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PHARMACOGNOSTIC STUDIES AND MONOGRAPHIC DEVELOPMENT OF *FICUS GLOMERATA* ROXB. FROM THE GREAT GANGETIC PLAIN

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ABSTRACT: In this study attempt has been made to develop the monographic parameters for *Ficus glomerata* Rox. leaves were collected from Gangetic plains (Lucknow). Under the study, pharmacognostic parameters were performed including macroscopic characters, microscopic characters, physicochemical parameters (extractive values, ash values, pH analysis, LOD), and preliminary phytochemical screening. Apart from pharmacognostic and phytochemical screening, HPTLC fingerprinting profile was also developed for *F. glomerata* Rox. Leaf extract. Pharmacologically active secondary metabolites like polyphenols and flavonoids were determined by using methods of phenolic content assay and total flavonoid content assay respectively. Leaf powder of *F. glomerata* was subjected to successive extraction with petroleum ether, chloroform, methanol and water. Different extracts were utilized for different parameters as per the requirement of protocols.

INTRODUCTION: *Ficus glomerata* Roxb. Also known as *F. racemosa* (Family: Moraceae), popularly called cluster Fig tree (English), gular or umrai (Hindi), udumbar (Sanskrit). It is a large tree indigenous to South-East Asia and commonly found in South India, Punjab, Bihar, Uttar Pradesh, Orissa, West Bengal and Rajasthan^{1,2}. The tree is average in height with quite rich green foliage. Leaves are ovate or elliptic in shape, sub-acute apex, and entire margin and with stalk. Figs are almost spherical, turned to red when ripe; occur in large clusters, on short branches without leaves emerging from the trunk and the main branches.

Different parts like root, bark, and stem have been used to obtain various chemical constituents. The leaf of *Ficus glomerata* possesses tetra triterpene, racemose acid and glauanol acetate. *F. glomerata* fruit has hentriacontane, glauanol, β -sitosterol, tiglic acid, cycloartenol, cycloeuphordenol, euphol, euphorbinol, isoeuphorbol, palmitic acid and so forth³. In a traditional system of medicine, *F. glomerata* is used for the treatment of variety of diseases. Its bark possesses good antidiuretic, hypolipidemic, ant cholinesterase and anthelmintic, memory enhancing and analgesic activities. It is also useful in urological disorders, dysentery, diabetes and piles.

Its root is used in the treatment of various inflammatory glandular enlargements, dysentery and diabetes. Its latex is useful for vaginal disorders, traumatic swelling and hemorrhoids. The leaf possesses anti-inflammatory, antihyperglycemic, hepatoprotective and antibacterial

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effects. Bark and leaf is used together like a mouth wash. Fruits have an astringent property and they are used in spleen and kidney disease^{4,5}. Though, it has a number of pharmacological activities, researchers are still interested in the leaf of *F. glomerata* because it has various bioactive chemical compounds. The objective of the present study is, to find and evaluate the pharmacognostical and phytochemical properties of the leaf of *F. glomerata* and analysis of its major bioactive polyphenols by HPTLC.

Scientific Classification:

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnoliopsida
Order	: Rosales
Family	: Moraceae
Genus	: Ficus
Species	: glomerata

Pharmacognostical Evaluation of *Ficus glomerata*: Generally the height of the tree is 18 m. Color of leaves is dark green (upper) light green (lower), the length is 7.5-10 cm and shape is ovate or elliptic. Apex is acute, the margin is wavy, leaf's surface is glabrous, the base is symmetric, the venation is reticulate and three prominent veins seem to be arising from the base of the lamina. The petiole is angular and reddish-brown. The fruit is arranged in large clusters, arising from the main trunk or large branches. The fruits look like the figs and when raw, they are of green, on ripening they become orange or dull reddish. The seeds are very small, large in number, grain-like. Bark has translucent flakes of grey to rusty brown color which can be easily removed, uniformly hard and non-brittle **Fig. 1, 2**.



FIG. 1: FICUS GLOMERATA IN FRUIT BEARING STAGE



FIG. 2: FICUS GLOMERATA LEAVES

MATERIALS AND METHODS:

Plant Material: The plant part, *i.e.* leaves of *Ficus glomerata*, was collected from Lucknow District, U.P, India **Fig. 1**. Identification and authentication of the plant were done by herbarium department, CSIR-NBRI (NBRI/CIF/679/2019). A voucher specimen has been submitted in the LWG herbarium.

Extraction and Fractionation: The leaves of *Ficus glomerata* were collected, washed with water. Then it was shade dried and powdered by using electric grinder at 60 mesh size. Soxhlet method is used for extraction. At first, the defatting was done by 250 ml of 98% petroleum ether for 6 hours using Soxhlet assembly. This is followed by 9 h Soxhletation with 250 ml of chloroform, methanol and water. All fractions obtained were passed through Whatman No. 1 filter paper. The filtrate obtained was concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4 °C for further use.

Quantitative Microscopy: Various parameters of quantitative microscopy were performed as per standard procedure (parameters are given in **Table 1**).

TABLE 1: RESULTS OF MICROSCOPIC PARAMETERS

Parameter	Mean value \pm SD
Stomatal index upper surface	5.5 \pm 0.5
Stomatal index lower surface	16.5 \pm 0.5
Palisade ratio	6 \pm 1
Vein islet number	13 \pm 0.5
Vein termination number	16 \pm 1

Physicochemical Parameters *Ficus glomerata*:

Extractive Values: The extracts obtained from crude drugs give the idea of approximate measures of their chemical constituents. As per the chemical nature and properties of constituents of drugs, various solvents are used to determine extractives.

The solvent used for extraction should be able to dissolve sufficient quantities of desired substances. *Ficus glomerata* leaves were washed separately to remove foreign particles and dust, crushed and shade dried to remove the moisture then it is coarsely powdered, the dried powder was used for extractive value determination using solvents with different polarity including alcohol and water.

Procedure: 5 gm (W_1) of the coarsely powdered drug was macerated with 100 ml of specified solvent in a closed container for 24 h. frequently shaken for 6 h then allow the mixture to stand for 18 h. Filter rapidly taking precautions against loss of solvent. Evaporate 25 ml of the filtrate to dryness in a tarred petri dish (W_2). Dry at 105 °C to constant weight (W_3). Calculate the percentage of solvent-soluble extractives with reference to the air-dried drug ⁶.

Calculation:

Percentage of solvent soluble extractive = $(W_3 - W_2) \times 100 / W_1 \times 25$

Where, W_1 = Weight of drug taken; W_2 = Weight of empty dish and W_3 = Weight of dish + extractive residue.

Ash Value Determination: After incineration of the crude drug, remained residue is known as ash. In this process, only inorganic salts are left behind. The quality and purity of the crude drug can be determined by ash value. This test is applicable for solid, semi-solid dosage form ⁷.

Total Ash Determination: About 2 to 3 gm (W_1) of the drug was accurately weighed and ground in a tarred silica crucible (W_2). Crucible is kept in a muffle furnace at a temperature of 450 °C-600 °C according to the physical property of the product) for 4 h.

Crucible is taken out from the furnace and cooled slowly in a desiccator and weigh (W_3). Calculate the percentage of ash with reference to the air-dried drug.

Calculation:

Percentage of ash = $(W_3 - W_2) \times 100 / W_1$

Where, W_3 = Weight of crucible with ash; W_2 = Weight of crucible and W_1 = Weight of drug taken.

Acid Insoluble Ash: Ash obtained from the above procedure, is transferred in a 250 ml beaker carefully to avoid loss of ash and 100 ml of dil. hydrochloric acid was added into the beaker. Crucible is washed with 10 ml of acid and transfers the washings to the beaker. The content was boiled for 5 min. The solution is filtered and the insoluble matter is collected on ashless filter paper. Content over the filter paper is washed with hot water until it's neutralized. Insoluble matter containing filter paper is transferred to the original crucible.

Content is dried on a hot plate and ignite at 600 °C in a muffle furnace (until becoming white ash). The residue is allowed to cool in desiccators and weighed. The same process is repeated until constant weight (W_3) is obtained. Acid insoluble ash is calculated with reference to the air-dried drug.

Calculation:

Percentage of acid insoluble ash: $W_3 - W_2 \times 100 / W_1$

Where, W_3 = Weight of crucible with ash; W_2 = Weight of crucible; W_1 = Weight of drug taken.

Loss on Drying: Moisture content is the amount of moisture present in a crude drug sample. Minimum the moisture content, minimum will be the decomposition of crude drugs either due to chemical change or microbial contamination ⁸.

Procedure Oven Method: Size reduction is done. 10 gm (W_1) of drug accurately weighed, up to the third decimal place (Without preliminary drying) in a tarred evaporating dish drug weight (W_2). Dry at 105 °C for 5 h and weigh (W_3). The process of the drying and weighing is repeated at 1 h interval until the difference between two successive weighing corresponds to not more than 0.25 percent. Constant weight is reached when two consecutive weighings after drying for 30 min.

Calculation:

Percentage of loss on drying = $W_2 - W_3 \times 100 / W_1$

Where, W_1 = Weight of drug is taken; W_2 = Weight of evaporating dish + drug before drying and W_3 = Weight of evaporating dish + drug after drying.

pH Analysis: The pH value gives an idea about the acidity or basicity of liquid in the negative

logarithm of the reciprocal of the hydrogen ion concentration in gram per liter⁹.

Procedure: Calibrate the pH meter by 4 and 7 at room temperature. Take 1.0 g of solid sample (test sample) in of distilled water (100 mL), macerate for 15 min. Then filter through filter paper. Dip the electrode of pH meter in clear liquid. Record the pH value when stable reading is obtained **Table 2**.

Total Phenolic Content Determination by using Folin-Ciocalteu's Reagent: Gallic acid is a polyphenolic compound and can be used for the quantitative estimation of total phenolics by the method of calibration curve equivalent factor with the help of UV-Visible spectrophotometer¹⁰.

Standard Gallic Acid Calibration Curve: Gallic acid (10 mg) was weighed and mixed in methanol (10 mL); 1 mL of this solution was transferred to

the volumetric flask (10 mL) and diluted with methanol up to 10 mL. Solution of the final concentration 0.1 mg/mL will be obtained in **Fig. 3**.

TABLE 2: RESULT OF PHYSICO-CHEMICAL PARAMETERS

S. no.	Physical parameters	Value
1	Alcohol soluble extractive value	7.6%
2	Water-soluble extractive value	9.3%
3	Loss on drying	7.5%
4	Total ash	Not more than 5.4%
5	Water soluble ash	Not more than 4%
6	Acid insoluble ash	Not more than 0.95%
7	pH value	5.3

Phytoconstituents of Different Extracts of *Ficus glomerata*: Standard methods were adopted for detection of phytoconstituents in **Table 3**.

TABLE 3: RESULTS OF PRELIMINARY PHYTO-CHEMICAL SCREENING

Tests	Petroleum ether	Chloroform	Methanol	Water
Tannins	-	-	+	-
Alkaloids	+	+	+	-
Steroids	+	+	-	-
Cardiac glycoside	-	-	+	+
Anthraquinone	-	-	-	-
Saponin	-	-	-	+
Flavonoids	-	-	+	+
Coumarin	-	-	-	-

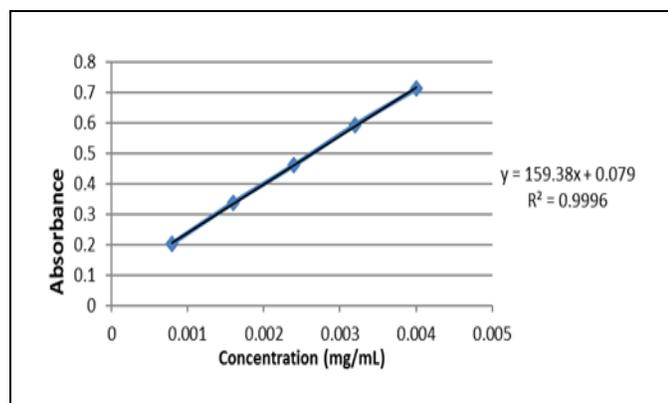


FIG. 3: STANDARD CALIBRATION CURVE

Dilutions: From above stock solution five different dilutions were prepared 0.2, 0.4, 0.6, 0.8 and 1 mL of stock solution was diluted as below procedure. Above stated different stock concentrations were taken in volumetric flask (25 mL), 10 mL distilled water and 1.5 mL folin-ciocalteu reagent was added, then after 5 min 4 mL Na₂CO₃ solution (20%) was added. Volume was adjusted to 25 mL with distilled water. Reaction mixture was kept for

30 min in dark then absorbance was taken at 765 nm at UV-Vis spectrophotometer.

Gallic acid is used for standard calibration curve (absorbance vs. concentration) obtained by UV-Vis spectrophotometer; sample content was measured by using the standard calibration curve.

Sample Preparation: Methanolic fraction of *F. glomerata* obtained during successive extraction was dissolved in methanol to obtain the concentration of 1 mg/mL, 1 mL of this solution was taken in the 25 mL volumetric flask.

10 mL distilled water and 1.5 mL folin-ciocalteu reagent was added, after 5 min 4 mL Na₂CO₃ solution (20%) was added and volume was adjusted up to 25 mL with distilled water.

Reaction mixture was kept for 30 min in dark and then absorbance was taken at 765 nm at UV-Vis spectrophotometer.

Calculation:

% of total phenolics or gallic acid equivalents = $C_0 \times \text{Purity of Std.} / C_1$

C_0 = Conc. obtained from calibration curve in mg/mL
 C_1 = Conc. applied on UV-Vis or final dilution factor in mg/mL.

Phenolic compounds are phytochemicals in which one or more hydroxyl groups containing an aromatic ring is present. A plant containing phenols is about 8000 and containing flavonoids is about 4000¹¹. Phenolics showed a wide spectrum of pharmacological activities like ant mutagenic, antioxidant, ant carcinogenic apart from this it has the ability to modify the gene expression¹². In plants or plant products, phenolics cover the largest portion of phytochemicals that is responsible for most of the antioxidant activity¹³.

Flavonoids occur in different plant parts both in Free State and as glycosides. These are the largest group of naturally occurring phenolic compounds. They have many pharmacological activities including antiulcer, antimicrobial, antiangiogenic, antiarthritic, anticancer, mitochondrial adhesion inhibition, protein kinase inhibition, etc¹⁴.

In the chemical structure of the flavonoids, two benzene rings are linked by a propane unit. Among phenolics flavones and flavonols are the most widely distributed¹⁵. Flavonoids can scavenge injurious free radicals such as superoxide and hydroxyl radicals because of their polyphenolic nature¹⁶. Various dietary plant flavonoids have shown tumor-inhibiting activity¹⁷. Apart from this; inhibition of the adhesion of blood platelets and the action of lens aldose reductase, the release of histamines, cardiac stimulant, blocking of the inflammatory effects of hepatotoxins shown by bioflavonoids¹⁸.

There is various strong evidence of biological activities of phenolic compounds on the basis of which experimental study was focused on the determination of total phenolics and flavonoids in *F glomerata*¹⁹.

Determination of Total Flavonoids Content: Estimation of total flavonoid in plant samples was performed by aluminum chloride colorimetric assay, with a quercetin calibration curve²⁰.

Quercetin Calibration Curve: Standard quercetin was weighed to prepare the stock solution of 0.1 mg/mL, then 0.2, 0.4, 0.6, 0.8 and 1 ml from the stock was taken in 10 mL volumetric flask, 1 mL AlCl_3 was added and volume was adjusted up to 10 mL with methanol. The reaction mixture was allowed to stand for 30 min. the absorbance was taken at 420 nm at UV-Visible spectrophotometer. Plot an Std. the curve of Abs. / conc. in the UV-Vis spectrophotometer, note the correlation coefficient and then measure the sample content by using that standard curve.

Sample Preparation: Methanolic fraction of *F. glomerata* obtained during successive extraction was dissolved in methanol to obtain the concentration of 1mg/ml for further testing. About 1 mL AlCl_3 was added to 1 ml methanolic extract, volume was adjusted up to 10 mL with methanol. The reaction mixture was allowed to stand for 30 min. the absorbance was taken at 420 nm at UV-visible spectrophotometer.

Calculation:

% of total flavonoids in sample crude drug = $\text{Conc. of the sample obtained by UV in mg} \times 10 \text{ ml} \times 100 / \text{Wt. of extract taken in mg}$

TABLE 4: RESULTS OF TOTAL PHENOLS AND TOTAL FLAVONOIDS

Parameters	Amount in methanolic extract of <i>Ficus glomerata</i> leaves (%)
Total phenolic content	12.94
Total flavonoid content	4.62

HPTLC Fingerprinting Profile:

Chemicals and Reagents: HPTLC analysis was performed on plates of Merck sized 20 cm × 10 cm HPTLC silica gel 60 F₂₅₄ (0.25 mm). Petroleum ether and n-hexane solvents were of instrumental grade from Merck, Germany. Other reagents were of analytical grade, used in the experiment and supplied by Merck, Darmstadt, Germany. The dried plant extracts were dissolved in methanol (98%) and stock solution of 10 mg/ml con, which is used for the application of spots on HPTLC plates.

Development of HPTLC Fingerprinting: Specifications are given in Table 5, fingerprinting profile at 366 nm given in Table 6 and fingerprinting profile after derivatization at the visible range is given in Table 7 and Fig. 4, 5.

TABLE 5: RESULTS OF INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

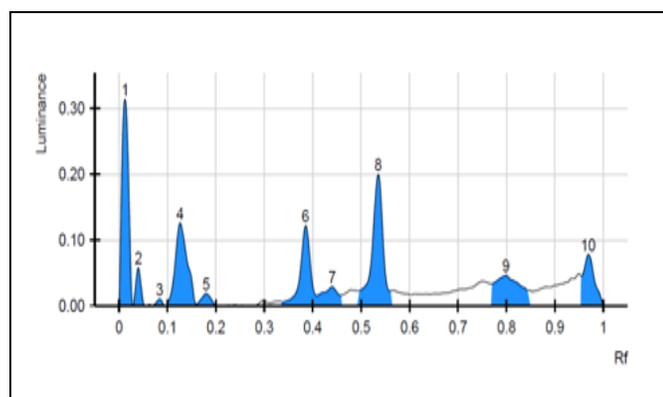
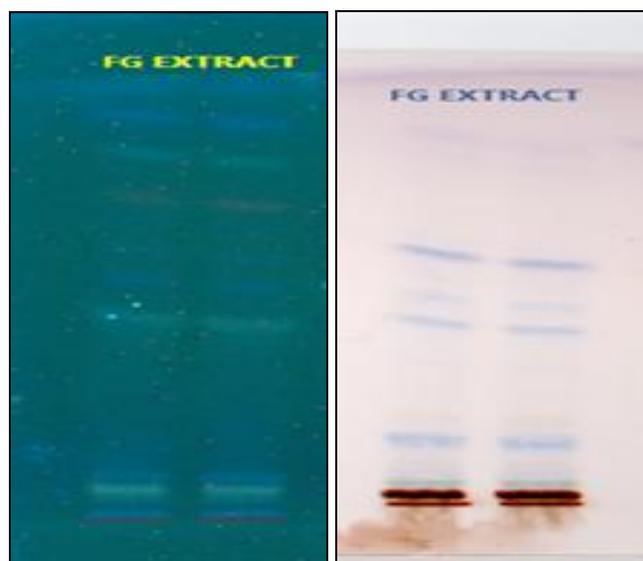
Parameters	Colour of spot
Stationary phase	Merck, HPTLC plates silica gel 60 F ₂₅₄
Mobile Phase	Toluene: ethyl acetate: formic acid 8.5: 1.5 : 0.5 v/v/v
Spotting device	Linomat V automatic sample applicator CAMAG
Syringe	100 µL hamilton (Bonaduz, Switzerland).
TLC chamber	Glass twin trough chamber (20 × 10 × 4 cm)
Densitometer	TLC Scanner 4 linked to win cats software V.4.06, CAMAG
Temperature	25 ± 2 °C
Relative humidity,	40%.
Scanning wavelength	600 nm
Visualizing agent	Anisaldehyde-sulfuric acid reagent
Scanning speed	10 mm s ⁻¹
Source of radiation	Deuterium lamp

TABLE 6: HPTLC FINGERPRINTING PROFILE AT 366 nm

S. no.	Color of spot	R _f
1	Yellow	0.1
2	Light blue	0.13
3	Yellow	0.42
4	blue	0.5
5	Light blue	0.55
6	Light blue	0.65
7	Red	0.7
8	Yellow	0.78
9	Blue	0.88

TABLE 7: HPTLC FINGERPRINTING PROFILE AFTER DERIVATIZATION AT VISIBLE RANGE

S. no.	Colour of spot	R _f
1	Light Brown	0.1
2	Blue	0.14
3	Blue	0.40
4	Light blue	0.45
5	Blue	0.55
6	Indigo	0.70

**FIG. 4: CHROMATOGRAM OF F. GLOMERATA EXTRACT AT 600 nm AFTER DERIVATIZATION****FIG. 5: HPTLC PROFILE OF FICUS GLOMERATA LEAF EXTRACT AT 366 nm WITHOUT DERIVATIZATION AND AT VISIBLE RANGE AFTER DERIVATIZATION****RESULTS:**

Quantitative Microscopy: Various parameters like palisade ratio, stomatal index, vein islet number, vein termination numbers were determined in leaf **Table 1**.

Physicochemical Parameters: Extractive values, total ash, acid insoluble, water soluble, loss on drying and pH was determined **Table 2**.

Phytoconstituents: Alkaloids and steroids were noted in petroleum ether and chloroform extract, tannin, alkaloid, cardiac glycoside, flavonoid in methanolic extract while cardiac glycosides, saponins and flavonoids were found in aqueous extract **Table 2**.

Total Phenolic Content: It was determined by using Folin- Ciocalteu's reagent. Gallic acid is a polyphenolic compound and was used for the quantitative estimation of total phenolics by method of calibration curve equivalent factor with the help of UV-Visible spectrophotometer **Table 4**.

Flavonoids Content: Estimation of total flavonoid in plant sample was performed by aluminium chloride colorimetric assay, with quercetin calibration curve **Table 4**.

HPTLC: In HPTLC fingerprinting at 366 nm and fingerprinting after derivatization at visible range shown nine and six peaks which denotes presence of different phytochemicals **Table 6, 7**.

DISCUSSION: *Ficus glomerata* commonly known as gular or Cluster fig tree, is native Indian medicinal plant, under this study monographic parameters were developed for *Ficus glomerata* Rox. leaves collected from Gangetic plains (Lucknow). Under the study pharmacognostic parameters were performed including macroscopic characters, microscopic characters, physico-chemical parameters (extractive values, ash values, pH analysis, LOD) and preliminary phytochemical screening. HPTLC fingerprinting profile was also developed for *F. glomerata* Rox. leaf extract which showed the presence of different phytochemicals in the form of spots (on HPTLC plate) and peaks (in chromatogram). At 366 nm total nine peaks were observed in the leaf extract which shows the presence of nine different phytochemicals. During the study, pharmacologically active secondary metabolites including total polyphenols and total flavonoids has been reported.

CONCLUSION: Pharmacognostic studies and monographic development of *Ficus glomerata* Roxb. leaves from the great Gangetic plain provides a collective data for the plant which can be utilized for the determination of purity of drug material available from various sources, data is useful for the detection of adulterants and other impurities and the results of various parameters are useful for the proper standardization of herbal formulations containing *Ficus glomerata* species as an ingredient.

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