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Research Article

STANDARDIZATION AND PHARMACOLOGICAL INVESTIGATION ON LEAVES OF FICUS BENGALENSIS

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ABSTRACT

Today the scenario of the World Health Organization estimated that in most developing countries the use of the medicinal plants. It is estimated that the 30% of the herbal medicine were dispensed in US contained different plant extracts or the active principles from the higher plants. Most of the medicines available in the market were derived from the natural products. Today these days the herbal medicines or the active principles obtained from the plants are used in the treatments of various diseases. Herbal medicines should be regarded as finished, labeled medicinal products that contain as active ingredients aerial or underground parts of plants, or other plant material. Plant material includes juices, gums, fatty oils, essential oils and any other substance of this nature. Herbal medicines may contain excipients in addition to the active ingredients. Medicines containing plant material combined with chemically defined active substances, isolated constituents of plants are not considered as the herbal medicines. In some countries herbal medicines may also contain natural organic or inorganic active ingredients which are not of plant origin. (Anonymous., 2002a). Multi-component botanical formulations can be standardized with newer techniques such as DNA fingerprinting, High Pressure Thin Layer chromatography, Liquid Chromatography and Mass Spectroscopy. The value of animal testing to establish safety and toxicity is not so critical if botanicals are used in traditional forms (Harborne., 1998).

INTRODUCTION

The matter of proper identification and appropriate quality, that is lack of adulteration, sophistication, or substitution, is an extremely important one in the field of herbal medicine. Many of today's widely used herbs were once the subject of official monographs in The United States Pharmacopoeia (USP) and The National Formulary (NF). These monographs established legal standards of identity and, subject to the limitations of the methods of the period, quality of the vegetable drugs.

No such standards exist today. Many of the herbs are collected in developing nations by persons who are not necessarily knowledgeable about the subtleties of plant taxonomy. They are usually sold to organizations that market them under their common names, not the recognized Latin binomial, which because of the lack of uniformity of the former can cause confusion.

Returning to the matter of standardization of herbs or herbal extracts, it must be noted that the concentration of active constituents in different lots of supposedly identical plant

material is highly variable. First of all, genetic variations exist. Just as one variety of apple tree will produce larger or tastier apples than another, so too will one variety of peppermint oil than another, even though the conditions of growth remain identical. These genetic variations in medicinal herbs, many of which are obtained from wild-growing plants, are not well understood.

Since few of these factors are precisely controlled even for cultivated plants, let alone those harvested from the wild, the most effective way to assure herbal quality is to assay, that is, to establish by some means, the amount of active constituents in the plant material. If the chemical identity of the constituent is known, it or a marker compound indicative of activity of the herb can usually be isolated and quantified by appropriate physical or chemical methods. (Robbers., 2002a).

General Guidelines in the use of Herbal Medicines

Generally speaking, consumers lack the background to diagnose most clinical conditions accurately. Because one disease can mimic another, potentially serious conditions can be misdiagnosed. Effective self-care requires highly informative and understandable package labeling and patient education materials that emphasize safe, appropriate, and effective use. Unfortunately, in the United States the FDA does not regulate herbal medicines as drugs but as dietary supplements; consequently, health or therapeutic claims cannot be placed on the package label. However, a vast hyperbolic advocacy literature has built up around them, providing product information designed to promote sales, not necessarily to inform. This situation increases the need for health care professional, especially pharmacists, to judge the quality of available products and to interpret the products' role in preventing and treating disease for the lay consumer. The FDA neither establishes nor regularly enforces any standards of quality for herbal products. This means that one rely upon the reputation of the producer for any quality assurance. Products are often misbranded, and often the quantities of the ingredients are not listed. In sufficient to render a therapeutic effect may be lacking. The consumer is best advised to purchase a preparation containing a specified amount of a standardized extract marketed by a reputable firm. Some herbal producers have responded to the question of quality by introducing entire lines of products, all standardized so that each unit of dosage contains a specific quantity of active constituents. Sales of some of these product lines are restricted by manufacturers to pharmacies in which patients can receive professional advice. In addition to standardization of the herbal preparation, another indicator of quality assurance is that the label should show the scientific name of the botanical, the name and address of the actual manufacturer, a batch or lot number, the date of manufacture, and the expiration date.

Most of the herbal medicines are technically unapproved drugs. They may have been used for centuries, but substantial data on the effectiveness and safety of long-term use are often lacking. Safety considerations include warnings and precautions relative to the use of a particular herb, drug-drug interactions between prescription medications and the herbal medication, and the fact that certain groups of individuals often experience a higher incidence of adverse drug effects which could have dire consequences. In the case of warnings and precautions, patients should cease taking an herb immediately if adverse effects (allergy, stomach upset, skin rash, headache) occur. Another important safety precaution is that herbal use is not recommended for pregnant women, lactating mothers, infants, or children under the age of six. In the pregnant woman, most drugs cross the placental barrier to some extent, and these expose the developing fetus to potential teratogenic effects of the drug. The first trimester, when organogenesis occurs, is the period of greatest teratogenic susceptibility and isthecritical period for inducing major anatomical malformations. In the lactating mother, the potential exists for the drug in the mother's milk, resulting in adverse effects in the nursing infant. The body and organ functions of infants and young-children are in a continuous state of development. Changes in the relative body composition (lipid content, protein binding, and body-water compartments) will produce a different drug distribution in the body than in adults. Also, many enzyme systems may not be fully developed in infants, particularly neonates, producing a slower drug metabolism. (Robbers., 2002b)

Standardization

Standardization is the process of delivering a product with a specified minimum level of one or more phytoconstituent (s), where we can make sure about the quality of the product; broadly it covers the qualitative and quantitative part of analysis. Qualitative analysis mainly covers the identification of the component(s) present in a particular compound, whereas the quantitative analysis is accomplished by measuring the level of a chemical in a crude herbal extract which are, present in that particular product and establishing a standard amount of that chemical for future production. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals.

Plant materials and herbal remedies derived from them represent a substantial proportion of global drug market and internationally recognized guidelines for quality assessment are necessary. For pharmaceutical purposes, the quality of the medicinal plant material must be as high as that of other medicinal preparations. However, it is impossible to assay for a specific chemical entity when the bioactive ingredient is not known. In practice, assay procedures are not carried for those medicinal plant materials where there are known active ingredients. (Mukherjee P., 2002a).

Physiochemical Parameters

Foreign organic matter: - To ensure the extent of contamination of extraneous matters such as filth and other parts of botanicals, not covered by definition of the herbal drug.

Ash value

This determination measures the presence of silica especially sand and siliceous matter.

Total ash

The total ash usually consists of carbonates, phosphates silicates and silica that include the physiological ash- which is derived from the plant tissue itself and non physiological ash which is the residue of the adhering material to the plant material e.g. sand and soil. While determining the total ash at very high temperatures (more than 600°C) may result in the conversion of carbonates to oxides.

Acid insoluble ash

Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the washed insoluble matter.

Extractive value

This method determines the amount of active constituents in a given amount of medicinal plant material when extracted with a solvent. These values provide an indication of the extent of polar, medium polar and non-polar components present in the plant material. It is employed for those plant materials for which no suitable or biological assay method exists. The extractive values are determined according to the method described in pharmacopoeia.

Water soluble extractive value

Water is used as the solvent.

Alcohol soluble extractive value

Ethanol is used as the solvent.

pH

It gives information whether drug is acidic or basic nature.

EXPERIMENTAL WORK

The present studies includes the evaluation of leaves and bark of *F. bengalensis* for

1. Pharmacognostic evaluation
2. Preliminary phytochemical analysis
3. Chromatographic analysis (HPTLC)
4. Evaluation of Anti-microbial and Anti-fungal activity of extracts of leaves and bark.

Plant Material

The leaves and bark of *F. bengalensis* were collected from botanical garden of Guru Nanak Dev University Amritsar (Punjab) and authenticated by Dr. Saroj Arora, Head, Department of Botanical and environmental Sciences, Guru Nanak Dev University, Amritsar (Punjab).

Determination of Water Soluble Extractive Value

5 g of the air-dried drug, coarsely powdered was macerated with 100 ml of purified water in a closed flask for 24 hours, kept in a mechanical shaker for first 6 hours and allowed to stand for 18 hours. There after filtered rapidly through whatman filter paper No.41. Evaporated 25 ml of the filtrate to dryness in a preweighed flat-bottomed petridish dried at 105ºC and weighed. Calculated the % w/w water-soluble extractive value with reference to the air-dried drug as follows

$$\text{Water soluble extractive value (\% w/w)} = \frac{\text{Weight of residue x 100}}{\text{Volume of extract evaporated x Weight of sample}}$$

Determination of Alcohol Soluble Extractive Value

5 g of the air-dried drug, coarsely powdered was macerated with 100 ml of alcohol (100% or 60%) in a closed flask for 24 hours, kept in a mechanical shaker for 6 hours and allowed to stand for 18 hours. There after filtered rapidly through whatman filter paper No. 41. Evaporated 25 ml of the filtrate to dryness in a preweighed flat-bottomed petridish, dried at 105ºC and weighed. The %w/w alcohol soluble extractive value with reference to the air-dried drug was calculated as follows

$$\text{Alcohol soluble extractive value (\% w/w)} = \frac{\text{Weight of residue x 100}}{\text{Volume of extract evaporated x Weight of sample}}$$

Determination of Ash Value (Anonymous., 1991)

a) Determination of Total ash

Taken about 1 g of the air-dried drug, coarsely powdered and accurately weighed in a previously ignited and tarred silica crucible. The material was spread uniformly and ignited gradually increasing the heat from 500 to 600ºC until white ash was formed then it was allowed to cool in dessicator for 30 min, weighed and calculated the %w/w total ash with respect to the air-dried material as follows:

Total ash (% w/w) = (Weight of ash/Weight of sample) x 100

b) Determination of Acid Insoluble ash

The above formed ash was taken in silica crucible and boiled with 25 ml of the 2 M HCl for 5 min. The solution was filtered through ashless filter paper (Whatman No.41) and then insoluble residue was collected, ignited the filter paper in silica crucible from 500 to 600ºC until white ash was formed then the residue was allowed to cool in dessicator for 30 min, weighed and then the %w/w acid insoluble ash was calculated with respect to the air-dried material as follows

Acid insoluble ash (% w/w) = (Weight of ash/ Weight of sample) x 100

c) Determination of water soluble ash

The total ash formed was taken in silica crucible and boiled with 25 ml of distilled water for 5 min. The solution was filtered through ashless filter paper (Whatman No. 41). The residue was washed twice with 5 ml distilled water. The insoluble residue left on filter paper was ignited in silica crucible at 450-500 ^{0}C until ash was formed and the residue was allowed to cool in dessicator for 30 min, weighed and the % w/w water soluble ash was calculated with respect to the air dried material as follows.

Water soluble ash (% w/w) = (Weight of ash- weight of insoluble ash/ Weight of sample) × 100

Preparation of successive extracts (Kokate., 1986)

Leaves and bark of *F. bengalensis* were dried in shade and powdered. The powdered leaves (100 g) were subjected to successive Soxhlet extraction by solvents in increasing order of polarity viz. petroleum ether (60-80 ^{0}C),

benzene, chloroform, ethyl acetate and methanol. Before each extraction the powdered material was dried in hot air-oven below 50 ^{0}C. Each extract was concentrated by distilling off the solvent and then evaporating to dryness on the water-bath.

Extracts were weighed and percentage was calculated in terms of the air-dried weight of the plant material

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Determination of percentage yield

The percentage yield of each extract was calculated by using following formula

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical screening

The various extracts of leaves and bark of *F. bengalensis* was subjected to qualitative chemical examination for the presence or absence of alkaloids, carbohydrates, flavanoids, proteins, saponins and tannins and phenolic compounds, glycoside. (Khandelwal., 1991)

Test for carbohydrates

Molish's test (General test)

To 2-3 ml. aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add cone. H_2S0_4 from sides of the test tube. Purple colour if produce indicates +ve test.

Inference

Purple colour shows presence of Carbohydrates.

Reduction of Fehling solution

Extract was treated with equal quantity of Fehlings soln A and Fehlings soln B and heated.

Inference

Brick red ppt shows presence of reducing sugar.

Tests for Glycosides

Determine free sugar content of the extract. Hydrolyse the extract with mineral acid (di, HCl / dil. H_2S0_4). Again determine the total sugar content of the hydrolised extract. Increase in sugar content indicated presence of glycoside in the extract.

High Performance Thin Layer Chromatography

a) Introduction

High performance thin layer chromatography, (HPTLC) is a modern, powerful analytical technique with separation power, performance and reproducibility superior to class TLC. HPTLC is still a better means to separate the various components of a mixture. HPTLC is rapidly gaining importance in biochemistry of natural products and in analysis of bio-fluid in the field of pharmacokinetics. The analytical profile for carotenoids, tropane alkaloids, flavonoids, steroidal compounds, anthracene aglycones, and lipids etc, have been developed by using this techniques (Sethi., 2001). For analysis of herbal drugs, HPTLC offers a number of advantages; the technique is especially suitable for comparison of sample based on finger prints. Fingerprint analysis of HPTLC is one of the most powerful tools to link the botanical identity to the chemical constituents profile of the plants. In combination with the microscopic investigations, the fingerprint provides the means for a convenient identity check. It can be used to detect adulteration in raw materials. From the constituents profile a number of marker compounds can be chosen, which might be used to further describe the quality of the herb or herbal preparation. High performance thin layer chromatography can also be employed for quantitative determination of such marker compounds. In HPTLC the smaller particle size, narrow particle size distribution, higher packing density, hence better surface homogeneity several advantages:

a) Smaller compact zones, better resolution, increased separation efficiency.
b) Shorter analysis time as migration distance is almost half as compared to that of classical TLC layer.
c) Increased detection sensitivity because of higher surface concentration of sample.
d) Efficiency decreases with increasing solvent migration; shorter development distance are mandatory in HPTLC, but not in conventional large particle TLC Layer.

Steps involved in HPTLC

I) Preparation of extract solution

The known amounts of the ethanol extract of *F. bengalensis* (0.25 gm) was dissolved in 25 ml of methanol respectively and further sonicated in ultrasonic water bath to dissolve and the volumes were made up to 25 ml with methanol and after filtration through Whatmann filter paper (44). The resulting solution was used as the extract solution.

II) Application of spot

Three spots of each extract of quantity 5µl, 10µl and 15µl of the solute were applied in silica gel $60F_{254}$ TLC plate with the help of Linomat5 applicator.

III) Development of chromatogram

The plate was placed in twin trough plate development chamber pre saturated with respective medium. The mobile phase was run up to about 80 mm. The developed chromo-plate was dried by hot air. The plate was then photo documented with the help of digital camera (CANON P).

IV) Detection and interpretation

The spots developed was scanned at wave length from 250 nm to 800 nm with help of Scanner 111. The software Wincats interpretated the intensity and R_f. Value of various peaks.

Antimicrobial activity of the plants can be detected by observing the growth response of various micro-organisms to those plants extracts which are placed in contact with them. Many methods for detecting such actions of organism are available but since they are not equally sensitive, the results obtained will be influenced by the method selected and the micro-organism used for the test.

I) Test organisms and inoculums

The following Freeze dried micro-organisms were obtained from the Institute of Microbial Technology, Chandigarh.

1*Bacillus subtilis* MTCC: *121 (Gram +ive Bacteria)

2.*Escherichia coli* MTCC: 40 (Gram –ive Bacteria)

3.*Aspergillus oryzae var. oryzae* MTCC: 40 (Fungi)

II) Media

The nutrient agar media was prepared in distilled deionized water. The composition of the media was as given under:

Table 4. 2: Composition of Media (Bacterial)

S.No.	Ingredients	Quantity Required For 1000 ml (in g)
1.	Peptone	5.0
2.	Sodium chloride	5.0
3.	Beef extract	1.0
4.	Yeast extract	2.0
5.	Agar	15.0

Table 4.3: Composition of Media (Fungal)

S.No.	Ingredients	Quantity Required For 1000 ml
1.	Czapek Concentrate	10.0 ml
2.	$K_2H\,PO_4$	5.0 g
3.	Sucrose	30.0 g
4.	Yeast extract	5.0 g
5.	Agar	15.0

Table 4.4: Composition of Czapek Concentrate.

S.No.	Ingredients	Quantity Required
1.	$NaNO_3$	30.0 g
2.	KCL	5.0 g
3.	$MgSO_4$. 7 H_2O	5.0 g
4.	$FeSO_4$. 7 H_2O	0.1 g
5.	Distilled Water	100 ml

III) Preparation of media

The various ingredients were accurately weighed and suspended in 1000 ml of distilled water in a conical flask. It was heated on a water bath to dissolve the medium completely. Direct heating was avoided as it may lead to charring of the medium components and render it useless for the purpose.

IV) Sterilization of media

The conical flask containing the nutrient agar medium was plugged with the help of a non-absorbent cotton plug. The mouth of the conical flask and the cotton plug were properly covered with aluminum foil. The medium was then sterilized by autoclaving at 15-lbs/ in^2 pressure for 20 minutes.

V) Preparation of test organisms inoculum

The freeze dried micro-organisms were revived by transferring them on recommended nutrient media in petri dish for sufficient long time (24 -48 hrs) at suitable temperature conditions (Bacteria at 37 ^oC, Fungi at 25 ^oC). Once micro-organisms were revived a loop full of microorganism were transferred to 10 ml of nutrient broth (Growth media without agar) aseptically. Nutrient broth was inoculated at recommended temperature condition for 24 hr. The test organisms were maintained on slants of medium and transferred to a fresh slant once a week. 1 ml of cultured nutrient broth was added 100 ml of sterilized saline (0.9 g/100ml NaCl in distilled water)

Temperature control

Thermostatic control is required in several stages of a microbial assay when culturing a micro-organism and preparing its inoculums and during inoculation.

Aseptic control

Aseptic condition must be maintained to prevent inter contamination from atmospheric micro-organisms. All the inoculums must be prepared under steam flow air from High Efficiency Particulate Affinity (HEPA) filter.

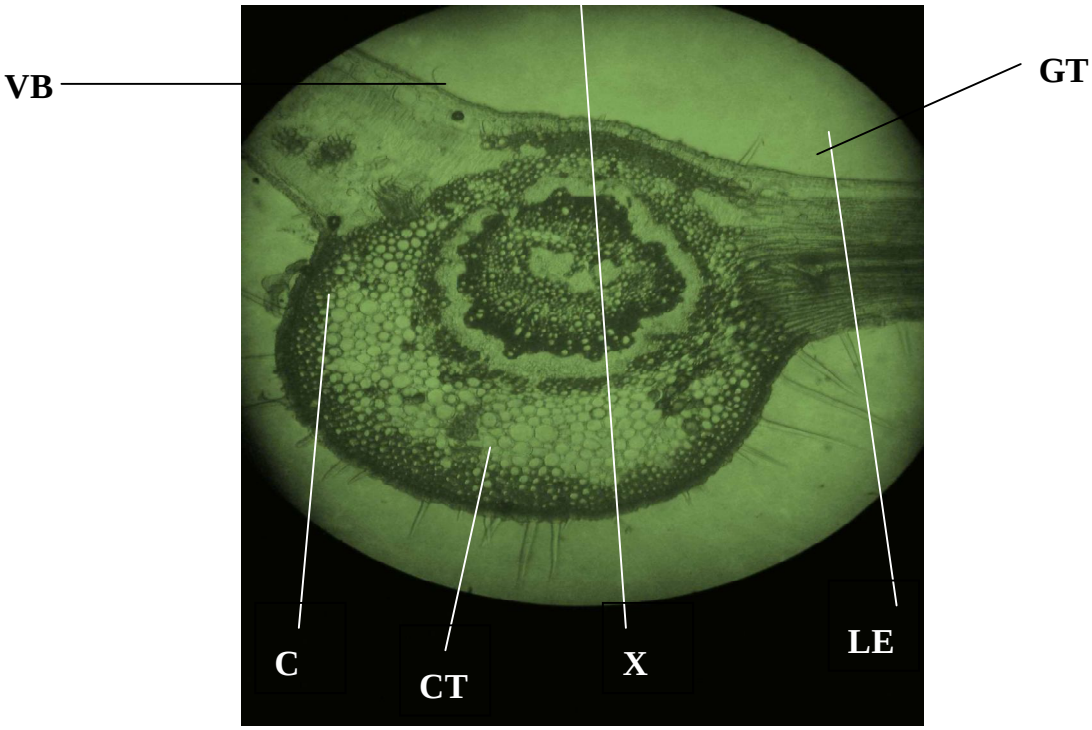
VI) Method

Disc diffusion technique (Kirby-Baur method)

A Previously liquefied and sterilized medium was poured in to plastic petri-plates of 100 mm size containing 0.1 ml of seed microbial inoculum. The inoculum and media were properly mixed by horizontal rotatory motion. Plates were prepared and kept for solidifying. Two dilutions of the ethanolic and aqueous extracts of leaves and bark were made having concentration of 25 mg and 50 mg, streptomycin (antibiotic) and clotrimazole (antifungal drug) were taken as standard at conc. 1 mg/ml and 5mg/ml respectively. Each group contains 8 plates for *E. coli*, B. *subtilis* and *A. orygae* (four for each concentration). Two test solutions were made in DMSO (Dimethyl sulphoxide) solvent which was used as control. Whattman filter paper disc of diameter 8 mm were impregnated in respective solution .The impregnated paper disc were carefully placed on solidified agar media in Petri dish at their respective places. They were incubated for about 24 hours at 37 ± 2^o C (bacteria) and 25 ± 2^o C (fungi) respectively. After 24 hrs the plates were examined and the diameter of zones of inhibition was accurately measured. (Bhattacharjee., 2006).

RESULTS and DISCUSSIONS

.Microscopic Evaluation



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Transvers section (TS) of *F. bengalensis* leave

Lower epidermis (LE), Covering trichome (C), Xylem vessels (X), Glandular Trichome (GT), Upper epidermis (UE), Collenchyma (C), Vascular Bundle (VB), Spongy parenchyma

Microscopy

Collencyhma is below the upper epidermis above the lower epidermis. In vascular bundle the xylem vessels are thick walled, lignified. Arc shaped spongy parenchyma and distinct cut vienlets are seen. Trichomes are mainly covering and glandular. The covering trichomes are unicellular and two celled and glandular trichomes are unicellular single stalk and two head. The upper epidermis single layered and the lower epidermis is similar to upper epidermis but stomata are present

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Physical Parameters of Powdered Leaves of *F. Bengalensis*.

Sample No.	Total ash (%w/w)	Water soluble Ash (%w/w/)	Acid insoluble ash (%w/w/)	Water soluble extractive value (%w/w/)	Alcohol soluble extractive value (%w/w/)	Foreign matter
1.	13.6	2.8	1.4	14.8	8.4	1.3
2.	11.8	3.8	1.3	15.4	9.2	1.6
3.	12.1	4.2	1.2	12.4	9.4	1.4
Average	12.5	3.6	1.3	14.2	9	1.4

Successive extraction

Various extracts of the powdered leaves and bark (5 gm) of *F. bengalensis* were prepared by successive extraction (using soxhlet apparatus).

Table 5.5: Successive Extraction of Leaves of *F. bengalensis*.

Extract	F. Bengalensis Leaves (%w/w)
Petroleum Ether	0.4%
Benzene	2.6%
Chloroform	3%
Ethylacetate	1.2%
Methanol	5.8%

Table 5.6: Successive Extraction of Bark *F. Bengalensis*.

Extract	F. bengalensis Bark
Petroleum Ether	0.2%
Benzene	0.3%
Chloroform	1.8%
Ethylacetate	0.56%
Methanol	5.2%

Preliminary Phytochemical Screening

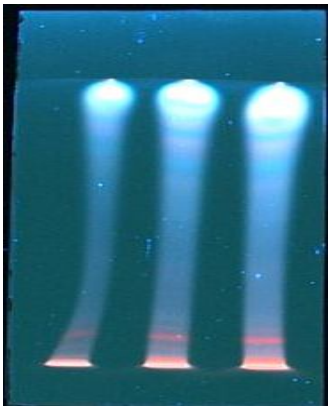
Table 5.9: Preliminary Phytochemical Screening of Leave extracts of *F. bengalensis*.

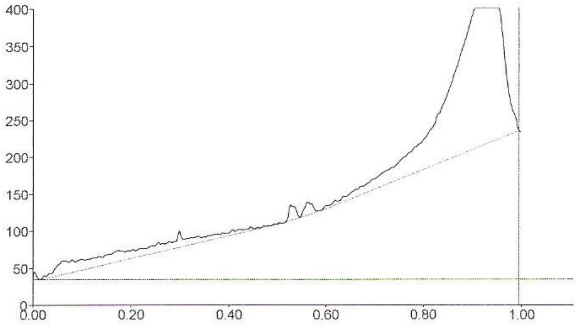
Plant Constituent / Test/Reagent Used	EXTRACTS				
	Petroleum Ether Extract	Benzene extract	Chloro-form Extract	Ethyl acetate extract	Methanol extract
1) Alkaloids					
Hager's reagent	+	–	–	+	+
Wagner's reagent	–	+	+	+	+
Mayer's reagent	+	–	+	–	–
Dragendorff's reagent	–	–	–	–	–
2) Carbohydrate					
Molisch's reagent	+	–	+	+	–
Fehling's solution	–	+	–	–	–
Benedict's reagent	–	–	+	+	+
3) Protiens and aminoacids					
Ninhydrin reagent	+	+	–	+	–
Biuret test	+	–	–	–	–
Millon's test	–	–	–	–	–
4) Phenolic compounds and tannins					
Ferric Chloride solution	+	–	–	+	+
Lead acetate test	+	+	–	–	+
Gelatin Solution	–	–	–	+	–
Bromine Water	–	–	+	–	–
Acetic Acid Solution	–	–	+	–	–
Pot. Dichromate	+	–	–	–	–
Dil. nitric acid	–	+	–	–	+

High Performance Thin Layer Chromatography

a) Leaf ethanol extract

Mobile Phase :- Toulene : Ethyl acetate :: 25 : 5





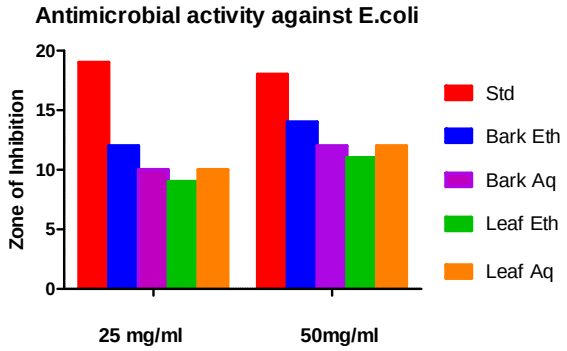
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.03	3.4	0.03	18.3	5.73	0.07	14.9	423.8	2.21
2	0.29	9.2	0.30	22.9	7.16	0.31	9.4	193.4	1.01
3	0.52	0.8	0.53	22.0	6.88	0.55	0.7	254.2	1.33
4	0.55	1.1	0.56	18.2	5.67	0.59	1.8	256.3	1.34
5	0.79	36.6	0.93	238.8	74.56	1.0	2.5	18021.3	94.11

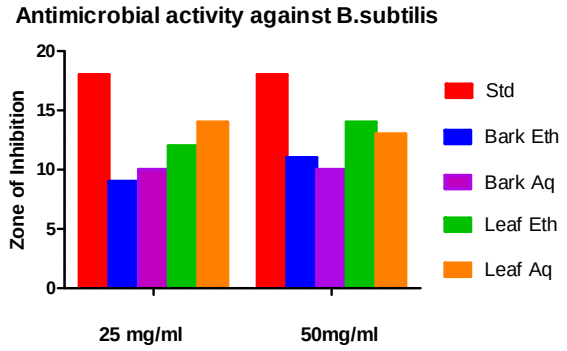
Antimicrobial activity

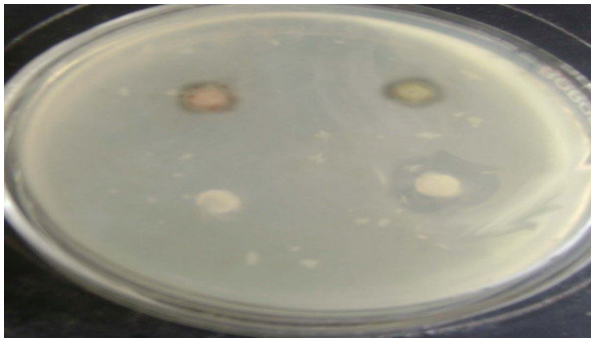
The aqueous and ethanol extracts of bark and leaves were examined for antimicrobial activity against *E. coli*, B. *subtilis* and A. oryzae The extracts showed significant antimicrobial activity in comparison to control drug. The following table comprise of Zone of Inbibition of various extracts.

Table 5.11: Antimicrobial Activity Against *E. coli* and *B. subtilis.*

Sample	Sample conc. (mg/ml)	Zone of inhibition (mm) *E. coli*	Zone of inhibition (mm) *B. subtilis*
Bark Ethanol	25	12	9
	50	14	11
Bark Aqueous	25	10	9
	50	12	10
Leave Ethanol	25	10	12
	50	11	12
Leave Aqueous	25	10	12
	50	12	13
Co	Control 1% DMSO	-	-
S	Streptomycin (1mg/ml)	20	18







Antimicrobial Activity against B. Subtilis

Bark ethanol 25 mg/ml (B1), Bark aqueous 25 mg/ml (B2), Control (C), Norfloxacin 1 mg/ml (S)

CONCLUSION

The standardization parameters were performed for the leaves and bark of the *F. bengalensis*. The HPTLC methods has been developed for finger printing profile for leave and bark extract. Finger printing profiles was developed and pure spols are required for Identification of various chemical components. Antimicrobial activity of the leave and bark extract of *F. bengalensis* against the *Bacilus subtilis; E. coil and Aspergillus oryzae* has shown significant results. Anti depressant activity of *F. bengalensis* was investigated by forced swim test and leaves ethanol, bark ethanol extract provided significant results**.**

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