections to the watch glass containing water with the help of a brush, reject thick and oblique one. Mount the section on glass slide, Transverse section of dried bark were stained with phloroglucinol: Hydrochloric acid (1:1), Sudan red III, and observed under microscope at 10X, 20X, 45X. The photomicrographs were taken using a digital biological microscope MOTIC223® CCD camera supported.[9]

Standardization of bark:

The standardization parameters like Foreign matter, Loss on Drying (LOD), ash value, extractive values in different solvents, foaming index were carried out for purity and identity of drug.[10,11]

Foaming index:

Accurately 1 gm of finely powdered drug transferred to a 500 ml conical flask containing 100ml of boiling water. This material wasboiled for 30 min. Cool and filter into a 100 ml volumetric flask and sufficient water added to make the volume to 100 ml. The above decoction were taken into 10 stoppered (height 16cm, diameter 16mm), graduated test-tubes in a series of successive portions of 1, 2, 3 upto 10 ml and the volume of the liquid in each test tube adjusted with water to 10 ml. These tubes shaked vertically for 15 seconds, 2 frequencies/ sec. and allowed to stand for 15 min and measured the height of the foam.

$$\text{Foaming index} = \frac{100}{a}$$

a = volume in ml of the decoction used for preparing dilution in the tube where foaming to a height of 1 cm is observed.[12]

Preparation of extract:

The air-dried bark of *Ficus bengalensis* Linn.powdered (40 size mesh) and around 500 gm of powder was subjected to extraction (soxhlet) with petroleum ether to defat the powder. This defatted powdered drug was macerated with methanol 70% and water 30% (Hydro-alcoholic) solvent for 6thdays, during maceration powdered drug was reflux for 4hrs on 1st and 6th day. After the effective extraction, solvent were concentrated using rotary evaporator and solvent was removed, the obtained extracts were subjected to phytochemical investigation.

Phytochemical screening:

The freshly prepared Hydro-alcoholic extract of *Ficus bengalensis* bark was qualitatively tested for the presence of major phytochemical constituents. This was carried out by the method described by J. B. Harborne, 1984.[13]

Isolation of Saponin:

The 2gm air-dried bark extract as per the method describe earlier were dissolved in 100ml of distilled water and hydrolyses this solution by addition of 10 ml dilute HCl. After acid hydrolysis extract further fractionated with butanol saturated with water, then butanol layer were concentrated to get crude saponins.[14]

Characterization by chromatographic method:

Thin layer chromatography (TLC) for Crude Saponin:

TLC for crude Saponin extract was performed for the presence of saponin. Various solvents such as chloroform, ethyl acetate, acetone, methanol, glacial acetic acid and water were used for optimizing mobile phase and aluminum plates precoated with silica gel 60F254 was used as stationary phase. The anisaldehyde-sulphuric acid reagent was used as derivatising agent.[15]

HPTLC finger print for crude saponin:

The completely dried isolated crude saponin extract were accurately weighed and stock solutions of 10 mg/ ml were prepared. Theses stock solutions were further diluted with methanol to get solutions of 1 mg/ml.The test solution of suitable concentration (100-500 ng/ml) was applied in doublets on precoated silica gel 60 F254 HPTLC plates (E. Merck), of uniform thickness of 0.2mm. The plates were developed in a solvent system of Toluene: methanol (9:1 v/v) in CAMAG twin trough chamber up to a distance of 8 cm. After development, the plate was dried in air and sprayed by using anisaldehyde-sulphuric acid reagent solution and subsequently heated at 120^0C for derivetization. These plates were scanned at 525nm absorbance / reflection mode using reflectance mode by CAMAG Scanner III and CATS software was used to analyze the plates.[16]The three spots were observed in UV chamber (violet, pink and slightly pink), Isolation of these saponin derivatives were done by preparative TLC.[17]

Characterization by UV and FTIR method:

The UV absorption spectrum of isolated compounds from crude saponin A, B, C was recorded on JASCO V 630 spectrophotometer, the FTIR absorption spectrum of isolated compounds A, B, C was recorded on shimandzu-8400U using KBr disc.

Results and Discussion:

Morphological & Microscopical Study:

The bark of old tree is 10-15 mm thick, brownish to grey in color, outer bark short in fracture whereas it fibrous from inner side. Taste of bark is faint, astringent. Externally bark having rough surface due to presence of lenticels (Fig. 1).

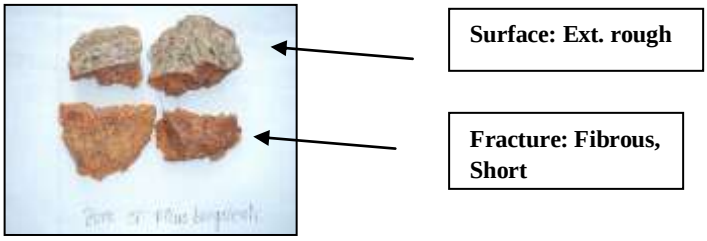
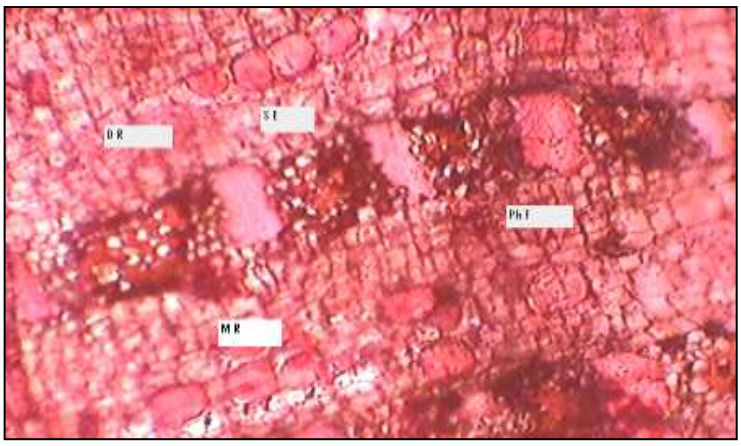


Fig. 1 Bark of *Ficus bengalensis* Linn.

In microscopy of mature bark transverse section shows medullary rays composed of thick walled cells, collapsed Secondary Phloem, pericyclic fibers cell, single isolated circular lignifiedphloem fibers, oil cells and short, narrow and straightsieve Tube(Fig. 2).



(DR- Dilated ray, PhF - Phloem fibers, SE- Sieve elements, MR-Medullary ray)

Fig. 2 T. S. of *Ficus bengalensis* Linn bark.

Standardization of bark:

The foreign organic matter and moisture content present in the bark was found to be 0.01% w/w and 4.64% w/w respectively. The total ash, water soluble ash and acid insoluble ash were present in the bark 5.0% w/w, 2.0% w/w and 1.0%w/w respectively. The foaming index of bark was observed 117.64%. Alcohol solubleExtractive Values, water solubleExtractive Values were recorded in Table 1.

Table 1.Evaluation parameter of *Ficus bengalensis* Linn bark.

Sr. No.	Evaluation Parameter	(% w / w)
1	Foreign organic matter	0.01 %
2	Moisture content	4.67 %
3	Total ash	5.0 %
4	Water- soluble ash	2.0 %
5	Acid insoluble ash	1.0%
6	Sulphated ash	4.0%
7	Alcohol solubleExtractive Values	1.9 %
8	Water solubleExtractive Values	1.74 %

Phytochemical screening:

Phytochemical analyses of the Hydro-alcoholic extract of *Ficus bengalensis* Linn.bark revealed the presence of major constituents like flavonoids, tannins, carbohydrates, saponin and steroids. The detail reports of phytochemical screening were shown inTable 2 and Table 3.

Table 2.Preliminary screening of extract from *Ficus bengalensis* Linn bark.

Sr. No.	Extract	Color	Consistency	Yield (%w/w)
1	Petroleum ether	Yellowish brown	Semisolid	0.46 %
2	Hydro-alcoholic (Methanol 70%+Water 30%)	Dark brown	Solid	1.68 %

Table 3. Preliminary phytochemical investigation of *Ficus bengalensis* Linn bark extract.

Sr. No.	Test Perform	Pet ether extract	Hydro-alcoholic extract
1	**Test for carbohydrate**		
	Molish's test	+	+
	Fehling test	+	+
	Benedict's test	+	+
	Barfoed's test	+	+
2	**Test for Proteins**		
	Biuret Test	-	-
	Millions Test	-	-
3	**Test for amino acids**		
	Ninhydrine test	-	-
4	**Test for Steroids**		
	Salkowski test	+	+
	Liebermann's test	+	+
	Libermann-Burchard reaction	+	+
5	**Test for Glycosides**		
	Cardiac	+	+
	Anthraquinone	-	-
	Cyanogenic	-	+
6	**Test for Saponin**		
	Foam test	-	+
	Haemolytic test	-	+
7	**Test for Flavonoids**		
	Shinoda test	-	+
	Lead acetate	-	+
	Sodium hydroxide	-	+
8	**Test for Alkaloids**		
	Dragendroff's test	-	-
	Mayer's test	-	-
	Hager's test	-	-
	Wagner's test	-	-
9	**Test for Tannins and phenolic compounds**		
	5% $FeCl_3$ test	-	+
	Lead acetate	-	+
	Dil.Potassium permanganate	-	+
	Bromine water	-	+
	Dil. Iodine solution	-	+
	Potassium dichromate	-	+
	Dil. HNO_3	-	+
10	**Test for Mucilage**	-	-

Where, (+) = Indicates Presence of phytochemicals, (-) = Indicates Absence of phytochemicals.

Isolation and characterization:

Crude saponins were isolated from the hydro-alcoholic extract of *Ficus bengalensis* Linn.Bark by chromatographic techniques. The percentage yield of isolated crude saponin was found to be 1.0% w/w. The isolated crude saponin from bark of *Ficus bengalensis* Linn were subjected to thin layer chromatography to detect the various constituents present in it.

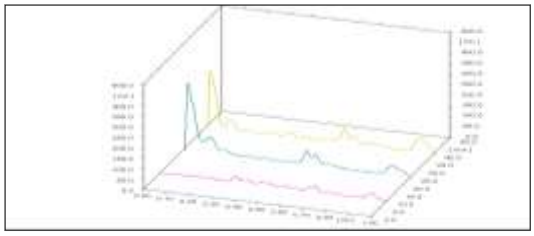
Thin layer chromatography (TLC):

TLC for crude Saponin extract was performed to further confirm the presence of saponins. The best separation was found in the mobile phase of Chloroform: Glacial acetic acid: Methanol: Water in the pro-portion 3:1.5:0.6:0.2 and Toluene: Methanol (9:1),the Rf values were found 0.25, 0.50, 0.60, 0.75 and 0.46 respectively (Fig. 3).

Fig. 3.Thin layer chromatography (TLC).

HPTLC finger print for isolated crude saponin:

The proposed HPTLC method was found accurate and sensitive; it is also found to reproducible, simple and rapid for the estimation of crude saponin in barks of *Ficus bengalensis* Linn. The Rf values of isolated crude saponins from developed HPTLC chromatogram were found 0.55 and 0.58. The Rf value of standard ß-sitosterol (0.52) were found to be closed to the Rf value of the isolated crude saponin (Graph 1). The isolation of three spots were done by preparative TLC method using toluene: methanol (9:1) as a mobile phase (Fig. 4).

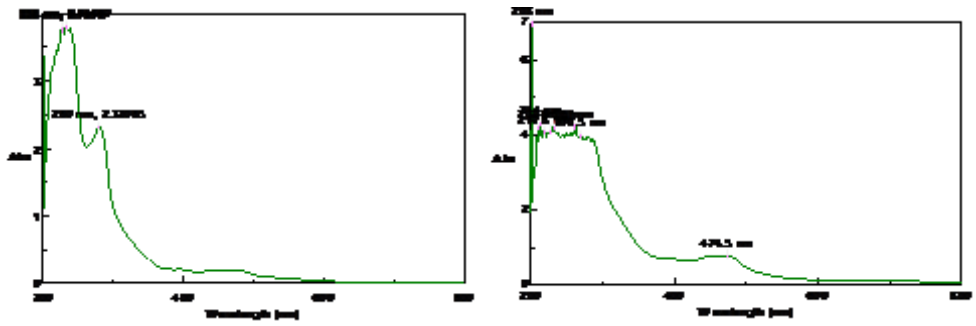


Graph 1.HPTLC profile of isolated crude saponin at 525nm.

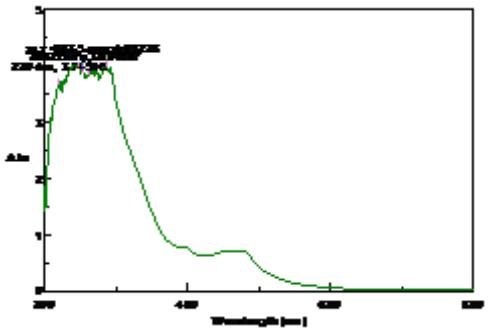
Fig. 4. Preparative TLC plate of crude Saponin.

Characterization by UV and FTIR method:

UV spectrum of three isolated compounds from crude saponin extracts A, B, C was recorded in JASCO V 630 spectrophotometer in AR-grade methanol.The UV spectrum of isolated compound were shows characteristic absorption at λ_{max} at 220nm, 280nm for compound A, 208nm, 215nm, 275nm and 475nm for compound B and 208nm, 220nm, 275nm, 310nm and 475nm for compound C respectively (Graph 2).



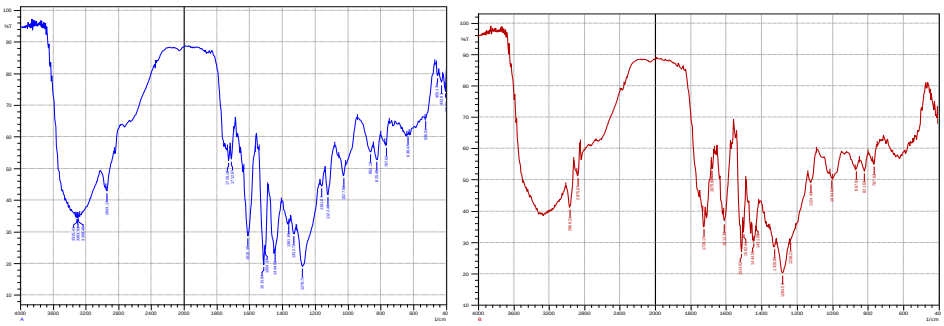
A B



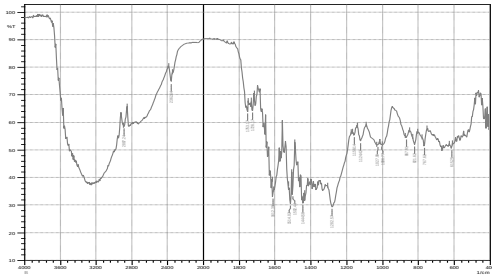
C

Graph 2.UV spectrum of isolated compound A, B, C.

The FTIR absorption spectrum of isolated compounds A, B, C was recorded in shimandzu-8400U spectrophotometer using KBr disc. The FT-IR spectrum of compound A was shows characteristic absorption frequencies (in cm-1) at 3315.41, 3303.83, 2943.17, 1712.67, 1610.43. The FT-IR spectrum of compound B was shows characteristic absorption frequencies (in cm-1) at 3387.54, 3356.45, 2966.31, 1726.17, 1612.38. The FT-IR spectrum of compound C was shows characteristic absorption frequencies (in cm-1) at 1612.38, 1726.17, 2887.24, 3347.24, 3368.34 (Graph 3).



A **B**



C

Graph 3.FTIR spectrum of isolated compound A, B, C.

Conclusion:

The results available from the pharmacognostic study of bark from *Ficus bengalensis* Linn. shows the purity and identity of the drug. The standardization parameters like foreign organic matter, moisture content, total ash, water soluble ash, acid insoluble ash, foaming index, alcohol soluble extractive value and water soluble extractive value will represents the quality of the drug. The preliminary phytochemical investigation of the Hydro-alcoholic extract of *Ficus bengalensis* Linn.bark revealed the presence of major constituents like flavonoids, tannins, carbohydrates, saponin and steroids. The isolated saponin derivatives characterized by using UV, FTIR techniques, however it need to characterized by further advanced techniques for confirmation of compound and evaluation of pharmacological potential for the same.

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