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"Pharmacognostical & Phytochemical Evaluation of *Ficus Racemosa* linn. Leaves (*Moraceae*)"

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ABSTRACT

These Pharmacognostical evaluations were carried out as organoleptic, macroscopic, microscopic, physicochemical, phytochemical using fresh or dried leaves. *Ficus racemosa* Linn. Leaves have simple, ovate-oblong or elliptic- lanceolate, apex acute, margin wavy, surface globous, texture membranous, base symmetric, venation reticulate, color of upper surface dark green and lower surface light green. Three prominent veins seem to be arising from the base of the lamina. Petiole is angular and reddish–brown and UV-vis spectral analysis is performed for FRL, Oleonic acid, Urosolic acid and β-sitosterol shows strong absorption maxima at 210, 215, 372 nm, respectively. The high-performance thin-layer chromatography analysis was performed on high performance thin-layer chromatography plates using Toluene: Chloroform: Acetone (4.5:4.5:1, v/v) as mobile phase. And well resolved bands for quercetin (R_f 0.38) and kaempferol (R_f 0.55). These studies confirm the presence of kaempferol and quercetin for *FRL.* This study is the first scientific reports that provides convincing macroscopic, microscopic, analytical, phytochemicals study.

Keywords: FRL- *Ficus racemosa* Linn
Pharmacognostical evaluations
Physicochemical evaluation
HPTLC
UV Spectroscopy

INTRODUCTION

The standardization is a vital step in establishing the correct identity, purity, safety and quality of crude drug and it should be established before the inclusion of crude drug in herbal pharmacopoeia.[1,6]The preliminary material should be under proper control to maintain the quality of herbal products. Recently, there has been an emphasis in standardization of medicinal plants of therapeutic potential. In spite of modern techniques, pharmacognostical evaluation is still more reliable for identification and evaluation of plants. World Health Organization (WHO) recommends that the macroscopic and microscopic evaluation is most important in establishing the identity and purity of the plant.[2]

The Phytochemical evaluation for plants are the rich source of bioactive chemical compounds (phytoconstituents). These phytoconstituents can modulate biochemical pathways of higher animals. Plant products are recognised to be harmless and therapeutically efficacious based on the traditional knowledge. This information can be used to build up plant based formulations to prevent diseases and for health management but through pharmacological and chemical studies their efficacy should be monitored. Several clinically applicable drugs were discovered from plants such as digoxin, digitoxin, morphine, quinine, reserpine, taxol, vinblastine, vincristine and others and their structure elucidation consequently led the pathway of synthesis and derivatisation of compounds with higher efficacy and lower adverse effects such as amiodarone, metformin, nabilone, oxycodone, teniposide, verapamil.[3] So, plants have always attracted scientists for the drug discovery.

Among various plant secondary metabolites, phenolic compounds have a vital role in health prophylaxis along with their ubiquitous nature in vascular plants which make them best subject for studying bioactive phytoconstituents. Phenolic compounds demonstrate various bioactivities such as antioxidant, anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-diabetic, cardioprotective, hepatoprotective and vasodilatory effects. Phenolic compounds are related with health benefits which are attributed to their antioxidant activity and found richly in vegetables, fruits, grains, bark, roots, flowers, seeds, tea and wine. Among these vegetables, fruits and beverages are the prominent sources of phenolic compounds in human diet. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations.[4,5]

MATERIALS AND METHODS

Collection and Authentication of the Plant:- *Ficus racemosa* Linn. leaves were collected from Rajasthan, Jaipur district, India, in the month of July. The plant was identified and authenticated by the Director of Chambal Agriculture Research and Training Centre, Kota, Rajasthan.

Pharmacognostical Evaluation:-

Pharmacognostical evaluation was carried out as organoleptic, macroscopic, microscopic, physicochemical using fresh plant parts along with the coarse powder of dried plant parts.

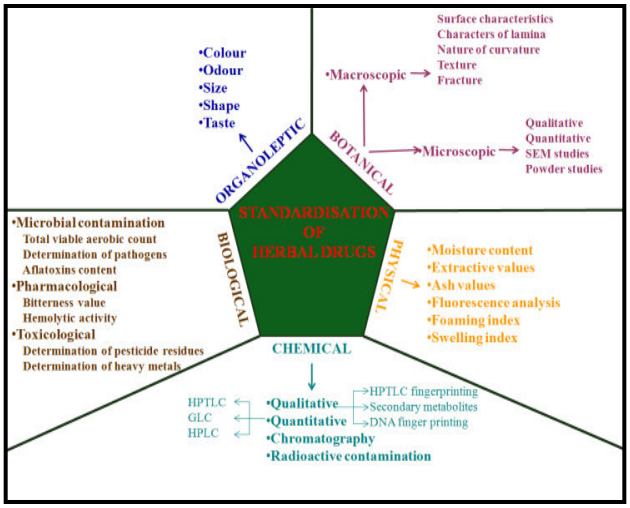

Figure1:-Various parameter regarding standardization of herbal drugs

Organoleptic evaluation

Various sensory parameters such as colour, odour, size, shape and taste were studied of fresh plant materials.

Macroscopic evaluation

Various macroscopic parameters of fresh leaves were recorded such as surface appearance, texture and characters of lamina. Lamina consists of various distinctive features such as shape, margin, incision, composition, apex, base and venation. Stem bark was studied for various morphological characters like nature of curvature, surface characteristics and fracture.

Microscopic evaluation

For microscopic evaluation, fresh leaves were fixed in fixing solution (containing formaldehyde (5 ml), glacial acetic acid (5 ml) and 70% alcohol (90 ml)) for three days before chloral hydrate solution treatment. Microscopic evaluation was carried out at both levels qualitatively and quantitatively. For qualitative microscopy of leaves a cube of pith was used and fine sections were obtained. Transverse sections of leaves were studied under microscope. The various characteristic features were studied of unstained sections of leaves under microscope and then photomicrography was done. In quantitative microscopy fresh leaves were used and leaves constants like stomata number, stomatal index, vein-islet number and vein termination number were studied as follows.[8]

a) Stomatal number*:* It is the average number of stomata per square millimeter (mm) of the epidermis of the leaves. Leaves were boiled with chloral hydrate solution. Then upper and lower epidermis was peeled separately using forceps and kept on slide. Put a drop of glycerine on the section. Slide was kept under the microscope. Epidermal cell and stomata were traced on paper using camera lucida on a 1 mm^2 by using stage micrometer. The stomata present in 1 mm^2 were counted along with the cell whose half of the area lied within the square. The result for each of the ten fields was calculated and average number of stomata per square millimeter was calculated for both the upper and lower surfaces.

(b) Stomatal index*:* It is the percentage which the number of stomata form to the total number of epidermal cells. Each stomata was counted as one cell. The stomata counted in stomatal number were used in calculating stomatal index also. The number of epidermal cells was also counted. Stomatal index was calculated using the formula for both the upper and lower surfaces:

Stomatal index = (S/(E+S)) × 100

Here, S = number of stomata per unit area, E = number of epidermal cells in the same unit area.

(c) Vein-islet number: A vein-islet is the small area of green tissue surrounded by the veinlets. The vein-islet number is the average number of vein-islets per square millimeter of a leaves surface. In this, leaves were cleared by boiling with chloral hydrate solution for thirty minutes. Camera lucida was fitted on the microscope and stage micrometer was placed on the microscope and 16 mm objective was used then by viewing through the microscope, a square of 1 mm^2 was drawn on the paper with the help of camera lucida. The cleared leaves was placed on the slide and kept on the stage of microscope. Veins were traced onto the paper within the square and outlines of those islets were completed which overlapped two adjacent sides of the square. The number of vein islets in the square millimeter was counted. Procedure was repeated for up to four mm^2 of counting. Average number of vein islets was counted from the four adjoining squares to find out the value of 1 mm^2.

(d) Veinlet termination number*:* It is defined as number of veinlet terminations per mm^2 of the leaves surface, midway between midrib and margin of the leaves. Leaves were placed on the microscope in the same way as that in vein-islet number. The number of veinlet terminations present within the square was counted. Procedure was repeated for three more squares and average number of vein terminations in 1 mm^2 was calculated.

Physicochemical evaluation

In this study, air dried plant material was used for quantitative determination of physicochemical values like total ash, acid insoluble ash, water soluble ash, moisture content (loss on drying), foaming

index, swelling index, crude fibre, total solid and extractive value according to the standard methods as follows.[9.10]. All the procedures were carried out in triplicate.

(a) Ash value:

(i) *Total ash value*: Air dried drug was weighed accurately (2 g) in a tared crucible and was incinerated at a temperature of 450ºC until the ash was white or nearly white. Percentage of ash was calculated with reference to air dried drug.

(ii) *Acid insoluble ash:* The ash obtained in total ash parameter was boiled with 25 ml of 2 M hydrochloric acid for 5 minutes. Then the insoluble matter was filtered onto an ashless filter paper and washed with hot water. The insoluble matter obtained was ignited at 450ºC and weighed. This weight was used to calculate acid insoluble ash in reference to air dried drug.

(iii) *Water soluble ash:* The ash obtained in the total ash parameter was put in 25 ml of water and boiled for 5 minutes. The insoluble matter was filtered and collected onto an ashless filter paper and ignited at 450ºC for 15 minutes. Then weigh the obtained residue and subtract it from the weight of total ash. This weight obtained was used to calculate water soluble ash against the weight of air dried drug.

(b) Moisture content:

(i) Loss on drying: Drug material (1 g) was placed in a tared china dish and put in hot air oven at 105ºC. Drug material was dried until constant weight was obtained. Constant weight obtained was subtracted from initial weight of air dried drug. This weight was used to calculate percentage loss on drying (% LOD) with reference to original weight of drug.

(c) Foaming index: Accurately weighed (1g) quantity of powdered plant material was transferred to a 500 ml conical flask having 100 ml boiling water. Then this was moderately boiled for 30 minutes and filtered after cooling into a 100 ml volumetric flask and volume was made up with water. The decoction was poured into 10 stoppered test tubes (height 16 cm, diameter 16 mm) in successive portions as 1 ml, 2 ml, 3 ml and so on then the volume was made up to 10 ml with water. Then these test tubes were stoppered and shaken in a lengthwise motion for 15 seconds. Further it was allowed to stand for 15 minutes and the height of foam was measured. The results were assessed as follows

- Foaming index was less than 100, if the height of the foam in every tube was less than 1cm.
- If a height of foam of 1cm was measured in any tube, the volume of the plant material decoction in this tube was used to determine the index. If this tube was the first or second tube in a series, an intermediate dilution was to be prepared in a similar manner to obtain a more precise result
- Foaming index was over 1000, if the height of the foam was more than 1cm in every tube. In this case, determination was repeated using a new series of dilution of the decoction to get the results.

Foaming index was calculated using the following formula:

$$1000/A$$

Here, A= the volume in ml of the decoction which was used for preparing the dilution in the tube where foaming to a height of 1 cm was observed.

(d) Swelling index: The specified quantity (1g) of the plant material was accurately weighed and was introduced into a 25ml glass stoppered measuring cylinder. Water (25 ml) was added to measuring cylinder. The mixture was then shaken thoroughly every 10 minutes for 1 hour. Then it was allowed to stand for 3 hours at room temperature. The volume (in ml) was measured which was occupied by the plant material including any sticky mucilage. The mean value of the individual determinations was calculated in reference to 1 g of plant material.

(g) Solvent extractive value: Air dried leaves were weighed (5 g) separately and macerated with 100 ml of a specified solvent in a closed flask for 24 hours. Then it was filtered and filtrate was evaporated at specified temperature. The residue obtained was weighed and percentage of it to the weight of original air dried drug was calculated. Different solvents used for extractive value were water, alcohol, acetone, chloroform and petroleum ether.

Extraction

The plant is known for the presence of phenolic compounds that are tannins and flavonoids. Also, on the basis of the results obtained for the extractive value in the pharmacognostical evaluation, the aqueous-alcoholic (hydroalcoholic) solvent was selected which is also supported by the literature as aqueous alcohol is considered the best solvent for extracting phenolic compounds from plant materials. Hence the hydroalcoholic solvent was selected for the extraction. In order to achieve complete and quick extraction of the polar fractions, the interaction between non-polar and polar compounds in plant material, the so-called "matrix effect" was broken up by a preliminary extraction with non-polar solvents like chloroform and n-hexane.[11]

Method

Standard procedure and analytical grade solvents were used for the extraction. 40 g of powdered plant parts (leaves) was preliminary extracted with chloroform and n-hexane, respectively by soxhlet apparatus separately and further with the same procedure extracted with hydroalcoholic solvent at temperature between 50-65ºC.

Phytochemical Evaluation

Plants are the rich source of bioactive chemical compounds (phyto-constituents). These phyto-constituents can modulate biochemical pathways of higher animals. Plant products are recognised to be harmless and therapeutically efficacious based on the traditional knowledge. This information can be used to build up plant based formulations to prevent diseases and for health management but through chemical studies their efficacy should be monitored. Several clinically applicable drugs were discovered from plants such as digoxin, digitoxin, morphine, quinine, reserpine, taxol, vinblastine, vincristine and others and their structure elucidation consequently led the pathway of synthesis and derivatization of compounds with higher efficacy and lower adverse effects such as amiodarone, metformin, nabilone, oxycodone, teniposide, verapamil.[3]

Among various plant secondary metabolites, phenolic compounds have a vital role in health prophylaxis along with their ubiquitous nature in vascular plants which make them best subject for studying bioactive phytoconstituents. Phenolic compounds demonstrate various bioactivities such as antioxidant, anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-diabetic, cardioprotective, hepatoprotective and vasodilatory effects. Phenolic compounds are related with health benefits which are attributed to their antioxidant activity and found richly in vegetables, fruits, grains, bark, roots, flowers, seeds, tea and wine. Among these vegetables, fruits and beverages are the prominent sources of phenolic compounds in human diet. The antioxidant activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations.[4,12]

Phytochemical Investigation

Preliminary phytochemical investigation of hydroalcoholic extracts of leaves were carried out according to standard procedure as follows.[8]

1. **Alkaloids-** Sample preparation: 50 mg of extract was shaken well with few ml of diluted hydrochloric acid (10%) and filtered. Filtrate was tested using following reagents:

a) Mayer's test- Few ml of filtrate + two drops of Mayer's reagent for White or cream precipitate.

b) Dragendorff's test- Few ml of filtrate + two drops of Dragendorff 's reagent for Orange brown precipitate.

c) Hager's test- Few ml of filtrate + two drops of Hager's reagent for Yellow precipitate.

d) Wagner's test- Few ml of filtrate + two drops of Wagner's reagent for Reddish brown precipitate.

2. Glycosides

(i) Anthraquinone glycosides

a) Borntrager's test-3 ml extract + diluted sulphuric acid and boiled, cooled and filtered. To 2 ml of filtrate + 2 ml chloroform. Shaken and separated chloroform Shaken and separated chloroform layer and added ammonia Pink or red colour.

(ii) Cardiac glycosides

a) Keller-Killiani test- Taken 2 ml of extract + 1 ml glacial acetic acid, 1 drop of 5% ferric chloride solution and concentrated sulphuric acid for Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish-green.

b) Legal's test- Extract + 1 ml pyridine. Added 1 ml sodium nitroprusside solution for Pink or red colour.

c) Baljet's test- 2 g of picric acid + 50 ml ethanol + 30 ml water. Shaken this mixture until all solid is dissolved. Then added 5 ml of 10% sodium hydroxide solution and made up volume to 100 ml with water (alkaline picric acid or sodium picrate solution). Then taken 3 ml of extract + 15 ml of sodium picrate solution for Yellow to orange colour.

(iii) Saponin glycosides

a) Foam test- Shaken extract vigorously with water for observation of persistant foam.

3. Carbohydrates

a) Molish's test- 2 ml of extract + 2 drops Molish's reagent. Then shaken the mixture well. Added 1 ml of concentrated sulphuric acid along sides of test tube. Allowed to stand for Violet ring formation at the junction of two liquids.

b) Fehling's solution A and B test - Mixed 1 ml of both solutions and heated. Added 1 ml of extract and heated for 5-10 minutes on water bath for First yellow then brick red precipitate formation.

c) Benedict's test- 1 ml of extract + 1 ml of Benedict's reagent. Mixture was heated on a boiling water bath for 5 minutes for Appearance of green, yellow or red solution.

4. Steroids

a) Liebermann-Burchard reaction - Mixed 2 ml of extract with chloroform and then added 1-2 ml of acetic anhydride and 2 drops of concentrated sulphuric acid from the side of test tube for First red then blue and finally green colour.

b) Salkowski reaction- Took 2 ml of extract + 2 ml of chloroform + added 2 ml of concentrated sulphuric acid. Shaken well for Red colour in chloroform layer and greenish yellow fluorescence in acid layer.

5. Oils and fats

a) Spot test- A small quantity of extract was pressed between two filter papers for Oil stain.

6. Phenolic compounds and tannins

a) Ferric chloride solution test- Extract + few drops of 5% ferric chloride solution for Deep blue-black colour.

b) Gelatin solution test- Extract + 2 ml of 1% solution of gelatin for White precipitate.

c) Lead acetate solution test- Extract + few drops of 10% lead acetate solution for White precipitate.

7. Proteins and amino acids

a) Millon's test- 3 ml of extract + 5 ml of Millon's reagent. Warmed after formation of white precipitate for White precipitate changes to brick red or dissolves to give red colouration.

b) Biuret test- 3 ml of extract + added 4% sodium hydroxide and few drops of 1% copper sulphate solution for Violet or pink colour.

c) Xanthoproteic test- Mixed 3 ml of extract with 1 ml concentrated sulphuric acid. Boiled. Formation of white precipitate and added ammonium hydroxide for White precipitate formation after boiling turns yellow and finally to orange colour on addition of ammonium hydroxide.

d) Ninhydrin test- Heated 3 ml of extract + 3 drops of (5%) ninhydrin solution in boiling water bath for 10 minutes for Purple or bluish colour.

8. Flavonoids

a) Sulphuric acid test- Extract + sulphuric acid (66%) for Yellow, orange or red colour.

b) Lead acetate solution- Extract + added lead acetate solution for Yellow precipitate.

UV Spectroscopy Weight accurately 0.1gm of powders FRL leaf extract. It dissolves in 20ml of ethanol and kept it for 15 minutes in sonicator, and makeup to volume 100ml. then subjected to UV from wavelength between 900-200nm. Record the UV spectra.

HPTLC

Preparation of Sample and Standard Solutions for *Ficus racemosa*

A. Standard preparation Standard stock solutions of kaempferol and quercetin were prepared. 2 mg of kaempferol was dissolved in petroleum ether in 10 mL volumetric fask and 2 mg of quercetin was dissolved in petroleum ether in 10 mL volumetric fask and volume was adjusted up to the mark with petroleum ether. For calibration curve of kaempferol, 1–7 µL solutions was applied on pre-coated TLC plates and 1–8 µL solutions was applied for quercetin.

B. Sample solution preparation Dried PEE was dissolved in 10 mL petroleum ether and 12 µL of PEE solution was applied to a TLC plate. Dried EE was dissolved in 10 mL Ethanol and 12 µL of EE solution was applied to a TLC plate.

Chromatographic method for Ficus racemosa HPTLC plates (20 cm×10 cm aluminium backed) coated with 0.3 mm layer of silica gel G 60 F254 (Merk, India) were used for performing HPTLC analysis. Before performing HPTLC analysis, the HPTLC plates were washed with methanol and activated in hot air oven at 110 °C. TLC sampler (CAMAG Linomat 5) was used for applying standard and sample solutions onto the HPTLC plates. CAMAG Automatic developing chamber 2 (ADC 2) was used. Toluene: Chloroform: Acetone 4.5:4.5:1 (v/v) was used as mobile phase. The development chamber was pre-saturated for 30 m before chromatogram development. After development, the HPTLC plates were air-dried for time duration of 5 m and scanned instantly at 254 nm wavelength. CAMAG TLC Scanner 4 was used to scan TLC plates. CAMAG-TLC Visualizer 2 software was employed for taking professional images and documentation of TLC/HPTLC chromatograms.

3. RESULTS & DISCUSSION

Pharmacognostical evaluation

Organoleptic evaluation

FRL Leaves simple, alternate, stipules 12-18 mm long, lanceolate, linear-lanceolate, pubescent, often persistent on young shoots; petiole 10-50 mm long, slender, grooved above, becoming brown scurfy.

Macroscopic Evaluation

FRL leaves are simple, 7.5-15 cm × 3.2-6.3 cm, ovate-oblong or elliptic- lanceolate, apex acute, margin wavy, surface globous, texture membranous, base symmetric, venation reticulate, color of upper surface dark green and lower surface light green. Three prominent veins seem to be arising from the base of the lamina. Petiole is angular and reddish – brown.

Microscopic Evaluation

Lamina of the transverse section shows a prominent hypodermis beneath the upper epidermis. Underlying the hypodermis are bi-layred, compact, radially elongated palisade cells followed by spongy mesophyll composed of 3-4 layers of loosely arranged parenchymatous cells with scattered calcium oxalate cluster crystals. Midrib consists of a well-developed collenchyma below upper epidermis and above lower epidermis. Ground tissue consists of loosely arranged polygonal parenchymatous cells having calcium oxalate prism and cluster crystals.

Vascular bundles are bi-collateral, crescent shaped, having patches bundles above the primary vascular bundle. Sheath of calcium crystal are present below the primary vascular. Starch grains are scattered throughout the ground tissue. Trichomes are covering, long, unicellular or bicellular, few having a hooked-top.

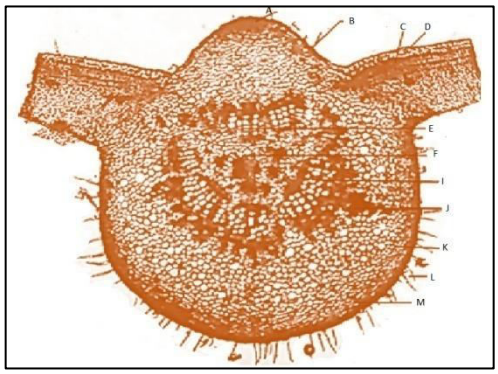

Figure.2: Ficus racemosa Linn. Leaves T.S (A, K - Collenchyma, B, M - Epidermis, C-Palisade, D- Hypodermis, E, I - Vascular bundle, F- Peri-medullary phloem, J- Calcium oxalate cluster crystals sheath, L- Trichomes

Quantitative Microscopy

Ficus racemosa has dorsiventral leaf, quantitative microscopy reveals that the leaves have anomocytic type of stomata. The characteristic microscopic features of leaves were observed as epidermal cells, palisade and spongy parenchyma, vascular bundles, xylem and phloem cells. The columnar (tubular or cylindrical) palisade cells were arranged compactly with their long axis at right angles to the leaf epidermis. This arrangement of palisade cells protects chloroplasts from excessive heat of sun rays.

Table 1: Leaf constants (at 100×)

S. No.	Parameters	FRL Value* (in 1 mm^2 area)
1	Stomata number, upper surface	106.52 ± 7.06
2	Stomata number, lower surface	158.33± 8.73
3	Stomatal index, upper surface	5.5 ± 0.5
4	Stomatal index, lower surface	16.5 ±0.5
5	Vein-islet number	13 ± 0.5
6	Veinlet termination number	16 ± 1

***Values are expressed as mean±SD**

Analytical study with all standardization parameter

Physicochemical evaluation- Physicochemical analysis of leaf powder of FRL viz. total ash, acid insoluble ash, water soluble ash, moisture content, foaming index, swelling index, are presented in Table 2.

Table 2: Physicochemical parameters

S. No.	Parameters	FRL*
1	Total ash (% w/w)	12.7±0.5
2	Acid insoluble ash (% w/w)	0.7±0.1
3	Water soluble ash (% w/w)	3.1±0.2
4	Moisture content (% w/w)	2.47±0.05
5	Foaming index	<100
6	Swelling index	00

***Values are expressed as mean±SD (n=3)**

Extraction

The plant leaves powder showed higher extractive value in water and alcohol than other solvents.

Table 3: Extractive value (in % w/w) in different solvents

S. No.	Solvents	FRL*
1	Water	16.3±0.3
2	Alcohol	4.3±0.2
3	Petroleum ether	1.7±0.2

Values are expressed as mean±SD (n=3)

The obtained extracts of FRL hydroalcoholic extract of leaves had percentage yield of 20.1%. Extracts were stored in sealed vials in a freezer until tested.

The results obtained from the extractive value in the pharmacognostical evaluation indicates the presence of more polar compounds as the higher extractive value was found in water and alcohol as solvents hence the aqueous-alcoholic (hydroalcoholic) solvent was selected for extraction.

PHYTOCHEMICAL EVALUATION

Qualitative Phytochemical investigation

S. No.	Test	Result (FRL)
1.	Alkaloids	**+++**
2.	Glycosides	**+++**
3.	Carbohydrates	**+++**
4.	Steroids	**+++**
5.	Oils and Fats	**---**
6.	Phenolic compounds and tannins	**+++**
7.	Proteins and Amino acids	**---**
8.	Flavonoids	**+++**

Here, '+++' = positive result, '---' = negative result

So, the major phytochemical present in the extracts were alkaloids, glycoside, flavonoids, phenolic compounds and tannins.

UV Spectroscopy

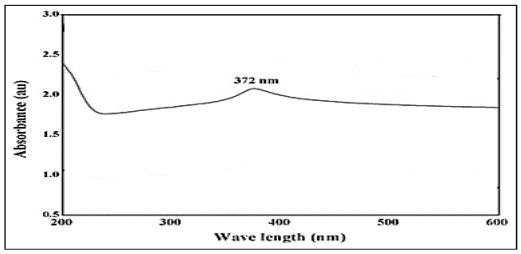

Figure 3: UV spectrum of Ficus racemosa Linn. Leaves

As per above spectra at 210nm, 215nm, 372nm, Oleonic acid, Uroslic acid and β – sitesterol was

observed, respectively. Above UV-vis spectra is characteristic for *Ficus racemosa* Linn. Leave hydroalcohalic extract.

Table.5: list of wavelengths of absorption peaks of molecule present in UV spectra of FRL

Sr. No	The wavelength of absorption peak (nm)	Molecule present
1	210	Oleonic acid
2	215	Ursolic acid
3	372	β-sitosterol

HPTLC

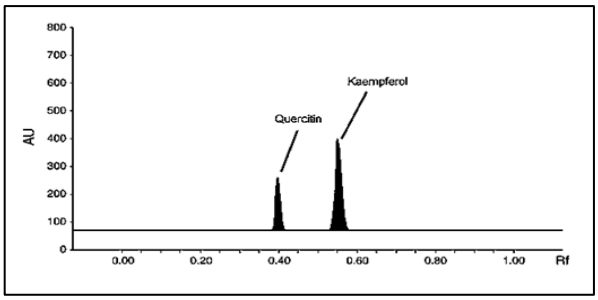

Figure 4: HPTLC chromatogram for Quercetin and Kaempferol

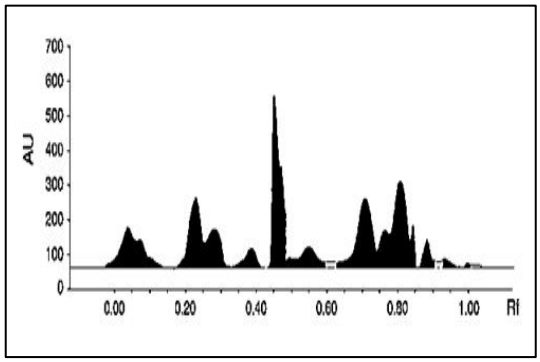

Figure.5: HPTLC chromatogram for *Ficus racemosa* Linn. Leaves.

The present work is the first report of simultaneous quantifcation of kaempferol and quercetin by HPTLC in Ficus racemosa L. The HPTLC densitometric chromatogram of reference standard kaempferol and quercetin in EE and PEE. The peaks resolving at R_f 0.55 and 0.38 in test solution were found to be superimposing with those of respective standards of kaempferol and quercetin.

The content of kaempferol and quercetin in the leaves of Ficus racemosa L. was calculated on the basis of peak area and was found to be 0.0298% and 0.0079% respectively in PEE and 0.0098% and 0.0024% respectively in EE. The calibration curves indicate that the peak-area response was a polynomial function of the amount of standards kaempferol and quercetin in the range of 200–1000 ng respectively. The correlation coefficient for quercetin and kaempferol was found to be 0.9876 and 0.9888 respectively.

4. SUMMARY

Experimental work includes the collection, authentication, pharmacognostical evaluation, extraction, phytochemical and pharmacological evaluation.

Pharmacognostical evaluation was carried out as organoleptic, macroscopic, microscopic, using fresh or dried leaves. *Ficus racemosa* Linn. Leaves have simple, ovate-oblong or elliptic- lanceolate, apex acute, margin wavy, surface globous, texture membranous, base symmetric, venation reticulate, color of upper surface dark green and lower surface light green. Three prominent veins seem to be arising from the base of the lamina. Petiole is angular and reddish – brown.

Microscopy reveals that the *Ficus racemosa* Linn leaves have prominent hypodermis beneath the upper epidermis. Underlying the hypodermis are bi-layered, compact, radially elongated palisade cells followed by spongy mesophyll composed of 3-4 layers of loosely arranged parenchymatous cells with scattered calcium oxalate cluster crystals. Midrib consists of a well-developed collenchyma below upper epidermis and above lower epidermis. Ground tissue consists of loosely arranged polygonal parenchymatous cells having calcium oxalate prism and cluster crystals.

Vascular bundles are bi-collateral, crescent shaped, having patches bundles above the primary vascular bundle. Sheath of calcium crystal are present below the primary vascular. Starch grains are scattered throughout the ground tissue. Trichomes are covering, long, unicellular or bicellular, few having a hooked-top.

Analytical study with all standardization parameter are still no attempt has been made for the pharmacognostical standardisation of leaves of *Ficus racemosa.* The standardisation is a vital step in establishing the correct identity, purity, safety and quality of crude drug and it should be established before the inclusion of crude drug in herbal pharmacopoeia[1]. The preliminary material should be under proper control, to maintain the quality of herbal products. Recently, there has been an emphasis in standardisation of medicinal plants of therapeutic potential. In spite of modern techniques, pharmacognostical evaluation is still more reliable for identification and evaluation of plants. World Health Organisation (WHO) recommends that the macroscopic and microscopic evaluation is most important in establishing the identity and purity of the plant[2]. Morphological, microscopic and physicochemical evaluation provides the simplest, quickest and cheapest means to establish the identity and purity of drug and also acts as a reliable tool for detecting adulteration. As the adulteration of the genuine raw material is the main cause of degradation of desired therapeutic effect of plant species used in traditional systems of medicine[2,13].

In conclusion, the parameters which are evaluated here can be considered as unique enough to identify and decide the authenticity of *F. racemosa* will serve in the development of pharmacopoeial standards for the future studies.

UV-vis spectral analysis is performed for FRL, Oleonic acid, Urosolic acid and β-sitosterol shows strong absorption maxima at 210, 215, 372 nm. UV-vis spectra is characteristic for respective plant leaf.

The high-performance thin-layer chromatography analysis was performed on high performance thin-layer chromatography plates using Toluene: Chloroform: Acetone (4.5:4.5:1, v/v) as mobile phase. and well resolved bands for quercetin (R_f 0.38) and kaempferol (R_f 0.55). these studies conform the presence of kaempferol and quercetin for *Ficus racemosa.*

Extraction was carried out by soxhlet apparatus using hydroalcoholic solvent. It is also supported by the literature as aqueous-alcohol is considered as the best solvent for extracting phenolic compounds from plant materials. Hence the hydroalcoholic solvent was selected for the extraction. Percentage yield was calculated after lyophilisation. Qualitative phytochemical investigation revealed the presence of major phytochemicals like alkaloids, flavonoids and phenolic compounds and tannins in extracts of *F. racemosa* leaves.

5. CONCLUSION:

Overall, the experimental studies concluded that the *F. racemosa* Linn. leaf extract in this study is the first scientific report that provides a convincing macroscopic, microscopic, and analytical study.

REFERENCS:-

1. Gupta, C.P., Sharma, N., Rao, C.V., (2012). Pharmacognostic studies of the leaves and stem of Careya arborea Roxb. Asian Pac. J. Trop. Biomed. 2, 404-408.
2. Kumar, D., Kumar, A., Prakash, O., (2012). Pharmacognostic evaluation of stem bark of Pongamia pinnata (L.) Pierre. Asian Pac. J. Trop. Biomed. 2, S543-S546.
3. Davis, S. N., & Granner, D. (2001). The effects of HDV-insulin on carbohydrate metabolism in Type 1 diabetic patients. J. of diabetes and its complications, 15(5), 227-233.
4. Balasundram, N., Sundram, K., Samman, S., (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem. 99, 191-203.
5. Bedoya, F. J., Solano, F., & Lucas, M. (1996). N-monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. Experientia, 52, 344-347.
6. Chakravarthy, B. K., Gupta, S., Gambhir, S. S., & Grode, K. D. (1980). Pancreatic beta cell regeneration. A novel anti-diabetic mechanism of Pterocarpus marsupium Robx. Indian J. of pharmacology, 12, 123- 127.
7. Deep P., Singh A., Ansari M. T., Raghav P. (2013). Pharmacological Potentials of Ficus racemosa - A Review; Int. J. Pharm. Sci. Rev. Res., 22(1), 29-34.
8. Khandelwal, K.R., (2010). Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan, Pune, India.
9. Mukherjee, P.K., (2012). Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. Business horizons, New Delhi, India.
10. Narendhira Kannan, R. T., Subramanian, S. K., & Kandaswamy, M. (2006). Biochemical evaluation of anti diabetogenic properties of some commonly used Indian Plants on Streptozotozin induced diabetes in experimental rats. Clinical Experimental Pharamacology and Physiology, 33, 1150-1157.
11. Maroo, J., Vasu, V. T., Aalinkeel, R, & Gupta, S. (2002). Glucose lowering effect of Enicostemma littorale Blume. in diabetes. A possible mechanism of action. J Ethno Pharmacol, 81, 317-320.
12. Begum, N., & Shanmuga Sundaram, K. R. (1978). Tissue Phosphates in experimental diabetes. A rozya. J Health Science, 4, 129-139.
13. Chandra, D., Chandra, S., Pallavi, & Sharma, A. K. (2016). Review of finger millet (Eleusine coracana (L.) Gaertn): A power house of health benefiting nutrients. Food Science and Human Wellness, 5, 149-55.
14. Anita Rami Shiksharthi, Stuti Mittal (2011). Ficus racemosa: Phytochemistry, Traditional Uses and Pharmacological Properties: A Review; International J. of Recent Advances in Pharmaceutical Research,: 4 : 6-15.
15. Dubey, N. K., Kumar, R., & Tripathi, P. (2004). Global promotion of herbal medicine: India‘s opportunity. *Current Science*, 86(1), 37-41.
16. Faiyaz Ahmed & Asna Urooj (2010). Traditional uses, medicinal properties, and phytopharmacology of *Ficus racemosa*: A review; Pharmaceutical Biology, 48(6): 672–681.
17. Faiyaz Ahmed, Asna Urooj (2011).Pharmacognostical studies on *Ficus racemosa* stem bark; *Pharmacognosy Journal*, 3(19), 19-21.
18. M. Ramila Devi and A. Manoharan(2011). Phytochemical Investigation of *Erythrina variegate* and *Ficus racemosa* leaves; J. of Chemical and Pharmaceutical Research; 3(6):166-172.
19. Paarakh P M (2009). *Ficus racemosa* Linn,- an overview, Natural Product Radiance, 8(1), 84-90.

20. Pahari N., Majumdar S., Karati D., Mazumder R. (2022). Exploring the Pharmacognostic properties and pharmacological activities of phytocompounds present in *Ficus racemosa* Linn. A concise review. Pharmacological Research. Modern Chinese Medicine 4, 01-10.
21. Pardia V. S., Karna N. (2022). Pharmaceutical Potential of *Ficus racemosa* Linn. International J. of Pharmaceutical Research and Application. 7 (3), 1816-1823.
22. Rajnish Kumar Yadav, Bankim Chandra Nandy, Siddhartha Maity, Srimanta Sarkar, Sudipta Saha (2015). Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*; Pharmacognosy Reviews, 9(17), 73-80.
23. Rodkey, F. L. (1965). Direct spectrophotometric determination of albumin in human serum. Clinical Chemistry, 11, 478-479.
24. Sajeeth, C.I., Manna, P.K., Manavalan, R., Jolly, C.I., 2010. Quantitative estimation of gallic acid, rutin and quercetin in certain herbal plants by HPTLC method. Der Chem. Sin.1, 80-85.
25. Salma Akter Sumi, Md. Afjalus Siraj, Amir Hossain, Md. SagirMia, Seagufta Afrin, and Md. MustafizurRahman (2016). Investigation of the Key Pharmacological Activities of *Ficus racemosa* and Analysis of Its Major Bioactive Polyphenols by HPLC-DAD; Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine, 1-9.
26. Shiksharthi A. R., Mittal S. (2011). *Ficus racemosa*: Phytochemistry, Traditional uses and Pharmacological Properties: A Review. International J. of Recent Advances in Pharmaceutical Research. 4, 06-15.
27. Tripathi., Poonam Pandey., Poonam Chaudhary., Mahendra Kumar Mishra., & Vandana Pathak. (2019). Quantitative Screening of Phytochemicals of Different Parts of Ficus benghelensis Linn. International J. of Advanced Scientific Research and Management, 5, 160-166.