PHYTOCHEMICAL SCREENING AND CHROMATOGRAPHIC EVALUATION OF FICUS BENGHALENSIS LEAVES

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ABSTRACT: Aim: To rationalize the macroscopical, anatomical and physico-chemical studies on leaves of Ficus benghalensis (Maraceae).

Materials & methods: The crude ethanolic extract of leaves of Ficus benghalensis (Maraceae) was using Physico-chemical parameters, and Preliminary photochemical investigation (TLC, HPTLC, & Column Chromatography). Results and conclusion: An attempt had been made to highlight this folk herbal medicine through dried crude samples anatomically and physico chemically. TLC fingerprint and HPTLC were carried out and the salient macroscopical qualitative and quantitative parameters were reported. These studies will provide referential information for correct information and help in checking adulteration in market sample medicines.

INTRODUCTION: Herbal medicine is the study and use of medicinal properties of plants. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions 1. And to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work 2. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects.

In 2001, researchers identified 122 compounds used in modern medicine which were derived from ethno medical plant sources 3. 80% of these compounds have had an ethno medical use identical to the current use of the active synthetic drugs 4. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including aspirin, digitalis, and quinine, opium. The use of herbs to treat disease is almost universal among non-industrialized societies 5, 6 and is often more affordable than purchasing expensive modern pharmaceuticals.

Researchers found that people in different parts of the world tended to use medicinal plants for
medical purposes. In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favour of drugs. Almost one fourth of pharmaceutical drugs are derived from botanicals.

The plant is a large evergreen tree distributed all over Southern and Eastern part of Nigeria but most common in deciduous forest South West. It is grown in gardens and road sides for shades. It is a member of four sacred trees meant to be planted around the home and temples. It is found throughout the year, grows in evergreen except in dry localities where it is a leafless for a short time. It is hardy and drought-resistant; it withstands mild frost. It is epiphytic when young. It develops from seeds dropped by birds on old walls or on other trees and is therefore, considered destructive to forest trees, walls and buildings.

The tree is commonly known as “Opoto” in Yoruba, “Banyan” tree in English, “Bar” in Hindi and as “Avaroha” in Sanskrit. The species of four *Ficus* yielding latex consist of Nyagrodha (*Ficus bengalensis*), Udumbara (*Ficus glomerata*/*Ficus racemosa*), Plaksha (*Ficus lacor*/*Ficus retusa*) and Ashvattha (*Ficus religiosa*). The bark and leaves of this group are used as astringent, haemostatic, anti-inflammatory, anti-septic; prescribed in diarrhoea, dysentery, and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia, deficient lactation.

A very large tree up to 30m in height leaves spirally arranged on branchlets up to 10-30cm long and 7-20cm wide, coriaceous, elliptic to ovate, apex obtuse, base rounded, with 5-7 basal nerves; petioles 2.5-5 cm long; stipules stout. Fruits receptacles sessile, axillary in pairs 1.3-1.9 cm in diameter globose silky pubescent, scarlet or brick red when ripe; bracts 4-5, copular, 6mm, shortly connate, obtuse persistent and sepals 3-5, Male flowers dispersed with female, stamen 1, another oblong, parallel, unequal, and shortly mucronate. Ovary-obovoid globose, 1.5×1mm, style erect or curved, tapering, gall flowers similar to female, pedicellate, achnes globose-ellipsoid, 2×1.5mm dark brown flowers during the summer and fruits the rainy season. Smooth bark, light grey-white, 1.27cm thick wood moderately hard, grey or grayish-white. In the traditional system of medicine, the plant is used for various health problems and diseases.

**Taxonomic classification of Ficus benghalensis:**

Kingdom : Plantae
Phylum : Traceophyta
Class : Magnoliopsida
Order : Urticales
Family : Moraceae
Genus : Ficus
Species : Ficus
Synonyms : *Ficus indica* L. and *Ficus banyan*

**MATERIALS AND METHOD:**

**Collection and Authentication:** The fresh leaf of wildly growing plant *F. benghalensis* were collected from the field areas of eastern Uttar Pradesh region during the month of September, 2015. The leaves were identified and authenticated by Dr. Gaurav Nigam (Department of Botany), from Bundelkhand University, Jhansi, with voucher Specimen no. **Bu/Bot./Phor./11-2015/02**. The fresh leaves were used for the study of macroscopical and microscopical characters. Whereas collected leaves were shade-dried and coarsely powdered. This coarse powder was used for the determination of ash values, extractive values and preliminary phytochemical investigation as per standard methods.

**Preparation of the materials:**

The materials were initially separated from the main plants body, rinsed with distilled water and air dried at room temperature in laboratory and then homogenized finely and stored in air tight bottles and was used for all the extraction process.

**Extract Preparation:**

Each of the dried leaf was weighed (100g) of *Ficus benghalensis* and packed in muslin cloth and subjected to soxhlet extractor for continuous hot
extraction with petroleum ether and ethanol for 8 hrs separately. Then the each extract were filtered and filtrate was evaporated to dryness. The percentage yield of the petroleum ether and ethanol extracts was calculated.

\[
\% \text{ yield} = \frac{\text{weight of extract}}{\text{weight of sample}} \times 100
\]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent</th>
<th>Wt. of Drug (gm)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pt. Ether (60-80%)</td>
<td>100</td>
<td>2.248</td>
</tr>
<tr>
<td>2.</td>
<td>Ethanol (100%)</td>
<td>100</td>
<td>18.384</td>
</tr>
</tbody>
</table>

**TABLE 1: EXTRACTION OF PLANT MATERIALS**

**Phytochemical Screening of Plants:**
Phytochemical analysis of plants was carried out for all the extracts as per the standard methods.

The various qualitative chemical tests of powder, ethanol extract, and petroleum ether extract indicates the presence of sterols, ß 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.

**TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF FICUS BENGALENSIS LINN**

<table>
<thead>
<tr>
<th>A. Tests</th>
<th>Pet ether extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alkaloids:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drangendroff’s test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td>Mayer’s test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td>Hager’s test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td>Wagner’s test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td><strong>Carbohydrates:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fehling’s test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Molish test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td><strong>Gums:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rheuthenium red + HCl</td>
<td>+ve</td>
<td>- ve</td>
</tr>
<tr>
<td><strong>Tannins:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aq. FeCl3 Test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td>Alc. FeCl3 Test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td><strong>Flavonoids:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td>Shinoda test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td>Alkaline test</td>
<td>-</td>
<td>+ ve</td>
</tr>
<tr>
<td><strong>Sterols:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salfowaski test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Liberman Burchad test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td><strong>Saponins:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foam test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td>Lead acetate test</td>
<td>+ ve</td>
<td>+ ve</td>
</tr>
<tr>
<td><strong>Glycosides:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baljet test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Legal’s test</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Killer lillani test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Bromine water test</td>
<td>+ve</td>
<td></td>
</tr>
</tbody>
</table>

**Macroscopical:**
The tree, often very large, up to 30 m tall, with many aerial roots which develop into new trunks so that the tree goes on spreading laterally indefinitely. The leaves are leathery, entire, ovate or elliptic, 20-40 cm wide, apex obtuse, base rounded with prominent lateral veins; petioles 2.5-5 cm long; stipules stout opposite arrangement and has reticular pinnate venation. The fruits are 1 to 2 cm in diameter, globose, without stalks, in pairs in leaf axils, and when ripe are bright red. The bark is grey, hard surfaced and uneven; 0.5-19 cm thick, on rubbing white papery flakes come out for the outer surface inner surface light brown fracture fibrous taste mucilaginous without any characteristics odour.
FIG. 1: A) MACROSCOPIC CHARACTERS OF *FICUS BENGHALENSIS*. B) TWIG SHOWING LEAF BUD (VATANKUR), LEAVES AND FRUITS

**Microscopic Characters:**

The transverse section of single leaf sheath shows upper and lower epidermal layers made up of rectangular cells and both the epidermal cells shows simple unicellular trichomes (tufts of trichomes). Epidermal cells were followed by many layered, rounded to polygonal, brown parenchymatous cells, compactly. These cells were filled with simple starch grains and rosette type of calcium oxalate crystals. In between the parenchymatous cells rounded to polygonal calcium oxalate crystals were present. Walls of the stone cells were highly lignified and lumen was broad and with pitted thickenings.

The leaf is uniform thick with smooth and even surfaces and fairly prominent midrib. The midrib region shows the upper and lower epidermis followed by two to three layers of collenchymatous cells and parenchymatous cells. Vascular bundles are feebly developed with xylem and phloem and contain small vascular bundles. Laminar region is also feebly developed with spongy and palisade parenchymatous tissue.

The diagnostic characters were the presence of tufts of uniserrate simple trichomes in sheath on both the surfaces. It also showed the presence of closely arranged thin walled rounded to tangentially elongate brown tannin colour parenchymatous cells and round to polygonal stone cells heavily lignified wall with pitted thickenings were also present. The parenchymatous cell contains simple starch grains, rosette calcium oxalate crystals and brown tannin content. Maceration of the whole leaf bud shows parenchymatous cells (rectangular) with tannin, helical to spiral vessel round to polygonal stone cells with heavily lignified cell walls with pitted thickenings unicellular simple trichomes.

Measurement of different tissues in microns.

1. Epidermis: 15-20-25 × 10-12-20 μ
4. Trichomes: 10-15-20 × 5-10-15 μ
5. Phloem: 5-10-15 × 3-5-10 μ

FIG. 2: TRANSVERSE SECTION (TS) OF *F. BENGHALENSIS* LEAVES

Lower epidermis (LE), Covering trichome (C), Xylem vessels (X), Glandular Trichome (GT), Upper epidermis (UE), Collenchyma (C), Vascular Bundle (VB), Spongy parenchyma
Standardization: Standardization is the process of delivering a product with a specified minimum level of one or more phytoconstituent 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 components present in a particular compound whereas the quantitative analysis is accomplished by measuring the level of a chemical in crude herbal extract which are, present is particular product and establishing a standard amount of that chemical for future production. The concept of standardized extract 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 the 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 where there are known active ingredients 19.

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 herbal drug.

Ash value:
This determination measure 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 send and soil. While determining the total ash at very high temperatures (more than 600ºc) may result in the conversion of carbonates to oxides.

Acid insoluble ash:
Acid insoluble ash is the 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. There values provide an indication of the extent of polar, medium polar and non-polar components present in the plants material. It is employed for those plant materials for which no suitable of 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 include the evaluation of leaves of F. benghalensis for
1. Pharmacognostic evaluation
2. Preliminary phytochemical analysis
3. Chromatographic analysis (HPTLC)
4. Chromatographic of column (IR, NMR, MASS)

Determination of water soluble extractive value:
5 g of the air-dried drug, coarsely powdered was macerate with 100 ml of purified water 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 No41. Evaporated 25 ml of the filtrate to dryness in a preweighed flat-bottomed Petridis dried at 105 ºc and weighed. Calculated the % w/w water-soluble extractive value with reference to the air-dried drug as follows
Water soluble extractive  

\[
\text{Weight of residue} \times 100 \div \text{Volume of extract evaporated} \times \text{weight of sample}
\]

**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 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 105 ºc and weighed. The %w/w alcohol soluble extractive value with reference to the air-dried drug was calculated as follows

Alcohol soluble extractive  

\[
\text{Weight of residue} \times 100 \div \text{Volume of extract evaporated} \times \text{weight of sample}
\]

**Determination of ash value:**  
Take 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 600 ºC until white ash was formed then it was allowed to cool in desiccator 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) × 100

**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 ash less filter paper whatman No.41. and the insoluble residue was collected, ignited the filter paper in silica crucible from 500 to 600 ºc 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) × 100

**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 ash less 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 ºc until ash was formed the residue was allowed to cool in desiccator for 30 min, weighed and then 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 extract:**  
Leaves of ficus benghalensis were dried in shade and powdered leaves (100 g) were subjected to successive soxhlet extraction by solvent in increasing order of polarity petroleum ether (60-80°C), benzene, chloroform, ethyl acetate and methanol ethanol, before each extraction the powdered material was dried in hot air-oven below 50 ºC. Each extract was concentrated by distilling off the solvent then evaporating to dryness on the water both. Extracts were weighed and percentage was calculated in term of the air dried weight of the plant material.

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

\[
\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of powder drug taken}} \times 100
\]

**Thin Layer Chromatography:**  
Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bond in

\[
R_f = \frac{\text{Distance travelled by solute front from origin line}}{\text{Distance travelled by solvent front from origin line}}
\]

Where R_f = Retention factor

The ethanolic extract of powdered of fruits of Ficus benghalensis was subjected to thin layer chromatography studies, to find the presence of number of compounds which support by the chemical test.
R_f value and colour of TLC spots, in solvent system of Chloroform: Benzene and few drops of Formic acid (5.5:4:5: Few drops).

### Table 3: TLC Fingerprinting of Ethanolic Extract of Leaves of Ficus Benghalensis Spots

<table>
<thead>
<tr>
<th>Extract</th>
<th>Solvent System</th>
<th>No. of Spots</th>
<th>Colour of Spots</th>
<th>R_f value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic Extract</td>
<td>Chloroform : Benzene: Formic acid (5.5:4:5: Few drops)</td>
<td>5</td>
<td>Green</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Green</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brown</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brown</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yellow</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**HPTLC finger printing:**

Ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system Chloroform : Benzene & few drops of Formic acid (5.5:4:5: Few drops) was ideal and gave well resolved sample peaks.
TABLE 4: \( R_f \) VALUES OF HPTLC FINGERPRINT PROFILE OF SAMPLE

<table>
<thead>
<tr>
<th>( R_f ) value</th>
<th>Before Derivatization</th>
<th>After Derivatization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>254nm</td>
<td>366nm</td>
</tr>
<tr>
<td>Rf1</td>
<td>0.20(Black)</td>
<td>0.09(Red)</td>
</tr>
<tr>
<td>Rf2</td>
<td>0.24(Black)</td>
<td>0.12(Red)</td>
</tr>
<tr>
<td>Rf3</td>
<td>0.28(Black)</td>
<td>0.14(Red)</td>
</tr>
<tr>
<td>Rf4</td>
<td>-</td>
<td>0.40(Faint blue)</td>
</tr>
<tr>
<td>Rf5</td>
<td>-</td>
<td>0.56(RED)</td>
</tr>
<tr>
<td>Rf6</td>
<td>-</td>
<td>0.66(RED)</td>
</tr>
<tr>
<td>Rf7</td>
<td>-</td>
<td>0.76(RED)</td>
</tr>
<tr>
<td>Rf8</td>
<td>-</td>
<td>0.82(RED)</td>
</tr>
<tr>
<td>Rf9</td>
<td>-</td>
<td>0.86(RED)</td>
</tr>
<tr>
<td>Rf10</td>
<td>-</td>
<td>0.89(RED)</td>
</tr>
</tbody>
</table>

**Column Chromatography:**
The basic principle lying in the Column Chromatography is adsorption of component at solid-liquid interface. For good separation, the component of mixture should have different degree of affinity for the solid support. The component having strong adsorption for column material is held up while that component having less affinity moves down the column at faster rate as the elute passes through the column.

Column Chromatography is separated into two categories depending on how the solvent flows down the column. If the solvent is allowed to flow down the column by gravity or percolation, it is called Gravity Column Chromatography. If the solvent is forced down the column by the air pressure, it is called Flash Chromatography. Data of column chromatography ethanolic extract of *Ficus benghalensis*.
**Result:** As a part of standardization study, the macroscopical examination of drug was studied. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The present extractives in different solvents indicated the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent.

**CONCLUSION:** It can be concluded that the present study on *F. benghalensis* can serve as...
an important source of information to ascertain the identity and to determine the quality and purity of the plant material available in market. This study is a substantial step and it further requires a long term study to evaluate therapeutic efficacy of leaves.

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