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PRILIMNARY PHYTOCHEMICAL INVESTIGATION AND *IN VITRO* ANTI BACTERIAL ACTIVITY OF AERIAL ROOTS OF *FICUS BENGHALENSIS* LINN

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ABSTRACT

Medicinal plants represent a rich source of anti bacterial agents. The traditional medicine involves the use of different plant extracts of bioactive constituents. Ficus is a huge tropical deciduous or evergreen tree with more than 800 species. **Objectives:** Bactericidal effect of ethnomedicinal important plant species *Ficus benghalensis* (Family-Moraceae) was screened against Gram +ve and Gram –ve bacteria. **Methods:** Anti bacterial activity of ethanolic extract of aerial roots was assessed by using Agar Well Diffusion Assay. Preliminary phytochemical analysis was also carried out. **Results:** Phytochemical evaluation showed the presence of Alkaloids, Flavonoids, Simple Phenolics, Steroids and Saponins. Result of the well diffusion study revealed that 95% ethanol extract have the resistance activity against all the tested bacterial strain. *Ficus benghalensis* roots showed significant anti bacterial activity. **Conclusion:** These findings suggest the excellent medicinal bioactivity of *Ficus benghalensis* and explain the popularity of this plant in the folk medicine as a remedy for bacterial disorders, thus supporting its folklore application as preventive remedy against bacterial diseases.

INTRODUCTION:

The plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. The plant kingdom is a virtual goldmine of potential drug targets and other active drug molecules waiting to be discovered. During the last decade, use of traditional medicine has expanded globally and gained popularity. Plant based drugs are having a revived interest now-a-days because of awareness of deleterious effects of modern synthetic drugs. Natural products can play a very crucial role in pharmaceutical industry as drug them or as drug carrier or bio-enhancers or excipients. The importance of herbal/plant medicines is well documented in Vedas, which proved to be the ancient literature. The properties of the plants and their remedies are given in detail and in fact Ayurveda is the very principle root for the emergence of Ancient medical science in India that gave origin to branches like Sushruta and Charka Samhita. According to WHO, today more than one billion people rely on herbal medicines to some extent. The WHO has listed 21,000 plants have reported medicinal uses around the world. India has a rich medicinal plant flora of some 2500 species, of these 2000 to

3000 atleast150 species are used commercially on a fairly large scale. Foreign researchers have always appreciated the traditional Indian healers. The healing powers of traditional herbal medicines have been realized since antiquities .About 34 percent of all pharmaceutical preparations come from higher plants and it goes to 60 percent when bacteria and fungi origins are taken into account. It is estimated that the country exports about 550 cores worth of herbal drugs but with the rich diverse botanical resource in our country, this is not an impressive export performance considering the world wide herbal market worth US 60 billion dollars. It is also necessary to integrate modern knowledge with traditional knowledge. The drugs and products of the industry are working on the scientifically defined techniques and explained with modern biological and chemical definitions and tools, and that alone will give a therapeutically active herbal original drug available for health care worldwide. The efficacy of some herbal products is beyond doubt, the most recent example being *Artemisia annua, Taxes brevifolia, and Silybium marianum, Hypericum perforatum, Allium sativum* and *Ginkgo biloba* are popularly used herbal remedies among people. All these herbals are standardized for active constituents. Standardization means adjusting the herbal drug preparation to a defined content of active constituent. Extract refers to a concentrated preparation of active constituent of a medicinal herb. The concept of standardization extracts definitely provides a solid platform for scientific validation of herbals.

Phytochemistry deals with the determination of chemical constituents in plant material. Most of the herbal extracts are made from crude herb they can vary in percentage of active constituents, which further influences the therapeutic activity of herbs depending upon the source. These medicinal herbs become popular due to single active constituents. Keeping in mind the overall scenario, only selected medicinal herbs have been used in standardized form and market analysis clearly favours them.

Taxonomic classification of *Ficus benghalensis* Linn
Kingdom: Plantae
Sub Kingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Hamamelidae
Order: Urticales
Family: Moraceae
Genus: Ficus
Species: *F. benghalensis*

Figure 1: *Ficus benghalensis* plant

Figure 2: *Ficus benghalensis* aerial roots

The present aim is to study of the physicochemical standards, phytochemical screening of extract through preliminary tests and anti bacterial activity of ethanolic *Ficus bengalensis* aerial root extract.

MATERIALS AND METHODS

Plant collection

Aerial roots of *Ficus benghalensis* were collected from local area of Medchal dist. Telangana, India.

Preparation of extract: Shade dried powdered extract of roots was subjected to successive Soxhlet extraction using 95%

ethanol solvent. After extraction solvent was removed under reduced pressure. Extracted material was stored in airtight container till use.

Physicochemical standards

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 drugs.

Determination of total ash

About 2g of powdered drug was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450 ^{0}C until free from carbon. The crucible was cooled and weighed for constant weight. The percentage of total ash was calculated with reference to the air-dried drug.

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 matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450 ^{0}C. The weight of the insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as the water -soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

Determination of acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 minutes. 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 reference to the air-dried drug.

Loss on drying

Five grams of the powdered crude drug was accurately weighed in a tarred dish and dried in an oven at 100 - 105 ^{0}C. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

Crude fiber content

About 2 g of drug was extracted with diethyl ether. The residue was transferred to a digestion flask containing 200 ml of 0.225N sulphuric acid fitted with the condenser and heated after 30 min the contents were filtered, washed with boiling water until the washings are basic. The residue was transferred to a flask with 200 ml of sodium hydroxide solution (0.13). The flask was connected with the reflux condenser and boiled for 30 min, then filtered through ash less filter paper (Whatmann No.41) followed by washing with water until free from alkali, it was washed with 15 ml of alcohol. The filter paper was transferred to a crucible and ignited at 450 ^{0}C. It was cooled in a desiccator and weighed. The loss in weight represents the crude fiber content.

Extractive values

The solvents obtained commercially (LR – Grade Extra pure) were purified by distillation methods prior to use for extraction and for phytochemical investigation. 1kg coarse powder was subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours by various solvents of increasing polarity (methanol, ethanol, water) by decanting and drying the marc after each extraction. The solvents were recovered by distillation of the extracts at 75 ^{0}C to 80 ^{0}C.

The extracts were dried under desiccator and percentage yield was calculated.

Fluorescence analysis

Fluorescence analysis of the powder was observed in day/visible light and UV light (Long wavelength – 365 nm and Short wave length – 265 nm). The drug powder was treated with various solvents like benzene, chloroform, ethyl acetate and 50% aqueous-ethanol and acids like 1M Hydrochloric acid and alkaline solutions like 1N sodium hydroxide. They were subjected to fluorescence analysis in daylight and in UV-light.

Phytochemical evaluation

Qualitative phytochemical evaluation

The different chemical tests were performed for establishing profile of the extract for its chemical composition; the following chemical tests for various phytoconstituents in the petroleum ether, chloroform, ethyl acetate, alcohol and water extracts were carried out as described below**.**

A) Test for alkaloids:

i) 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.

ii) 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.

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

iv) Hager's Test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

(B)Test for tri terpenoids: i) Salkowski test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink colour indicates the presence of Terpenoids.

ii) Hirshonn reaction: When the substance was heated with trichloro acetic acid, red to purple colour was observed.

(C)Test for steroids:

i) 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.

(D)Test for coumarins:

i) 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.

(E)Test for tannins:

i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

(F)Test for saponins:

i) 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.

(G)Test for flavones:

i) Shinoda Test: To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red colour showed the presence of flavones.

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow colour shows the presence of flavones.

(H)Test for quinones:

i) To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.

(I)Test for flavanones:

i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.

ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

(J)Test for anthocyanins:

i) To the extract, 10% sodium hydroxide was added, and the blue colour shows the presence of anthocyanins.

ii) To the extract, conc. sulphuric acid was added, and the yellowish orange colour confirms the presence of anthocyanins.

(K)Test for anthraquinones:

i) Borntrager's Test: The extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet colour in the aqueous layer after shaking indicates the presence of anthraquinones.

(L) Test for phenols:

i) Ferric chloride test: To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green colour indicates the presence of phenols.

(M) Test for proteins:

i) Biuret Test: To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

ii) Xanthoprotein Test: To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed; it is then boiled and cooled. Then, 20% sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

iii) Tannic Acid Test: To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

(N)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 colour 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.

(O)Test for glycosides:

i) The extract was mixed with a little anthrone on a watch glass. One drop of concentrated sulphuric acid was added and made into a paste, warmed gently over water bath. The presence of glycosides was identified by dark green colour.

(P)Test for amino acids:

i) Ninhydrin test: Two drops of ninhydrin solution were added to the extract, a characteristic purple colour indicates the presence of amino acids.

(Q) Test for fixed oils and fats:

i) Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

Table 1: **Preliminary phytochemical screening of *Ficus benghalensis* root**

Constituents	Ethanol extract
Alkaloids	+
Tri terpenoids	-
Steroids	+
Coumarins	-
Tannins	+
Saponins	+
Flavones	+
Quinones	-
Flavonones	+
Anthocyanins	-
Anthraquinones	-
Phenols	+
Proteins	+
Carbohydrates	+
Glycosides	-
Amino acids	+
Fixed oils and fats	-

(+) Present, (-) Absent

Table 2: Physicochemical analysis of *Ficus benghalensis* root

S.No.	Parameter	Value (% w/w)
1.	Total Ash	13.2
2.	Acid Insoluble Ash	2.0
3.	Water Soluble Ash	7.02
4.	Crude Fiber Content	1.5
5.	Loss on Drying	8.6
6.	Extractive Value	
	a) Methanol	2.2
	b) Ethanol	2.63
	c) Water	1.65

Table 3: Fluorescence analysis of *Ficus benghalensis* root

S. no.	Treatment	Day Light	UV Light (254 nm)	UV Light (365 nm)
1.	Drug Powder	Light green	Green	Brown
2.	Powder + 1N NaOH (aq)	Light green	Light Green	Brown
3.	Powder + 1N NaOH (al)	Light green	Green	Light Brown
4.	Powder + 1N HCl	Light green	Light Green	Brown
5.	Powder + 50% H_2SO_4	Light green	Light Green	Brown
6.	Powder + 50% HNO_3	Light Brown	Light Green	Dark Brown
7.	Powder + Picric acid	Light Yellow	Light Green	Pale Brown
8.	Powder + Acetic Acid	Light Brown	Light Green	Brown
9.	Powder + $FeCl_3$	Pale Green	Light Green	Brown
10.	Powder + HNO_3 + NH_3	Light Brown	Light Green	Brown

Table 4: Anti bacterial activity of 95% ethanolic extract of aerial roots of *Ficus benghalensis* on bacterial pathogens

Sample extract	Concentration (mg/ml DMSO)	*S. aureus*	*Pseudomonas aeruginosa*	*E. coli*	*S. typhi*
95% ethanolic extract of aerial roots of *Ficus benghalensis*	25	3.2	3.2	3.0	0.00
	50	4.7	4.5	4.2	0.00
	75	7.3	7.1	6.1	0.00
	100	10.0	8.2	7.4	3.0

Anti bacterial activity screening:

The anti bacterial activity of 95% ethanolic extracts of *Ficus benghalensis* aerial roots was examined using agar well diffusion assay as described by known concentrations of ethanolic extracts of *Ficus benghalensis* aerial roots i.e. 25, 50, 75, 100 mg/ml DMSO were prepared. To the solidified Nutrient agar medium (composition in g/L: peptone:5; sodium chloride:5; beef extract:3; agar agar:17; pH: 7.2) 5mm wells were cut. An uninoculated plate served as media control and test bacteria swab inoculated was used as a organism control. A plate swab inoculated with test bacteria with a well filled with DMSO was used as treatment control. To the test plates an aliquot of each concentration of ethanolic extracts of *Ficus benghalensis* aerial roots was filled after swab inoculation of test bacteria viz. *Staphylococcus aureus* and gram negative bacteria i. e. *Pseudomonas aeruginosa, E. coli and Salmonella typhi*. All the plates were allowed to incubate at 37 ^{0}C for 24-48h in bacteriological incubator. After incubation the clear zone around the well was measured in mm scale defined as zone of inhibition of the test bacteria.

RESULTS AND DISSCUSSION

Preliminary Phytochemical Screening:

Our observation revealed that in the preliminary phytochemical screening was found that the ethanolic dried aerial root extract contain alkaloids, phytosteroids, terpenoids, saponins, flavonoids and proteins. The preliminary phytochemical screening results are illustrated in Table 1. The physiochemical screening results are illustrated in Table 2 and 3.

Plants are important source of pharmacophore which will function as new chemotherapeutic agents. The first step to develop a chemotherapeutic agent from plants would be the assay of *in vitro* anti bacterial activity. The extracts thus found active will help to identify the active compounds responsible for the activities from the plant. In recent years multi drug resistance is seen in pathogenic bacteria which has revived interest in the search of new antibacterial agents from natural sources. In fact, gram negative bacteria *P. aeruginosa* are frequently reported to have developed multi drug resistance to many of the antibiotics. But, the extracts especially the polar ones show a good activity against *P. aeruginosa*. The anti bacterial agents from natural sources also eliminate the side effects of synthetic or semi synthetic anti bacterial agents. The antibacterial activity of the plant extract was variable with various organisms. 95% ethanolic extract of aerial roots of *Ficus benghalensis* inhibited *S. aureus, P. aeruginosa and E. coli* with increasing seed extract concentration as 25, 50, 75 and 100mg/ml while the extract concentration of 25, 50, and 75mg/ml were unable to show zone of inhibition against *S. typhi* and the pathogen was inhibited at 100mg/ml concentration of the extract with a zone of inhibition of 3.0mm. Among the four concentrations 100mg/ml of extract concentration of aerial roots of *Ficus benghalensis* had highest zone of inhibition against all pathogenic bacteria tested (Table 4). The anti bacterial potential exhibited by roots extract may be contributed to the presence of alkaloids, saponins, tannins,

flavonoids and anthraquinones in preliminary phytochemical investigations. Further study is needed to characterize the active principles.

CONCLUSION:

From the above study, it is concluded that the aerial roots of *Ficus benghalensis* may represent a new source of anti-bacterial with stable, biologically active components that can establish a scientific base for the use of this in modern medicine. These local 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 anti bacterial agent from *Ficus benghalensis*.

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