Original Article

PHARMACOGONOSTICAL, PHYTOCHEMICAL, AND ANTI MICROBIAL ACTIVITY OF LEAVES OF FICUS MOLLIS VAHL

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Abstract

Ficus mollis vahl. is an deciduous tree under the family Moraceae. This study was carried out with an objective to investigate pharmacogonostical, phytochemical analysis of leaf extracts of a medicinal plant, F. mollis and their antimicrobial activities against pure microbial cultures of Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger. Leaves crude drug for microscopic, organoleptic and behavior of the leaf powder upon treatment with different chemical reagents was observed and reported. The preliminary phytochemical investigation for the leaves extracts proved the presence of pharmacologically active compounds such as alkaloids, phenols, flavonoides, glycosides, tannins, lignins, saponins and steroids. The maximum antimicrobial activity was observed in methanol and aqueous extracts against A. niger and P. aeruginosa with zone of inhibition 25.75 and 25.00 mm. It is also reported that there is no activity in benzene extract on all organisms. Minimum inhibitory concentrations on all organisms with leaf extracts ranges from 0.32 to 3.62 mg/ml compared to 10 mg/ml of Nystatin. Antimicrobial activity of leaves extracts proved the plant contains antimicrobial compound which can be further developed as phytomedicine for the therapy of infection. All these findings serve as preliminary data for further studies on F. mollis.

Keywords: Ficus mollis Vahl., Moraceae, Phytoconstituents, Pharmacognostical, and Antimicrobial activity.

INTRODUCTION

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. The Moraceae often called the mulberry family or fig family — is a family of flowering plants comprising about 40 genera and over 1,000 species. Many Ficus species have long been used in folk medicine as astringents, carminatives, stomachics, vermicides, hypotensives, anthelmintics and anti-dysentery drugs [1]. It is believed that some Ficus species can be used as a remedy for visceral obstructive disorders, diabetes, leprosy, respiratory disorders and certain skin diseases, [2] and as an absorbent for inflammatory swellings and burns [3].

F. mollis vahl. commonly called as soft fig is a medium sized tree it is found only in india and srilanka [4]. The trees produce three types of flower; male, a long-styled female and a short-styled female flower, often called the gall flower. It is used as medicine in ayurveda and folk medicine for treating different ailments. The crushed leaves are applied as a poultice a bark to treat boils. Applied externally decoction is used as a wash and un cler and as gargle in salivation [5]. A paste made from the bark is applied as an ointment in the treatment of cuts and wounds [6]. The white wood is moderately hard, with alternate bands of soft pale and firm dark tissue, very regular and concentric [7]. The Latex is used for caulking boats and water proofing.

MATERIALS AND METHODS:

PHARMACOGNOSTICAL STUDIES

Collection and identification of plant material:

The leaf material of F. mollis was collected during September 2018 – February 2019 from Talakona forest in Tirupati, Andhra Pradesh, India. The taxonomic identification of the plant is confirmed by Prof. N. Yasodamma.

The voucher specimen B.K: 4 were deposited in the herbarium, (RUK) Department of Botany, Rayalaseema University, Kurnool for future reference as per standard methods [8]. The present work was carried out in the Department of Botany, Rayalaseema University, Kurnool. Plant material was thoroughly washed and then dried under shade for one week. The dried leaves were grind in a mixer grinder and sieved. The leaves powders were stored in air sealed polythene bags at room temperature until further use.

MACROSCOPIC / MORPHOLOGICAL AND ORGANOLEPTIC CHARACTERS

Habit, morphology; colour, odour, taste, texture, of leaves were observed [9].

HISTOLOGY

Small fragments of the fresh leaves were embedded in paraffin and transverse sections (4-5 µm) were obtained. Good sections were collected and placed on a grease free microscopic slide along with a drop of glycerin and water (1:1). The sections were covered with clean cover slip and observed under the compound microscope at 10x magnification and the sections were suitably traced out 10&11].

PHYSICOCHEMICAL ANALYSIS

Determination of ash values:

Ash values such as total ash, acid insoluble ash, water soluble ash, sulphated ash and moisture content/loose of weight on drying, values were determined with the powders of leaves [12].

Total Ash:

1g of air dried powders was taken separately in a previously ignited and weighed silicacrucible. The powder was spreaded in an even layer and ignited by gradually increasing the heat up to 500 - 600^{0}C until it becomes white, indicating the absence of carbon. Then crucible was cooled in desiccator. The ash was weighed and percentage of total ash with reference to air dried powder was calculated.

$$\% \text{ of Total Ash Value} = \frac{\text{Weight of the Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Acid insoluble ash:

25ml of hydrochloric acid (70 g/l) was added to the crucible containing the total ash and boiled gently for 5 minutes. The insoluble matter was collected on the ash less filter paper and washed with hot water until the filtrate is neutral. The filter paper was transferred to the original crucible and ignited to a constant weight. The residue was allowed to cool in a suitable dessicator for 30 min. The ash was weighed without delay and percentage of acid-insoluble ash with reference to air dried powder was calculated.

$$\% \text{ of Acid insoluble Ash} = \frac{\text{Weight of the acid insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Water soluble ash:

25 ml of water was added to the crucible containing the total ash and boiled for 5 min. The insoluble matter was collected on the ash less filter paper and washed with hot water. The filter paper was transferred to the original crucible and ignited to a constant weight at a temperature not exceeding 450^{0}C. The residue was allowed to cool in a suitable desiccator for 30 minutes. The weight of the residue was subtracted from the weight of total ash. The ash was weighed without delay and percentage of water soluble ash with reference to air dried powder was calculated.

$$\% \text{ of Water Solube Ash Value} = \frac{\text{Weight of the Total Ash-Weight of the water insoluble ash}}{\text{Weight of the crude drug taken}} \times 100$$

Sulphated ash:

A silica crucible was heated to red for 10 minutes and was allowed to cool in a desiccator and weighed. A gram of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until white fumes are no longer evolved and ignited at 800^{0}C ± 25^{0}C until all black particles have disappeared. The ignition

was conducted in a protected place from air currents. The crucible was allowed to cool. A few drops of concentrated sulphuric acid was added and heated. Ignited as before and was allowed to cool and weighed. The operation was repeated until two successive weighing do not differ by more than 0.5 mg.

$$\% \text{ of Sulphated Ash Value} = \frac{\text{Weight of the Sulphated Ash}}{\text{Weight of the crude drug taken}} \times 100$$

Moisture content / Loss on drying:

Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter can be driven off under specified conditions [13]. 2 g of dried powder of tubers and leaves were accurately weighed and placed in a previously dried weighing bottle. The sample was heated at 100 - 105^{0}C until two consecutive weighing does not differ by more than 5 mg. The loss of weight in mg material was calculated.

$$\% \text{ of Moisture Content} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample taken}} \times 100$$

Extractive value determination:

Fifty grams of coarsely powdered air-dried material of leaves were macerated with 250 ml of each solvents, placed in a glass stoppered conical flask (Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether) shaking frequently, and then allowing it to stand for 18 hrs. Filter it rapidly through what man No.1 filter paper, taking care not to lose any solvent. Transfer 25 ml of filtrate to flat- bottom dish and evaporate the solvent on a water bath. Dry at 105^{0}C for 6 hours, cool in a desiccator for 30 minutes and weigh it immediately. Calculate the content of extractable matter in % of air-dried material [14 &15].

$$\text{Extraction yield}(\%) = \frac{W_1}{W_2} \times 100$$

Where W_1 = Net weight of the extract in gm after extraction.

W_2 = Weight of the total powder taken in gms.

Histochemistry:

Hand sections of fresh leaves were stained with a series of histochemical reagents as a) Safranine (1% safranine in 50% alcohol) for lignins b) Iodine solution for cellulose c) Ruthenium red for mucilage d) Iodine for starch e) 20% aq. NaOH for sugars f) Wagner's reagent for alkaloids g) Dilute $FeCl_3$ solution for tannins h) Conc. H_2SO_4 for saponins 20% aq. NaOH for sugars i) heating with strong KOH along with sulphuric acid for suberins [16, 17 & 18].

PHYTOCHEMICAL STUDY

Preliminary Phytochemical Screening:

To detect the different classes of secondary metabolites in the crude extracts of leaves of F. mollis a preliminary phytochemical analysis was undertaken by adopting standard qualitative methods [19, 20 & 21].

Crude drug preparation of aqueous and organic solvent extracts:

Dried leaves powder (50g in 250ml) were extracted with Aqueous, Acetone, Alcohol, Benzene, Chloroform, Ethyl acetate, Methanol and Petroleum ether. The drug was soaked for 72 hrs. and the filtered extract was dried on water bath stored at 4^{0}C in refrigerator.

Preparation of test solutions:

The preliminary tests for the detection of secondary metabolites was carried out for all the extracts (Methanol, ethanol, ethyl acetate, chloroform, benzene, acetone petroleum ether and aqueous) of root. 500 mg of each extract was dissolved in 100 ml of the respective solvent and filtered through Whatman filter paper No.1. Thus the filtrate obtained was used as test solution for the following preliminary phytochemical screening tests.

Tests for Alkaloids: The test sample (crude extract) was dissolved in chloroform and the solution was extracted with dil. H_2SO_4 or dil. HCl and acid layer was taken and tested for presence of alkaloids.

1) **Mayer's test**: To the acidic solution, Mayer's reagent (Potassium mercuric iodide solution) was added. Cream colored precipitate indicates the presence of alkaloids.
2) **Wagner's test**: To the acidic solution, Wagner's reagent (Iodine in potassium iodide) was added. The formation of reddish brown precipitate indicated the presence of alkaloids.

Tests for Flavonoids: The test solution of the extract was dissolved in one ml of alcohol and then subjected to the following tests:

1) **Ferric Chloride test**: A few drops of neutral ferric chloride solution were added to one ml each of above alcoholic solution. Formation of blackish red colour indicates the presence of flavonoids.
2) **Shinoda's test**: To one ml of alcoholic extract, a small piece of magnesium ribbon or magnesium foil was added and few drops of conc. HCl were added. Change in colour (from red to pink) shows the presence of flavonoids.
3) **Zinc-HCl reduction test**: A pinch of zinc dust and a few drops of conc. HCl were added to alcoholic extract. Magenta colour indicates the presence of flavonoids.
4) **Lead acetate test:** To one ml of alcoholic extract, a few drops of aqueous basic lead acetate solution were added solution was added. Reddish brown bulky precipitate indicates the presence of flavonoids.

Test for Phenols:

1) **Phenol test**: A positive reaction is the development of intense colour by the addition of ferric chloride solution to the test solution.
2) **Ellagic acid test:** Test solution of the crude extract was treated with a few drops of 5% acetic acid and few drops of 5% sodium nitrate solution. Formation of muddy or niger brown precipitate indicates the presence of phenols.

Test for Glycosides:

1) **KellarKilani test**: The test solution of the extract was dissolved in glacial acetic acid and after cooling, 2 drops of ferric chloride solution is added to it. These contents are transferred to a test tube containing 2 ml of concentrated sulphuric acid. A reddish brown colour ring was observed at the junction of two layers.

Test for Tannins: The test solution of the extract was dissolved in minimum amount of water, filtered and the filtrates were thus subjected to the following test:

1) **Ferric chloride test:** To the filtrate, a few drops of ferric chloride solution were added. A blackish precipitate indicates the presence of tannins.
2) **Gelatin test:** To the filtrate, gelatin (Gelatin dissolves in warm water immediately) solution was added. Formation of white precipitate indicates the presence of tannins.
3) **Lead acetate test:** To the filtrate, a few drops of aqueous basic lead acetate solution were added Reddish brown bulky precipitate indicates the presence of tannins.

Test for steroids: The test solution of the extract was dissolved in 5ml of chloroform separately and was subjected to the following tests:

1) **Salkowski test**: One ml of conc. sulphuric acid was added to the above solution and allowed to stand for 5 minutes after shaking. Lower layer turning into golden yellow colour indicates the presence of steroids.
2) **Liebermann Burchard test:** To one ml of the extract treated with chloroform, a few drops of acetic anhydride, one ml of conc. H_2SO_4 were added from the sides of the test tube and allowed to stand for 5 minutes. Formation of brown ring at the junction of the two layers and the upper layer turning green indicates the presence of steroids.

Test for Quinones: The test solution of the extract was treated separately with alcoholic potassium hydroxide solution quinones give coloration from red to blue.

Test for Lignins:

1) **Labat test:** The test solution is mixed with gallic acid, it develops olive green colour indicating the positive reaction for lignins.

2) **Lignin test:** Formation of red colour, when 2% (W/V)furfuraldehyde is added to the test solution indicates the presence of lignin.

Test for Saponins: The test solution was separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Foam formation indicates the presence of saponins.

Test for Fixed oils:

1) The test solution of the extract was pressed separately between two filter papers. Formation of transparent spot indicates the presence of fixed oils.
2) A few drops of 0.5N alcoholic potassium hydroxide was added to the solution of the extract with a few drops of phenolphthalein as indicator and mixture were heated for 1 - 2 hrs soap formation shows the presence of fixed oils.

ANTIMICROBIAL ACTIVITY

Test organisms:

Pure bacterial cultures of Bacillus subtilis (MTCC-441) and Pseudomonas aeruginosa (MTCC-741) and fungal cultures of Candida albicans (ATCC-10231) and Aspergillus niger (ATCC-16404) were procured from department of microbiology, S.V. University and Sri Venkateswara institute of medical sciences, Tirupati. These were further maintained on nutrient agar slants at 4^0C until further use.

Preparation of the bacterial medium:

To prepare 1 lit of nutrient agar medium 5 gm of beef extract, 3 gm of Sodium chloride, 3g of peptone, 15 gm of agar were accurately weighed using digital electronic balance and dissolved in 1 liter of distilled water before the addition of agar, the P^H of the medium was adjusted to 7.2 by adding few drops of 0.1N NaOH/HCl using digital P^H meter. Later this medium was transferred to conical flasks and plugged with non absorbent cotton. These were then sterilized by autoclaving at 15 lbs for 20 minutes, cooled to 40^0C and used for the study.

Preparation of the fungal medium:

200 g of potato slices were boiled with distilled water. The potato infusion was used as water source of media preparation. 20 g of dextrose was mixed with potato infusion. 20 grams of agar was added as a solidifying agent. These constituents were mixed and autoclaved at 15 lbs for 20 minutes cooled to 40^0C and used for further study.

Agar well diffusion method:

The antibacterial activity of plant extracts was determined by using agar well diffusion method [22]. 18 hrs old bacterial and 48h old fungal broth cultures were used as inoculums after adjusting their population to 10^6 CFU/ml (colony forming units) using 0.9% (w/v) sterile saline as described by standard method [23]. 0.5 ml of standard inoculums were pipetted into a sterile petriplate, 20 ml of melted agar medium is then added in each plate and mixed well by gently swirling on the table top. The seeded plates are allowed to solidify. Four agar wells (9 mm, diameter) were made in each plate equidistantly by cutting out the media using sterile broad end (8.5 mm) of micropipette tip, in order to load test solutions and are filled with 10 mg/well of the extracts in quadruplicates. The standard antibiotic (Ampicillin -1mg/welfor bacteria and Nystatin for fungil) was used as positive control and kept for 24 hours for bacteria and 48h for fungi incubation at 37^0C to note down the zone of inhibition. The inhibition was compared with control Ampicillin /Nystatin at 10 mg/well.

Statistical analysis:

The results were analyzed for statistical significance using **One way ANOVA** followed by Dunnet's test. The $p < 0.01$ and $p < 0.05$ was considered significant.

Evaluation of minimum inhibitory concentration (MIC):

Minimum Inhibitory Concentration was determined by broth dilution method [24 & 25]. Extracts to be tested were taken ranging from 10 mg/ml. It involves a series of nine tubes for each test extract against each strain. To the first assay tube 4 ml of broth agar for bacteria and potato dextrose for fungi was transferred and then 4 ml of

test extracts of 10 mg/4 ml was added and mixed thoroughly. To the remaining nine assay tubes, from the first tube 4 ml of the content was pipette out into second test tube and this was mixed thoroughly. This twofold serial dilution was repeated up to ninth tube. 0.2 ml of the inoculums was added to all test tubes and also to the control tubes were taken aseptically and incubated for 24 hrs for bacteria and 48 hrs for fungi. The absorbance was measured by calorimeter at 600 nm after 24h for bacteria and fur fungal at 530 nm after 48h with a calorimeter. Absorbance of the tubes is compared with the control Ampicillin (10 mg/ml) for bacteria and fungi the control Nystatin (10 mg/ml) and minimum inhibitory concentration mg/ml was determined.

RESULT:

ABOUT THE SELECTED MEDICINAL PLANT

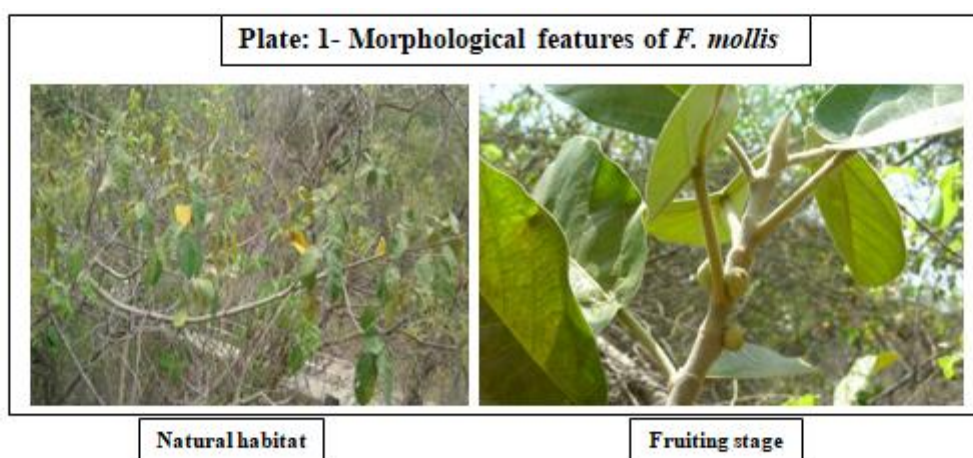
Common name: Soft fig

Vernacular names: Kalijuvvi, kallatthi, kondakalajuvvi, kondamarri and pittamarri

Taxonomic classification:

Kingdom: Plantae
Division: Magnoliophyta
Class: Dicotyledons
Sub class: Polypetalae
Series: Calasiflorae
Order: Rosales
Family: Moraceae
Genus: Ficus
Species: mollis vahl.

Plate: 1- Morphological features of *F. mollis*



Natural habitat

Fruiting stage

Distribution:

This species has a restricted global distribution occurring only in India and Sri Lanka. Within India, it has been recorded in Karnataka and Tamil Nadu.

Description: (Plate: 1)

Deciduous trees, epiphytic or independent, to 15 m high; aerial roots few or more; bark 5-6 mm thick, surface greyish-white, smooth. Leaves simple, alternate spiral; stipule lateral, ovate-lanceolate, densely appressed, brown-strigose, cauducous, leaving an annular scar; petiole 10-50 mm long, stout, tomentose, often with a subglandular patch on the back at the11 junction with the midrib below; lamina 6-15 x 3-9 cm, elliptic, oblong, obovate, elliptic-ovate, elliptic-obovate, ovate-oblong, base round or cordate, apex obtuse, subacute or obtusely tomentose on both sides when young, becoming glabrescent above and tomentose beneath when mature, coriaceous; 3-7-ribbed from base; lateral nerves 5-6 pairs, pinnate, prominent beneath, intercostae reticulate, faint. Flowers unisexual; inflorescence a syconia, sessile, in axillary pairs, subglobose, tomentose, orifice

cicular, slightly raised, closed by 3 glabrous apical bracts in a disc; internal bristles sparse to abundant; basal bracts 3, 4-5 mm long, half covering the body of syconia, ovate, acute, often split, velvety tomentose without; flowers of 4 kinds; male flowers disperse; pedicel 0.7 mm long, tepals 4, reddish, free, lanceolate, stamen 1, filament 0.3 mm, anther oblong; female flowers sessile; tepals 4, shorter than ovary, ovary superior, ellipsoid-globose, 1 mm, brown; style 1.5 mm, tapering; gall flowers sessile or pedicellate; tepals 4, shorter than the smooth ovary; style short. Fruit a syconium 5-8 mm across, fleshy, grey, tomentose; achenes smooth.

PHARMACOGNOSTICAL STUDIES

Organoleptic Studies:

Table-1: Macroscopic / Organoleptic Studies:

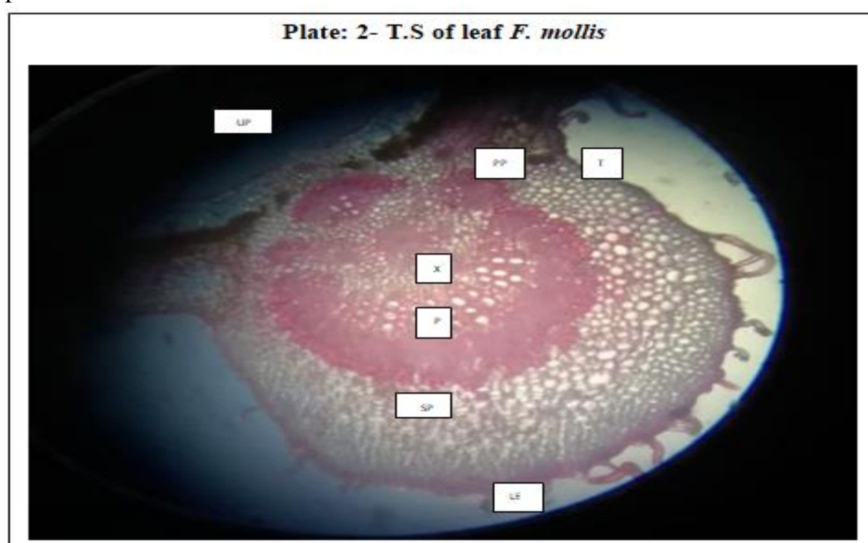
Characters	Leaf
Colour	Green
Odour	Characteristic
Taste	Bitter
Texture	Fine
Fracture	Smooth

Color: Leaves green; **Odour**: Characteristic; **Taste**: Bitter; **Texture:** Fine; **Fracture:** Smooth. Morphological studies and physiochemical constants help in the standardization of the crude drugs. Study of organoleptic characteristics provides firsthand information about the quality of raw material used for the study.

Microscopic evaluation of Transverse section of leaf: (Plate: 2)

Transverse section of leaves showed is bifacial with distinct adaxial and abaxial faces. In transverse section, the lamina shows an adaxial/upper epidermis consists of a single layer of cells found on the upper surface of leaf. It is covered by a thick cuticle. The abaxial/ lower epidermis made up of rectangular / barrel shaped cells with thick cuticle which distinctly forms on outer and inner ledges on the guard cells and the subsidiary cells of the stomata. The type of the stomata is anomocytic. In these stomata, accessory cells are absent. The guard cells are surrounded by ordinary epidermal cells, numerous unicellular, uniserrate and grandular covering trichomes. mesophyll region is distinctly differentiated into palisade and spongy tissues. Cells of palisade parenchyma are very compactly arranged in 5-6 layers. The vascular tissue system is composed of vascular bundles which are usually collateral and closed. But the bundles entering the leaf occupy such a position that xylem occurs on the upper side and phloem on the lower.

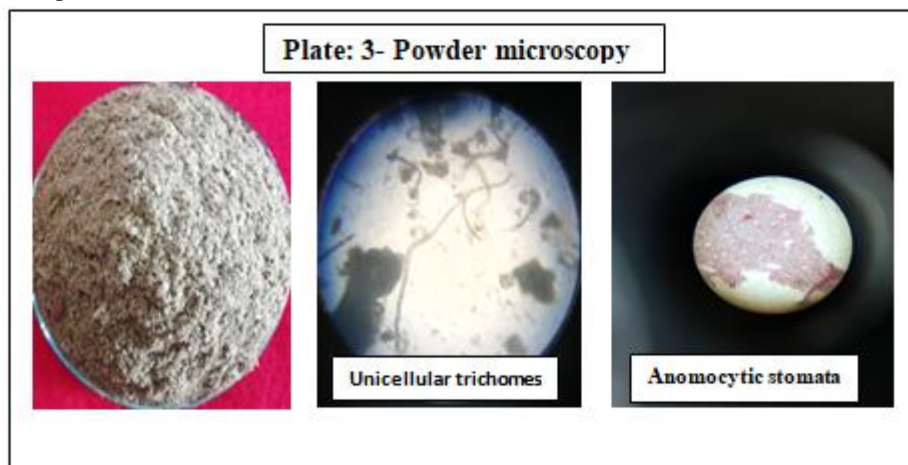
Plate: 2- T.S of leaf *F. mollis*



Note: UE-Upper Epidermis, PP-Palisade Parenchyma, SP- Spongy Parenchyma, X-Xylem, P-Phloem, LP-Lower Epidermis, T- Trichomes

Poweder microscopy of leaves: (Plate: 3)

The leaf powder is green in colour with an aromatic odour on microscopial examination, powder of leaves showed the presence of unicellular and glandular trichomes. Both upper and lower epidermis compries of polygonal cells. Each stomata is surrounded by normal epidermal cells .Stomatal index of upper epidermis is more than lower epidemis.



Stomatal study: (Plate- 3)

Table 2: Stomata number and index:

S.No	Leaf constant	Values
1	**Stomatal number**	
	Upper epidermis	10
	Lower epidermis	20
2	**Stomatal index**	
	Upper epidermis	33.0
	Lower epdermis	25.0

Histochemical study:

Table 3: Histochemical studies of leaves:

Test	Reagent used	Nature/% of change		Histological zone
Lignins	Safranine (1%)	Red	++	Endodermis.
Cellulose	Iodine solution	Pale yellow	+	Epidermis, cortex and protoxylem.
Mucilage	Ruthenium red	Pink	++	Trichomes, cuticle and epidermis.
Starch	Iodine	Blue	+	Xylem.
Sugars	20% Aq. NaOH	Yellow	++	Xylem and phloem
Alkaloids	Wagner's reagent	Brown	++	Cortex and phloem.
Tannins	Dil. $FeCl_3$ solution	Blackish blue	++	Epidermis and phloem,
Saponins	Conc. H_2So_4	Light yellow	+	Epidermis, stele and cortex.
Suberins	KOH+ H_2So_4	Light brown	+	Endodermis and stele.

Lignins are localized in endodermis with red; **Cellulose** in epidermis, cortex, protoxylem with pale yellow; **Mucilage** in trichomes cuticle and epidermis with pink; **Starch** in xylem with pink; **Sugars** in xylem and phloem yellow in colour; **Alkaloids** in cortex and phloem with brown; **Tannins** in epidermis and phloem with

blackish blue. **Saponins** in epidermis, cortex and stele with light yellow; **Suberins** in endodermis and stele with light brown colour.

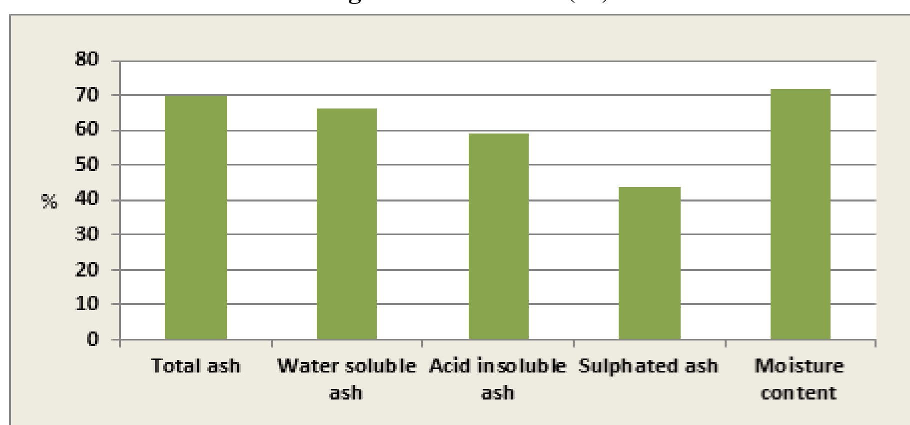
PHYSICO CHEMICAL STUDIES

Powdered drug Ash values (%):

Table- 4: Ash values: Powered Drug: (%)

S.No.	Parameters	Yield
1	Total ash	70.0
2	Water soluble ash	66.40
3	Acid insoluble ash	59.23
4	Sulphated ash	44.20
5	Moisture content	72.00

Figure-1: Ash values (%)



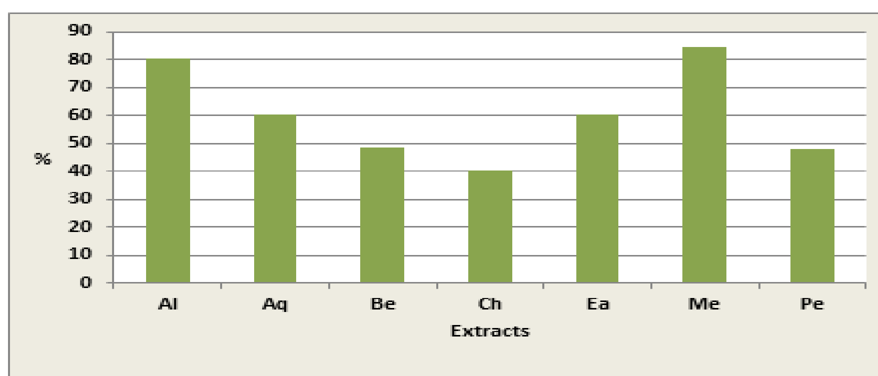
Ash values of any crude drug gives an idea about the presence of earthy matter and /or inorganic composition and /or other impurities present along with the crude drug. In the present study **Total ash** 70**%, water soluble ash** 66.40%, **Acid insoluble ash** 59.23% **Sulphated ash** 44.20% and **Moisture content** 72% were reported.

Extractive Values:

Table-5: Extractive Values (%w/w)

Extracts	Extraction	Filtration colour	Extract nature and colour
Al	80.20	Dark green	Smooth & Green
Aq	60.60	Golden yellow	Solid & Orange
Be	48.50	Green	Sticky & Dark green
Ch	40.50	Dark green	Sticky & Dark green
Ea	60.35	Black green	Smooth & Blackish green
Me	84.25	Dark green	Smooth & Green
Pe	48.20	Light green	Smooth & Yellowish green

Figure-2: Extractive Values (%)



Extractive values of leaves yielded highest amount in methanol **84.25w/w,** Filtrate color of leaves powder exhibit dark green residue color and nature is smooth green. Lowest in chloroform **48.20w/w**, light green residue color and nature is smooth yellowish green extract. Extractive values represented the presence of compounds in polar and non-polar solvents. It is useful for the diversity of chemical nature and property of drug contents.

PHYTOCHEMICAL STUDIES

Table-06: Preliminary Phytochemical screening

Test	**Ac**	**Aq**	**Al**	**Be**	**Ch**	**Ea**	**Me**	**Pe**
Alkaloids								
Mayers	-	++	++	+	++	++	-	-
Wagner's	-	++	++	++	++	++	++	++
Flavonoids								
Shinodons	+	++	+	-	-	-	++	-
$FeCl_3$	-	++	-	-	+	-	++	-
Phenols								
$FeCl_3$	+	++	+	+	+	++	++	++
Ellagic acid	-	++	-	-	++	-	++	-
Glycosides								
Keller –Kilani	+	+	-	++	+	+	_	-
Tannins								
$FeCl_3$	-	++	-	-	++	-	+	-
Steroids								
Salkowski	-	++	-	-	+	-	+	+
Quinones	-	-	-	-	-	-	-	-
Lignins								
Labat test	-	-	-	-	++	++	-	-
Saponins	-	++	-	++	++	-	+	++

"++" indicates -Abundant presence; **"+" indicates -** (Slightly presence); "**– "indicates** – Absent

Ac: Acetone, **Al:** Alcohol, **Aq:** Aqueous, **Be:** Benzene, **Ch:** Chloroform, **Ea:** Ethyl acetate, **Me:** Methanol, **Pe:** Petroleum ether

Preliminary Phytochemical screening:

Wide variety of natural compounds like alkaloids, glycosides, saponins, phytosterols, phenolics, terpenoids, flavonoids, coumarins,quinines, saponins and tannins which exert physiological activity as synthesized in plants. Leaves yielded highest amounts and more number of secondary metabolites as alkaloids, flavonoides, phenols, glycosides, tannins, lignins saponins and steroids in most of the extracts. Alkaloids and phenols are abundant in most extracts. Quinones are totally absent in all extracts.

Antibacterial Activity (Zone of inhibition in mm) (Plate-04)

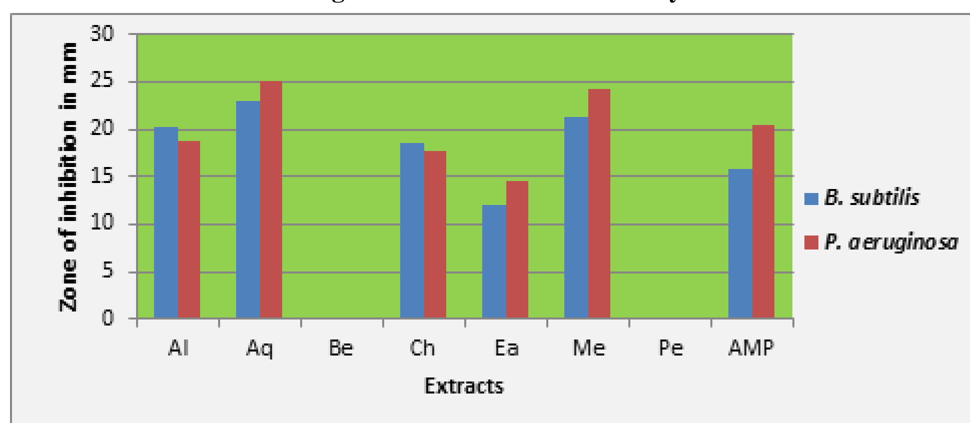
Table-07: Antibacterial Activity (Zone of Inhibition in mm)

Extracts	B. subtilis	P. aeruginosa
Al	20.18±0.43**	18.75±0.50**
Aq	23.00±0.00	25.00±0.50**
Be	0.00±0.00	0.00±0.00
Ch	18.50±0.50**	17.75±0.16*
Ea	12.00±0.50*	14.50±0.00
Me	21.20±0.20*	24.25±0.43**
Pe	0.00±0.00	0.00±0.00
AMP (Control)	15.83±0.14**	20.33±0.28**

Ac: Acetone, **Al:** Alcohol, **Aq:** Aqueous, **Be:** Benzene, **Ch:** Chloroform, **Ea:** Ethyl acetate, **Me:** Methanol, **Pe:** Petroleum ether

All the data are expressed as mean ±SEM: **p<0.01,* p<0.05 as compared to control group, n=4: (One –way ANOVA followed by Dunnett's test).

Figure-03: Antibacterial Activity



Antibacterial activity of leaves aqueous and methanol extracts showing more effective activity on P. aeruginosa with 25.00; 24.25 mm zone of Inhibition than other extracts. Ethyl acetate extract shows lowest zone of inhibition on B. subtilis 12 mm. It is also observed that there is no activity in petroleum ether extracts on all organisms. It is also observed that P. aeruginosa is more resistance and **B. subtilis** is least susceptible.

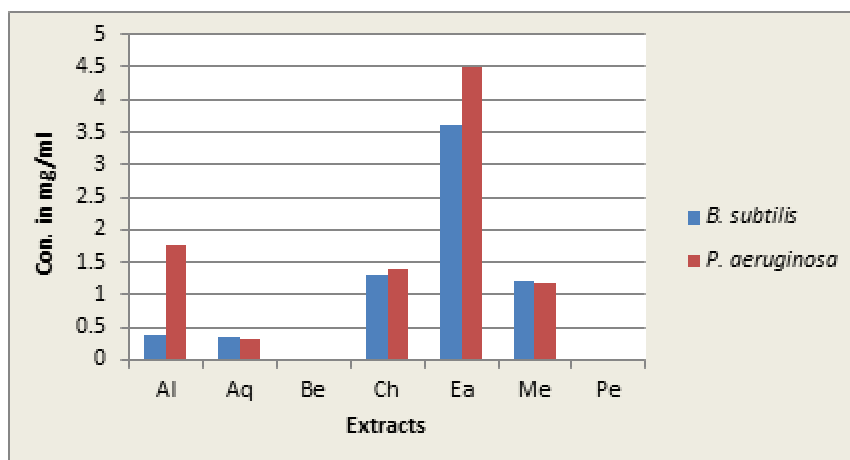
MIC for Antibacterial Activity:

Table-08: MIC for Antibacterial Activity (mg/ml)

Extracts	B. subtilis	P. aeruginosa
Al	0.39	1.75
Aq	0.36	0.32
Be	0.00	0.00
Ch	1.30	1.40
Ea	3.62	4.50
Me	1.20	1.18
Pe	0.00	0.00

Al: Alcohol, **Aq:** Aqueous, **Be:** Benzene, **Ch:** Chloroform, **Ea:** Ethyl acetate, **Me:** Methanol, **Pe:** Petroleum ether.

Figure-04 MIC for Antibacterial Activity



Minimum Inhibitory Concentrations with leaves extracts at 0.32 to 3.62 mg/ml compared to that of the 10 mg of Ampicillin.

Antifungal Activity: (Plate- 5)

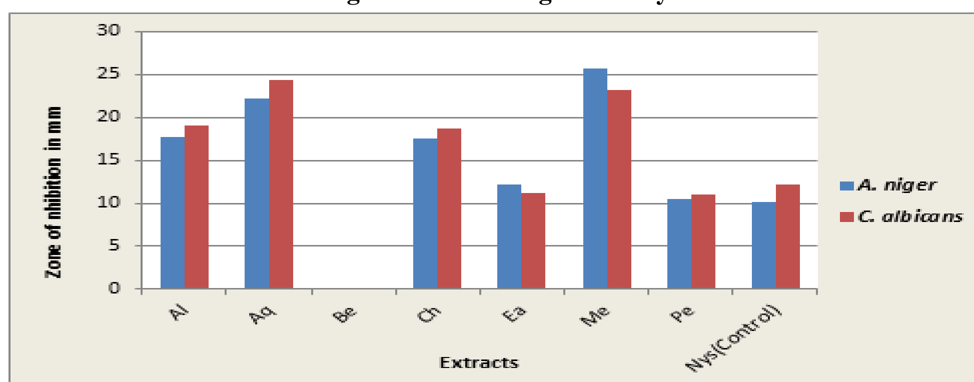
Table-09: Antifungal Activity (Zone of Inhibition in mm)

Extracts	A. niger	C. albicans
Al	17.75±0.00	19.00±0.00
Aq	22.25±0.00	24.30±0.00
Be	0.00±0.00	0.00±0.00
Ch	17.5±0.50**	18.75±0.43*
Ea	12.25±0.43**	11.25±0.50*
Me	25.75±0.00	23.25±0.00
Pe	10.5±0.50*	11.00±0.00
Nys (Control)	10.20±0.20**	12.10±0.16**

Ac: Acetone, **Al:** Alcohol, **Aq:** Aqueous, **Be:** Benzene, **Ch:** Chloroform, **Ea:** Ethyl acetate, **Me:** Methanol, **Pe:** Petroleum ether

All the data are expressed as mean ±S EM: **p<0.01,* p<0.05 as compared to control group, n=4: (One – way ANOVA followed by Dunnett's test).

Figure-05: Antifungal Activity

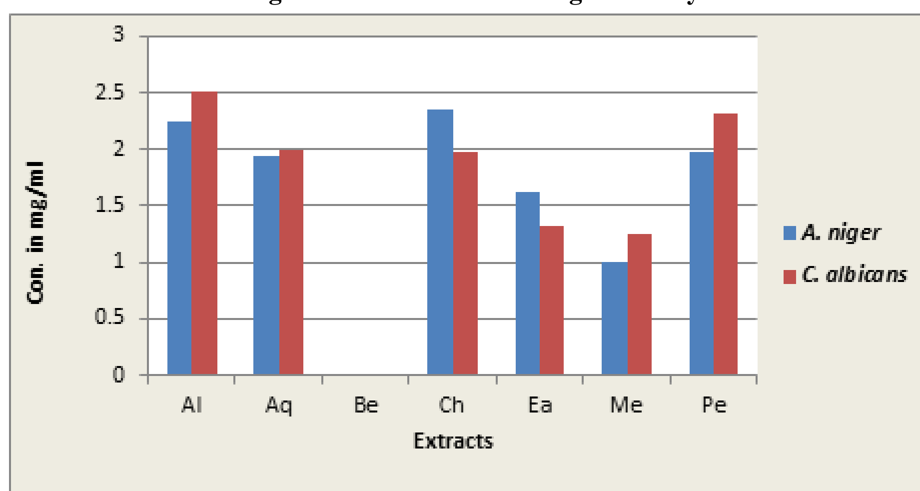


Antifungal activity of leaves methanol on extracts was more effective on A. niger 25.75 mm zone of inhibition than C. albicans when compared to Nystatin the control drug at 10 mg/well with 10.20 to 12.10 mm of zone of inhibition. Petroleum ether extracts shows lowest zone of inhibition. Benzene extracts has not shown any antifungal activity on both organisms.

Table-10: MIC for Antifungal Activity (mg/ml)

Extracts	A. niger	C. albicans
Al	2.25	2.50
Aq	1.95	2.00
Be	0.00	0.00
Ch	2.35	1.98
Ea	1.62	1.32
Me	1	1.25
Pe	1.98	2.32

Figure-06: MIC for Antifungal Activity



MIC for Antifungal Activity:

Fungal Minimum Inhibitory Concentrations on both organisms with different leaves extracts ranges from 1.00 mg to 2.32 mg/ml compared to 10 mg of Nystatin.

DISCUSSION:

Transverse section and powder microscopic of F. mollis leaves reveals the presence of upper and lower epidermal layers, anomoytic type of stomata, unicellular and glandular trichomes. Mesophyll is differenciated in to palisade and spongy parenchyma, xylem vessels, phloem surrounded by bundle sheath cells and collechyma. The behavior of the leaf powder upon treatment with different chemical reagents was also observed and reported . Physicochemical standards discussed here, can be considered as identifying parameters to substantiate and authenticate the drug. The preliminary phytochemical investigation for the leaves extracts proved the presence of pharmacologically active compounds such as alkaloids, flavonoides, phenols, glycosides, tannins, lignins, saponins and steroids.

Antimicrobial activity of leaves extracts proved the plant contains antimicrobial compound which can be further developed as phytomedicine for the therapy of infection. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule at the onset of drug discovery will pay off later in drug development. Lastly, to conclude the extracts were found to inhibit the growth of Gram-positive bacteria as well as the Gram negative bacteria and the fungal species

CONCLUSION:

The results of the present research work explore the presence of medicinally active compounds in the F. mollis leaves and their mechanisms in controlling pathogenic bacteria and fungal sp. This study provides referential information for identification and characterization of F. mollis leaf and its extracts.

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CONFLICT OF INTEREST: I declare that, I have no conflict of interest.

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