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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

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medical purposes. In the early 19th century, when chemical analysis first became available^{7, 8} 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 a grown in gardens and road sides for shades⁹. 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 buildings¹⁰.

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 benghalensis*), Udumbara (*Ficus glomerata/Ficus racemosa*), Plaksha (*Ficus lacor/Ficus retusa*) and Ashvattha (*Ficus religiosa*)¹¹. 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 lactation¹².

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 stout¹³. 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 erects 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^{14, 15}. In the traditional system of medicine, the plant is used for various health problems and diseases.

Taxonomic classification of *Ficus benghalensis*:

Kingdom : Plantae

Phylum : Tracheophyta

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 wildy 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 1: EXTRACTION OF PLANT MATERIALS

S. No.	Solvent	Wt. of Drug (gm)	% Yield
1.	Pt. Ether (60-80%)	100	2.248
2.	Ethanol (100%)	100	18.384

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, β 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

A. Tests	Pet ether extract	Ethanol extract
Alkaloids:		
Dragendorff's test	-	+ ve
Mayer's test	-	+ ve
Hager's test	-	+ ve
Wagner's test	+ ve	+ ve
Carbohydrates:		
Fehling's test	+ ve	+ ve
Molish test	+ ve	+ ve
Gums:		
Rheuthenium red + HCl	+ve	- ve - ve
Tannins:		
Aq. FeCl ₃ Test	-	+ ve
Alc. FeCl ₃ Test	-	+ ve
Flavonoids:		
Lead acetate test	-	+ ve
Shinoda test	-	+ ve
Alkaline test	-	+ ve
Sterols:		
Salfowaski test	+ ve	+ ve
Liberman Burchad test	+ ve	+ ve
Saponins:		
Foam test	+ ve	+ ve
Lead acetate test	+ ve	+ ve
Glycosides:		
Baljet test	+ve	
Legal's test	-ve	
Killer lillani test	+ve	
Bromine water test	+ve	

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 indefinitely¹⁷. 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 odour¹⁸.



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: $15-20-25 \times 10-12-20 \mu$
2. Parenchyma: $25-38-45 \times 15-20-35 \mu$
3. Stone cells: $20-25-35 \times 15-20-30 \mu$
4. Trichomes: $10-15-20 \times 5-10-15 \mu$
5. Phloem: $5-10-15 \times 3-5-10 \mu$

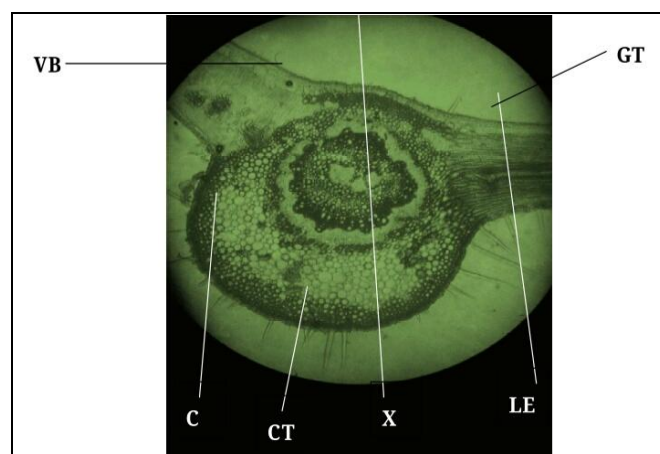


FIG. 2: TRANSVERSE SECTION (TS) OF *F. BENGHALENSIS* LEAVES
Lower epidermis (LE), Covering trichome (C), Xylem vessels (X), Glandular Trichome (GT), Upper epidermis (UE), Collenchyma (C), Vascular Bundle (VB), Spongy parenchyma

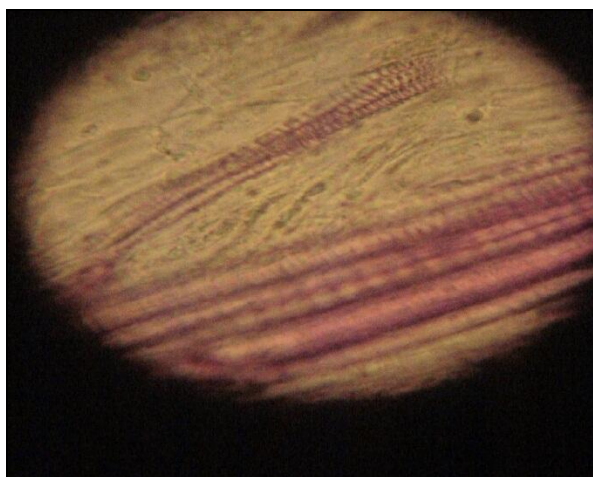


FIG. 3: SPIRAL VESSELS OF *F. BENGALENSIS*

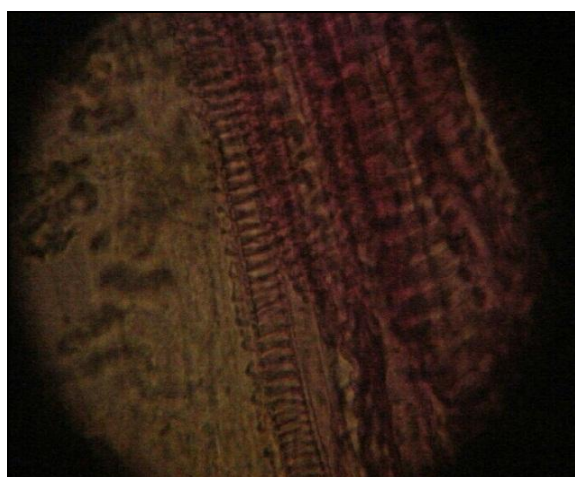


FIG. 4: PARENCHYMATOUS CELLS OF *F. BENGALENSIS*

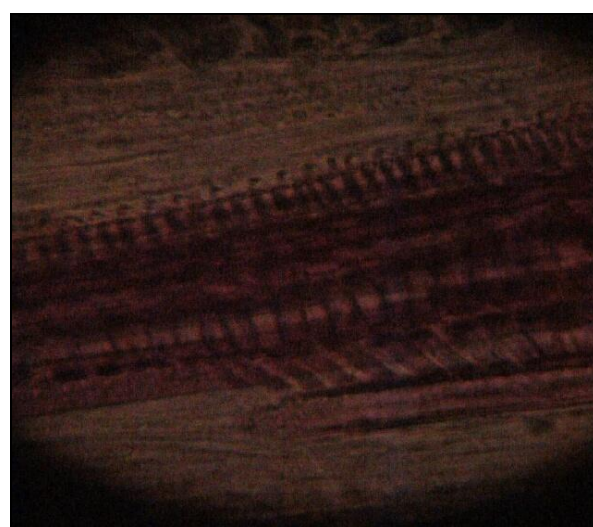


FIG. 5: UNICELLULAR TRICHOMES OF *F. BENGALENSIS*

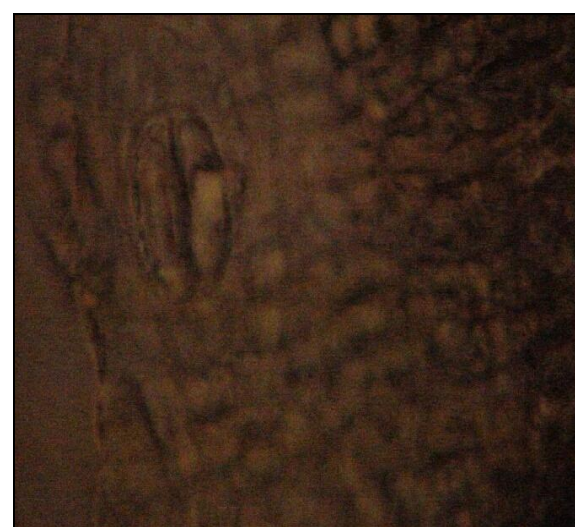


FIG. 6: STOMATA OF *F. BENGALENSIS*

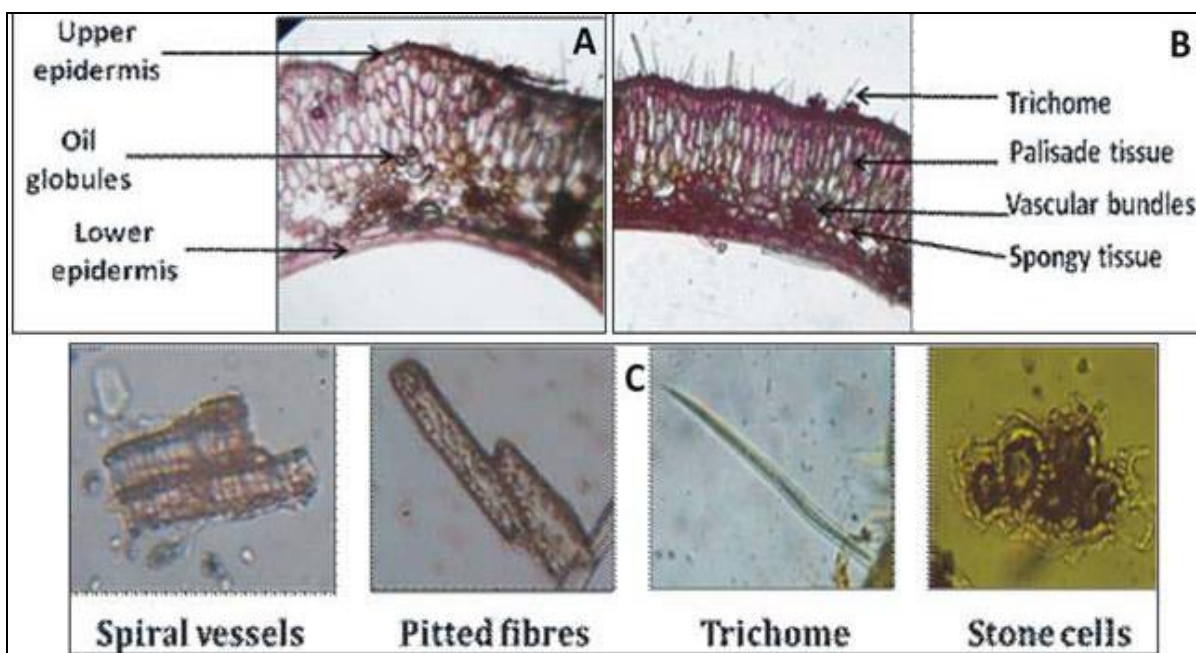


FIG. 7: TRANSVERSE SECTION OF VATANKUR (FRESH) SHOWING A) UPPER EPIDERMIS, OIL GLOBULES AND LOWER EPIDERMIS B) TRICHOME, PALISADE TISSUE, SPONGY TISSUE AND VASCULAR BUNDLES C) POWDER MICROSCOPY OF VATANKUR

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 in 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 ingredients¹⁹.

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 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 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 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. Then 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 °C and weighed. Calculated the % w/w water-soluble extractive value with reference to the air-dried drug as follows

Water soluble extractive =

$$\frac{\text{Weight of residue} \times 100}{\text{Volume of extract evaporated} \times \text{weight of sample}}$$

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

Alcohol soluble extractive =

$$\frac{\text{Weight of residue} \times 100}{\text{Volume of extract evaporated} \times \text{weight of sample}} \text{ value (\%w/w)}$$

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 °C 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.

$$\text{Total ash (\%w/w)} = (\text{weight of ash/weight of sample}) \times 100$$

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 °c until white ash was formed then the residue was allowed to cool in dessicator for 30 min, weighed and then the %w/w to the air-dried material as follows.

$$\text{Acid insoluble ash (\%w/w)} = (\text{weight of ash/weight of sample}) \times 100$$

Determination of water soluble ash:

The total ash formed was taken in silica crucible and boiled wit 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 °c 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.

$$\text{Water soluble ash (\%w/w)} = (\text{weight of ash} - \text{weight of insoluble ash} / \text{weight of sample}) \times 100$$

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 °C. Each extract was concentrated by distilling off the solvent then evaporating to dryness on the water bath. Extracts were weighed and percentage was calculated in term of the air dried weight of the plant material ²⁰.

Determination of percentage yield:

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

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).



FIG. 8: TLC FINGER PRINTING OF ETHANOLIC EXTRACT ON LEAVES OF *FICUS BENGHALENSIS*

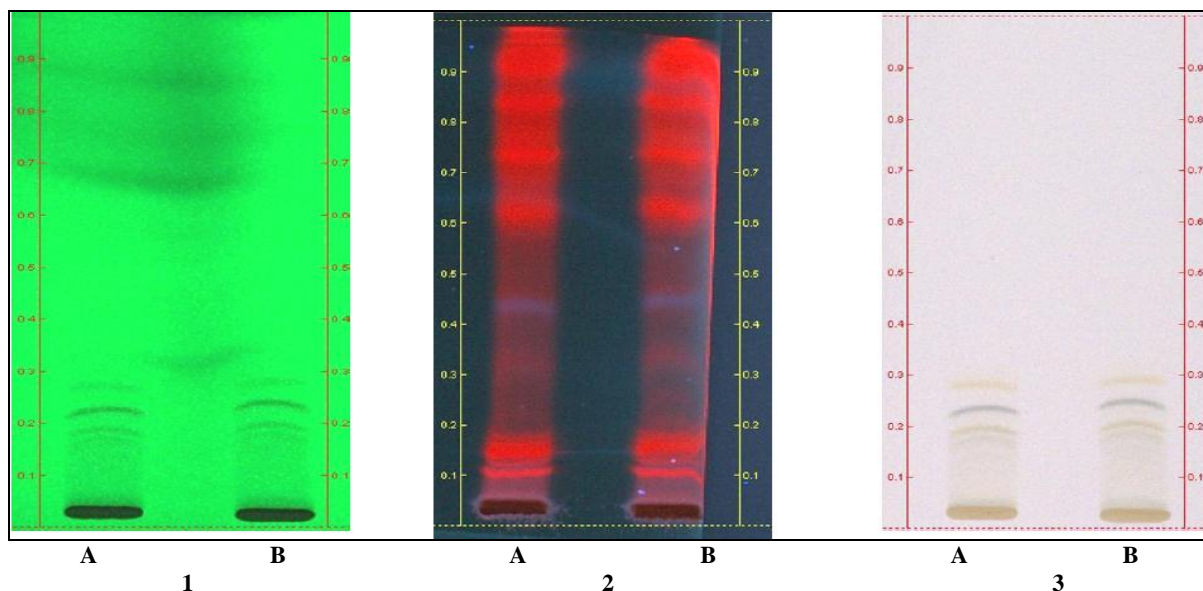
TABLE 3: TLC FINGER PRINTING OF ETHANOLIC EXTRACT OF LEAVES OF *FICUS BENGHALENSIS* SPOTS

Extract	Solvent System	No. of Spots	Colour of Spots	R _f value
Ethanolic Extract	Chloroform : Benzene: Formic acid (5.5:4:5: Few drops)	5	Green	0.88
			Green	0.74
			Brown	0.62
			Brown	0.49
			Yellow	0.29

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.



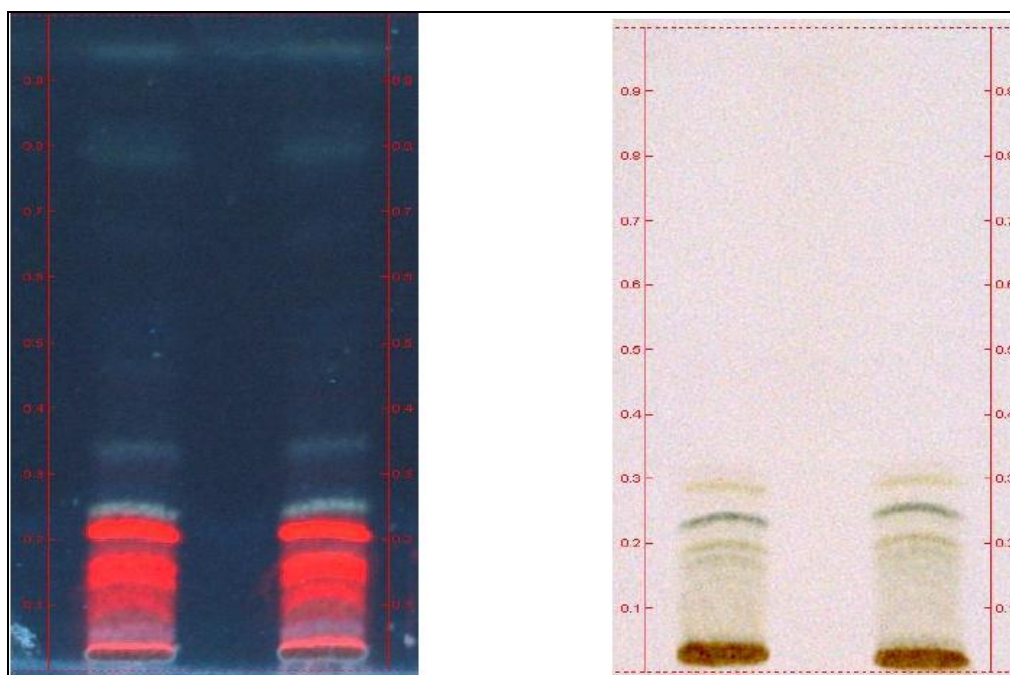


FIG.9: HPTLC FINGERPRINTING

TABLE 4: R_F VALUES OF HPTLC FINGERPRINT PROFILE OF SAMPLE

Rf value	Before Derivatization			After Derivatization	
	254nm	366nm	Visible light	366nm	Visible light
Rf1	0.20(Black)	0.09(Red)	0.18	0.04(Pink)	0.21(Brown)
Rf2	0.24(Black)	0.12(Red)	0.20(Golden brown)	0.12	0.25(Green)
Rf3	0.28(Black)	0.14(Red)	0.25(Violet)	0.16	0.29(Brown)
Rf4	-	0.40(Faint blue)	0.29(Golden brown)	0.21(Red)	-
Rf5	-	0.56(Red)	-	0.25(Faint white)	-
Rf6	-	0.66(Red)	-	0.34(Faint white)	-
Rf7	-	0.76(Red)	-	0.78(Faint white)	-
Rf8	-	0.82(Red)	-	0.94(Faint white)	-
Rf9	-	0.86(Red)	-	-	-
Rf10	-	0.89(Red)	-	-	-

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*.

FIG. 10: COLUMN CHROMATOGRAPHY ETHANOLIC EXTRACT OF *F.BENGHALENSIS* LEAVESTABLE 5: DATA OF COLUMN CHROMATOGRAPHY ETHANOLIC EXTRACT OF *F.BENGHALENSIS* LEAVES

Column Fraction No.	Eluent	TLC Solvent system	Colour of fraction	No. of Spots	R _f value & Code
1.	1(1-5)	n-Hexane	100	-	-
2.	2(6-10)	n-Hexane: Benzene	98:2	-	-
3.	3(11-15)	n-Hexane: Benzene	95:5	-	-
4.	3(16-20)	n-Hexane: Benzene	95:5	-	-
5.	4(21-25)	n-Hexane: Benzene	85:15	-	-
6.	4(26-30)	n-Hexane: Benzene	85:15	-	-
7.	5(31-35)	n-Hexane: Benzene	75:25	-	-
8.	5(36-40)	n-Hexane: Benzene	75:25	-	-
9.	6(41-45)	n-Hexane: Benzene	65:35	2	0.86,0.76
10.	7(46-50)	n-Hexane: Benzene	55:45	2	0.86,0.76
11.	8(51-55)	n-Hexane: Benzene	45:55	2	0.86,0.76
12.	9(56-60)	n-Hexane: Benzene	35:65	2	0.86,0.76
13.	10(61-65)	n-Hexane: Benzene	25:75	2	0.86,0.76
14.	11(66-70)	n-Hexane: Benzene	15:85	2	0.86,0.76
15.	12(71-75)	n-Hexane: Benzene	10:90	2	0.86,0.76
16.	13(76-80)	Benzene	100	1	0.86,0.76
17.	14(81-85)	Benzene	98:2	1	0.86,0.76
18.	15(86-90)	Benzene	98:2	1	0.86,0.76
19.	16(91-95)	Benzene	98:2	1	0.76,V1
20.	17(96-100)	Benzene	98:2	1	0.76,V1

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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