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Aishwarya J Bhange
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India

Rutuja D Walke
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India

Yogita B Shinde
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India

Dnyanesh A Galande
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India

Corresponding Author:
Aishwarya J Bhange
PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India

Pharmacognostic and phytochemical evaluation on aerial roots of *Ficus benghalensis* Linn

Aishwarya J Bhange, Rutuja D Walke, Yogita B Shinde and Dnyanesh A Galande

Abstract
The aerial roots of *Ficus benghalensis* Linn have been reported to have immunomodulatory, anti-bacterial and hair growth promoting activities. There are no reports on pharmacognostical and phytochemical investigations of these aerial roots. The aim of the present study was evaluation of sectional microscopy, powder characteristics, preliminary phytochemical screening of successive solvent extracts (petroleum ether, toluene, dichloromethane, methanol, ethyl acetate and aqueous extracts) of the aerial roots. The results of sectional microscopy indicated the presence of cork cells, phloem and xylem. The powder characteristics indicated the presence of calcium oxalate crystals, lignified fibres. Preliminary phytochemical screening indicated the presence of flavonoids, phenolics and saponins was done.

Keywords: *Ficus benghalensis*, phytochemical screening, anti-bacterial, hair growth

Introduction
It is great to the credit of the people of India that they have been gifted with larger number of medicinal plants than the natives of any country on the face of earth. Natural products have served as an important source of drugs since ancient times and about half of the useful are derived from natural sources. India is being sitting on a goldmine of well-recorded and traditionally well-practiced knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. It is generally estimated that over 6000 plants in India are used in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the third world countries [1].
The effective use of automated procedures and data bases in the isolation, identification and biological profiling of bioactive compounds from natural sources will be a best guarantee to the continued discovery of novel chemo nature. Natural products research continues to explore a variety of lead structure, which may be used as template for the development of new drugs by the pharmaceutical industry, while microbial products have been the mainstay of industrial natural product discovery. In recent years, phytochemistry has again become a field of active interest.
The banyan tree is a large, long-lived, fast-growing evergreen tree up to 20 (-25 m) tall. It has a wide leafy crown of horizontal branches covering up to 100 m around, and surrounded by aerial roots. The banyan starts as an epiphyte. Its seeds can be laid by birds at branch forks and germinate on the host tree (US Forest Service, 2014). Its aerial roots grow around the host and may strangle it, hence the name "strangler fig". The trunk is massive, fluted. The bark is smooth, softly puberulous when young. The leaves are large, 8-25 cm long x 6-20 cm broad, stoutly petiolated. The lamina is coriaceous, nerved, ovate to obovate in shape. The lamina is glabrous on the upper face and finely pubescent beneath. The banyan tree is monoecious, male flowers and female flowers are distinctly borne on the tree. The inflorescence is hollow, consisting in a variable number of flowers in a pear-shaped receptacle. Gall flowers, a third kind of (sterile) flower exists. Gall flowers are produced in a large amount by the fig wasp, a fig tree pollinator. The fruit is a globose to depressed-globose fig, 1.5-2.5 cm in diameter and pinkish red in colour [2].

Review literature
1. Analgesic activity study
All the animals (albino mice) of 25-30 g weight were obtained. The animals were kept under standard laboratory conditions at room temperature with normal humidity. They were given standard diet and water ad libitum. The animals have fasted for 12 h with the availability of normal water before the experiment. The animals were divided into four groups (n=6), control, standard, test 1, and test 2 of two different concentrations of ethyl acetate extract [3].

2. A study on *in vitro* antibacterial activity of *Ficus benghalensis* Linn

It is concluded that *Ficus benghalensis* Linn extracts can be used as an effective antibacterial agent against dental caries and have activity against both Streptococcus mutans and actinomyces viscous [4].

3. Wound healing activity

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (The deepest skin layer) begin to increase collagen (Connective tissue) production. Later, the epithelial tissue (The outer skin) is regenerated. There are three stages to the process of wound healing: inflammation, proliferation, and remodeling. Traditionally, *Ficus benghalensis* is used for wound healing. Since no detailed scientific data are available regarding the wound-healing activity of *F. benghalensis*, the present study was designed to explore the same. The wound-healing efficacy of ethanolic and aqueous extracts of *F. benghalensis* was evaluated in excision and incision wound models [5].

4. Antimutagenic and antioxidant activity

Aqueous extract of heat treated stem bark was used to determine antimutagenic and antioxidant activity by using Ames test (standard plate incorporation assay) and ex-vivo inhibition of lipid peroxidation in liver microsomes of rats respectively. Extract concentration of 500 µg inhibited mutagenic activity of sodium azide (NaN_3) in *Salmonella typhimurium* with IC_{50} value of 70.24 µg/ml and inhibited microsomal lipid peroxidation with value of 80 ug/ml [6].

Material

Collected from Aerial roots of *Ficus benghalensis* from herbal garden in PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre Kharadi Pune 411014.

Chemicals: Sulphuric acid, Glacial acetic acid, Ferric chloride, Sodium hydroxide in Loba Chemie Pvt. Ltd.

Methodology

A) Microscopy

1) Microscopy of Aerial Roots

Generally, the light is passed through a condenser to focus it on the sample to have maximum brightness. After the light has passed through the sample, it goes through the objective lens to magnify the image of the sample & then to the oculars, where the enlarged image is viewed. After TS of buff colored aerial roots was taken [7].

2) Microscopy of Powder

The fresh aerial roots collected, were dried in oven at 60°C for 4-6 hours to make them moisture free and ground using an electric grinder. The resultant powder was passed through sieve no. 60 and was examined microscopically by mounting in chloral hydrate solution and phloroglucinol solution. This was done under 10x magnification [7].

B) Physical analysis

1) Ash value

The ash content of the crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration. Ash value varies with narrow limits in case of the individual drug but varies considerably in case of different drug [8].

2) Determination of water soluble ash

The ash obtained in the determination of total ash was boiled for 5 Minutes with 25 ml of water. The insoluble was collected on an ash less filter paper and washed with hot water. The ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450 °C. The weight of the insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as water soluble ash [9].

3) Determination of acid insoluble ash

The ash obtained was described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 min. The insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into pre-weighed silica crucible. The percentage of acid insoluble ash was calculated with dried drug [10].

C) Determination of extractive value

1) Procedure of extractive value

The dry powdered plant material was extracted with water, methanol. Ethanol, acetone, chloroform and petroleum ether using a maceration process. 2 gm of the coarsely powdered plant material was weighed in a weighing bottle and transferred into a dry 250 ml conical flask. Then the flask was filled with different solvents 30 ml separately. The flasks were corked and kept aside for 24 hrs at room temperature, shaking frequently. The mixtures were filtered through Whatmann No. I filter paper into a 50 ml measuring cylinder. After the filtrate has obtained, it was then transferred into a weighed poetry plates. The obtained extracts were concentrated to dryness by keeping filtrate for complete evaporation of solvent. The extractive value in percentage was calculated by using following formula and recorded.

Extractive value = Weight of dried extract/Weight of plant material ×100

2) Procedure of Aerial Roots extract

The *Ficus benghalensis* aerial roots were separated and cleaned well. Cleaned roots were then dried under shade. The drying was done until all the water molecules evaporated and aerial roots became well-dried for grinding. After drying, the aerial roots were ground well using mechanical blender into fine powder and transferred into air-tight container with proper labelling for further use. The dry and roper labelling for further use. The dried and powdered *Ficus benghalensis* fruits were extracted sequentially with Hydroalcohol using soxhlet apparatus. 500 g of dried aerial roots of benghalensis powder was weighed and successively extracted with 2.5 litres of solvent like hydroalcohol by soxhelation for a period of 72 hours. The extract was concentrated by heating the mixture on the heating mantle the dried mass of the extract was used for carrying out Qualitative Phytochemical Screening for different phytochemical constituents' *viz*., carbohydrate, reducing sugars, amino acids, protein, steroid, flavonoids, saponins, alkaloids, tannins, phenol, vitamin C, terpenoids, glycosides, phlobatannins, anthroquinones and chloride. The method employed to analyses the phytochemicals are described below [11].

D) Qualitative Phytochemical Evaluation

1) Test for alkaloids

a) **Dragendroff's Test:** In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and

the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

b) Wagner's Test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

c) Mayer's Test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids [12].

2) Test for Tri- terpenoids

Salkowski Test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink color indicates presence of terpenoids [12].

3) Test for steroids

Liebermann Burchard Test: To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids [13].

4) Test for coumarins

To 1 ml of extract, 1 ml of 10% sodium Hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour [14].

5) Test for tannins

1. To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.
2. The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins [15].

6) Test for saponins

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

7) Test for carbohydrates

i) Molisch's Test: To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.

ii) Fehling's Test: To the extract, equal quantities of Fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

iii) Benedict's Test: To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates [16].

8) Test for phenols

Ferric chloride test: To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green color indicates color of phenol [17].

Result and Discussion

A) Microscopy

1) Microscopy of Aerial Roots

The transverse section of aerial roots indicated the presence of thick brownish continuous layer of cork cells. The cork cells were mostly uniform in shape and polygonal in appearance. A single attened layer of epidermis was seen below this. Below the cork cells, there was a thick layer of cortex which appeared to be polygonal in shape. The phloem was characterized by the presence of thick walled oval shaped cells. Horizontal tracks of cambium were observed followed by double walled polygonal cells of xylem.

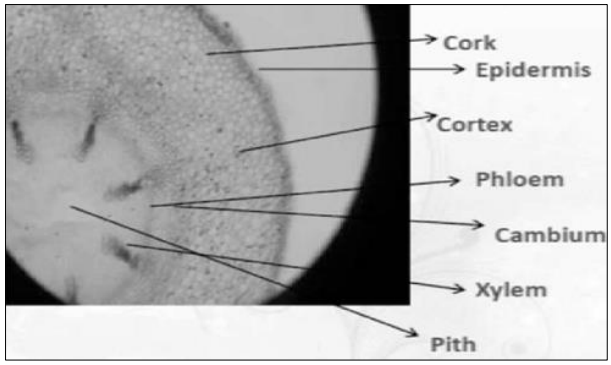

Fig 1: Microscopy of Aerial Roots of F. Benghalensis

2) Microscopy of Powder

1. Aerial root powder
2. Border pitted vessels
3. Border pitted
4. Stone cell with lumen
5. Simple starch grain
6. Compound starch grains
7. Prismatic crystal
8. Fiber with tannin content
9. Stone cell filled with tannin
10. Tannin content
11. Rhomboidal crystal
12. Septate fibers
13. Pitted scleroids
14. Pitted stone cells
15. Parenchyma with tannin content
16. Lignified fibers through medullary rays
17. Cork in surface view
18. Stone cells with tannin

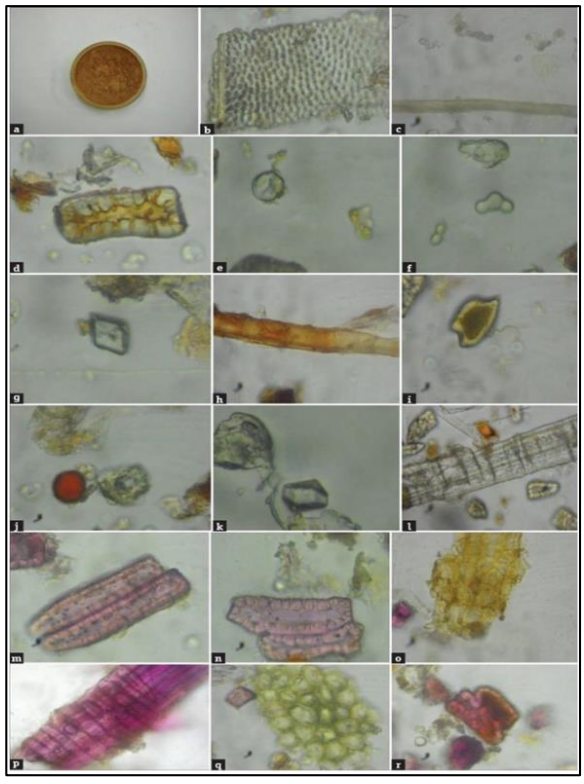

Fig 2: Microscopy of Powder

B) Physical Analysis
Ash value

Fig 3: Determination of Ash value

Table 1: Determination of Ash value

Total ash	**3.32**
Acid insoluble ash	2.71
Water soluble ash	2.92
Heavy metals	No turbidity shown

B) Determination of Extractive value

Table 2: Determination of Extractive value

Solvent	**Weight of plant material**	**Color of extract**	**Extractive value**
Water	2	Dark brown	7.6
Methanol	2	Yellowish green	10
Ethanol	2	Green	8.6
Acetone	2	Green	1.5
Chloroform	2	Light	2
Petroleum ether	2	Colorless	0.5

E) Qualitative Phytochemical Evaluation

Table 3: Quantitative Phytochemical Evaluation

Phytochemical Extract	**n- Hexane Extract**	**Ethyl Acetate Extract**	**Methanol Extract**
Alkaloids	_	_	+
Terpenoids	+	+	+
Steroid	_	+	+
Tannin	_	+	+
Saponin	+	+	+
Carbohydrate	_	_	_
Phenol	_	+	+

Conclusion

From the above study, it is concluded that aerial roots of *Ficus benghalensis* may represent a new source for biologically active components that can establish a scientific base for the use of this constituent in modern medicine. These alocal ethno-medical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind. These findings suggest a new pathway in elucidating a potent agent from *Ficus benghalensis*.

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