

Determination of Flavanoidal Content by *Ficus religiosa* Linn Leaf Extract by TLC and HPTLC

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ABSTRACT

Herbs have always been the principal form of medicine in India. *Ficus religiosa* (L.), commonly known as peepal belonging to the family Moraceae, used to cure some of the diseases like Asthma, Diabetes, Epilepsy, Gastric problems, etc., Plant parts used are leaves, bark, bud and fruit. Leaves mainly used for skin diseases. Chemical constituents present in *Ficus religiosa* are Beta-sitosterol, n-octacosanol, methyl oleanolate, lanosterol, stigmasterol, lupeol. The extraction done by cold maceration process by using methanol as solvent. Thin layer chromatography is done by using silica gel as adsorbant and benzene:chloroform (7:3) as mobile phase. Spotting is done by Ethyl acetate, diethyl ether, n-butanol, chloroform, pet-ether, benzene extracts, and water. Spots are detected under long UV at 365nm in UV chamber, then R_F values are calculated. HPTLC method is done to know percentage content present and it is most reliable method. It has no toxic effect on human consumption when taken in prescribed dosage.

Keywords: *Ficus religiosa*, Flavanoidal content, TLC, HPTLC.

INTRODUCTION

Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are found as secondary plant metabolites in one or more parts of these plants. *Ficus religiosa* (L.) is a large perennial tree, glabrous when young, found throughout the plains of India upto 170m altitude in the Himalayas. The stem bark and leaves of *F. religiosa* are reported phytoconstituents of phenols, tannins, steroids, lanosterol, stigmasterol, lupen-3-one. The active constituent from the root bark *F. religiosa* was found to be -sitosterol-D-glucoside. The seeds contain phytosterol, -sitosterol, and its glycoside, albuminoids. The fruit of *F. religiosa* contained appreciable amounts of total phenolic contents, total flavonoid¹. The leaves can be used to alleviate fevers, bleeding wounds, constipation, dysentery, bruises, boils and mumps.

MATERIALS AND METHODS

Plant material: The plant of *Ficus religiosa* (Linn) leaves were collected in the month of Jan 2009 in and around of Rajampet. These were authenticated by Dr.K.Madhava chetty, prof, dept.of botony,S.V.University, Thirupathi.

Chemicals: Ethyl acetate, Diethyl ether, n-butanol, Chloroform, Pet ether, Benzene

Instruments: Weighing balance, Hot air oven, Heating mantle, HPTLC

EXPERIMENTAL INVESTIGATION

Macroscopical and physiochemical evaluation
Macroscopical parameters: The Macroscopic evaluation was carried out to know the shape, size, colour, odour, taste

and fracture of the drug. Different physiochemical values such as Ash values, Loss on drying, Foreign organic matter were determined.

Physiochemical parameters: Foreign organic matter (FOM): Weigh accurately 50gm of the original sample and spread it out in a thin layer. Inspect the sample with unaided eye or with the use of a 6 X lens and separate the foreign organic matter manually as completely as possible. **Determination of Moisture (Loss on drying [LOD]):** Weigh about 1gm of the powdered crude drug in to a weighed flat and thin porcelain dish. Dry in the oven at 100°C – 105°C for half an hour. Cool and weigh the contents. Keep the contents in a hot air oven and repeat the drying at 100°C – 105°C for half an hour (care should be taken that the contents should not get charred off). Cool the contents and its weight. Repeat the drying and weighing procedure until two concordant weights are noted².

Determination Total Ash: Heat a silica or platinum crucible to red heat for 30min, Allow to cool in a Dessicator and weigh. Weigh accurately about 2gm of the substance being examined and evenly distribute in the crucible. Dry at 100°C to 105°C for one hour and ignite to constant weight in a muffle furnace at 600±25°C. Allow the crucible to cool in a desiccator after each ignition. The material should not catch fire at any time during the procedure. If after prolonged ignition a carbon free ash cannot be obtained, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, Incinerate the residue and filter paper until the ash becomes white or nearly so. Calculate the percentage of ash with reference to air dried drug. The result were shown in the tables.

Macroscopic characters of *Ficus religiosa*.Linn leafPhysiochemical Parameters of *Ficus religiosa* Linn leaf powder

S.No	Parameters	Percentage Yield % w/w
1	Foreign organic matter	2.3
2	Loss on drying	0.1
3	Total ash	6.8
4	Acid insoluble ash	3.9
5	Sulphated ash	0.25
6	Water soluble ash	3.0

Fluorescence analysis study of *Ficus religiosa* Linn leaf powder

S.No	Sample	Colour in Day light	Colour in UV
1	Powder	Pale green	Green
2	Powder+ 0.1N sodium Hydroxide	Green	Dark green
3	Powder+ Acetic anhydride	Pale green	Dark green
4	Powder+ Acetic acid	Green	Brownish green
5	Powder+0.1N Hydrochloric acid	Pale grey	Dark green
6	Powder+Water	Slight Yellowish green	Green

Determination of Acid-insoluble Ash: The total ash obtained is boiled for five minutes with 25ml of 2M hydrochloric acid and filtered through an ashless filter paper. The filter paper is ignited in the silica crucible, Cooled and then acid insoluble ash is weighed.

Determination of Water-soluble Ash: Water soluble ash is the difference in weight between the total ash and the residue obtained after boiling the total ash in water. The total ash obtained, should be boiled for five minutes with 25 ml of water. The insoluble matter may be collected on a Gooch crucible or on ash-less filter paper. It should be washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The total weight of insoluble matter should be subtracted from the weight of the ash. This difference in weights represents the water soluble ash. The percentage of water soluble ash should be calculated with reference to the air dried drug.

Determination of Sulphated Ash: The total ash obtained is boiled for five minutes with 1ml of sulphuric acid, until the fumes are no longer evolved and ignite at $600^{\circ} \pm 25^{\circ} \text{C}$ until black particles have disappeared. Allow the crucible to cool, Add a few drops of sulphuric acid and heat. Ignite and Allow to cool and weigh^{3,4}. Repeat the procedure until two successive weighing don't differ by more than

0.05mg. Calculate the percentage difference between total ash and Sulphated ash.

Fluorescence analysis study of *Ficus religiosa*(Linn) leaves powder: Fluorescence analysis study of powdered drug material with different reagents was carried out to observe the color reactions..

Preparation of Extracts from *Ficus religiosa*(Linn) leaf: The leaves were dried by shade dry method. Powdered the crude drug and passed through 40meshes size and stored in closed vessel for further use. Weighed accurately 50gms of crude drug packed in a thimble of soxhlet apparatus and successively extracted by 250ml of solvents in increasing polarity order for 72 hrs by Hexane, Pet ether, Chloroform, Ethyl acetate, Methanol and Water. Each extract was concentrated by distilling off the solvent and then evaporating the solvent to dryness to become residue. Then calculate the percentage yield of the extract.

Phytochemical Evaluation: Test for Alkaloids: The extract was dissolved with methanol, add 1% HCL, shake well and filtered. The filtrate was taken for the following tests: Small quantity of filtrate with 6 drops of Mayer's reagents was added. Same procedure was followed for Wagner's reagent, Dragendroff's reagents, Hagers reagent, observe the colour change of the precipitate.

Percentage yield calculation for Extracts

S.no	Extract	Percentage yield
1	Hexane	4.0
2	Pet.ether	3.8
3	Chloroform	5.2
4	Ethyl acetate	4.4
5	Methanol	5.0
6	Water	5.2

General chemical tests for Leaf powder of *Ficus religiosa* Linn

S.No	Name of chemical Test	Observation
1	Alkaloids	+
2	Flavanoids	-
3	Glycosides	+
4	Steroids	+
5	Triterpenoids	+
6	Tannins	+

+Present -Absent

Preliminary phytochemical Analysis of Extracts of *Ficus religiosa* Linn leaf

S.No	Phytoconstituents	Results of Extracts					
		H.E	P.E	C.E	E.E	M.E	A.E
1	Alkaloids	+	+	+	+	+	+
2	Flavanoids	-	+	+	-	-	-
3	Glycosides	+	-	+	+	+	+
4	Steroids	+	-	+	-	-	-
5	Triterpenoids	+	+	-	+	+	+
6	Tannins	+	+	+	-	+	+

+ = Present, - = Absent

H.E – Hexane extract

P.E—Pet ether extract

C.E—Chloroform extract

E.E – Ethyl acetate extract

M.E—Methanol extract

A.E—Aqueous extract

Test for Glycosides: Bortrager's test: To 2ml of extract, add dilute sulphuric acid boil and filter. To cold filtrate, add equal volume benzene or chloroform. Shake well and separate the organic solvent, then add ammonia. Ammonical layer turns pink or red.

Modified Bortrager's test: To 5ml extract, add 5ml 5% Ferric chloride and 5ml dilute hydrochloric acid. Heat for 5 mins in boiling water bath. Cool and add benzene or any organic solvent. Shake well, separate organic layer and add equal volume of dilute ammonia. Ammonical layer shows pinkish red colour⁵.

Test for Flavonoids: A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3min. The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow coloration was observed indicating a positive test for flavonoids.

To the test solution in 10ml ethanol add conc. HCl and magnesium ribbon and it develops a pink-tomato red colour indicates the presence of flavonoids.

Shinada's Test: To the extract add 5-10 drops of dilute HCl was added followed by a small amount of Mg and the solution was boiled in a water bath for a few minutes. Appearance of Meijanta colour shows the presence of flavanoids.

Test for Triterpenoids: Salkowski test: Five ml of each extract was mixed in 2ml of chloroform, and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Noller's Test: One ml of extract with tin and thionyl chloride [1ml] were added. Heat in a water bath. Purple colour shows the presence of triterpenoids⁶.

Test for Steroids: Liebermann – Bur chard's Test: One ml of extract 0.5ml of Chloroform, 5ml of acetic acid, heat, add 5ml of acetic anhydride and add conc. Sulphuric acid. Green Colour shows the presence of Steroids.

Test for Tannin: One ml of extract was diluted to 5ml with distilled water in a tube and to this a few drops of lead acetate solution (1%) was added. A white precipitate indicates the presence of tannin.

TLC Procedure : Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance⁷. Silicagel G used as adsorbent and benzene:chloroform in the ratio of 7:3 used as a mobile phase. Plate was prepared by pouring silica gel on glass plate and activated by heating at 110°C for 30 min. Then the sample was spotted on the plate and the plate was kept

TLC PLATES



HEXANE



PET ETHER



CHLOROFORM



ETHYL ACETATE



DIETHYL ETHER



WATER

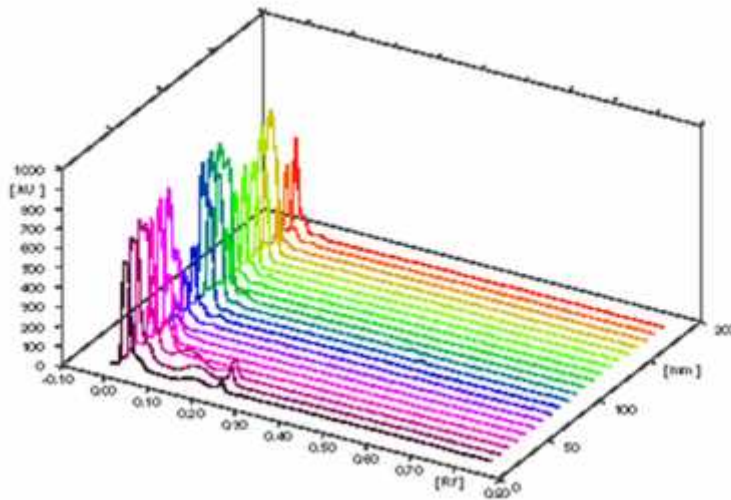
R_F Values for TLC Plates

Name of the extract	R _F value	No of spots	Observation
Hexane	0.163	2	Blue
Pet ether	0.33	1	Blue
Chloroform	0.274	1	Blue
Etyl acetate	0.375	1	Blue
Diethyl ether	0.107	2	Blue
N-butanonol	0.625	1	Light blue
Water	-	-	-

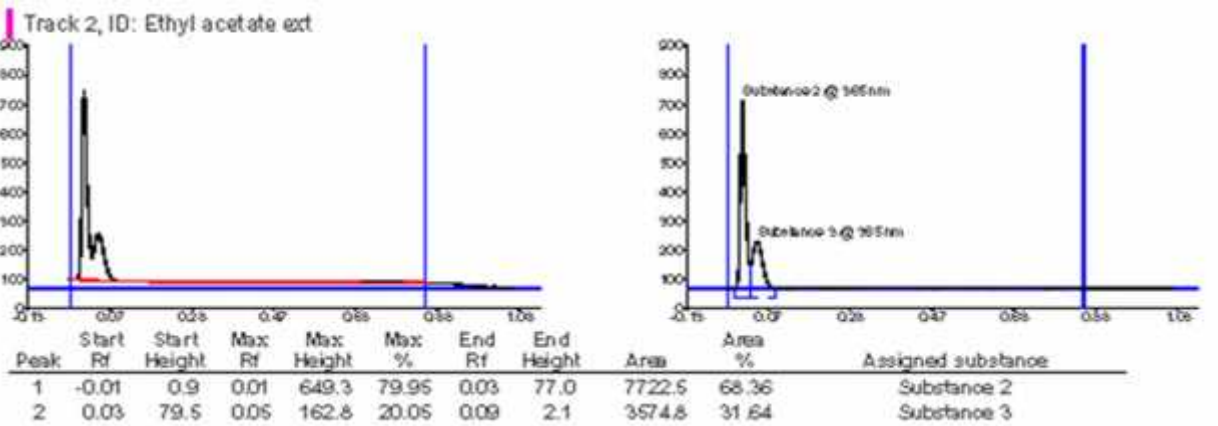
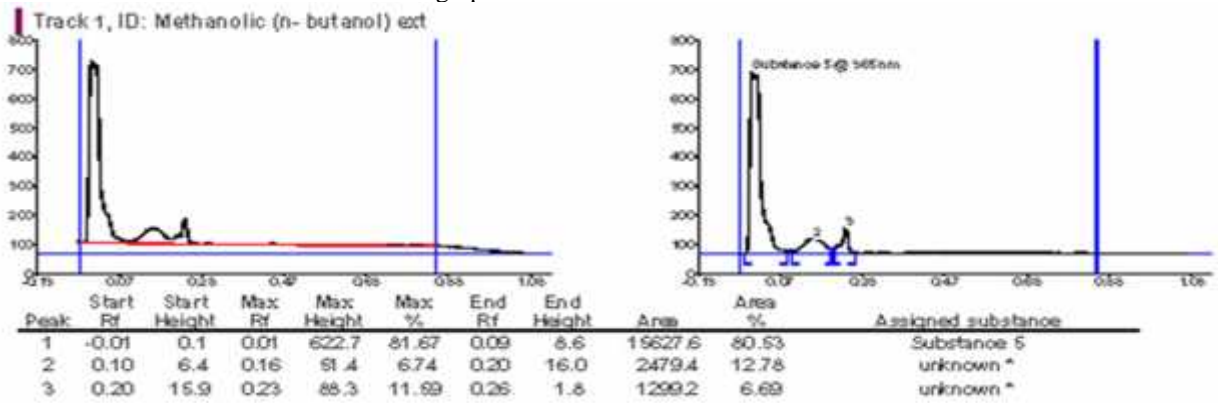
in the solvent system until it reaches to 3/4 th of the plate. The spots are detected under long uv at 365 nm and R_f values are calculated³. High Performance Thin Layer Chromatography: HPTLC is characterized by efficient

separation used either for identification or quantitation of chemical substances. Aluminium plates are normally used. Silica gel is the most widely used adsorbent. HPTLC plates are produced from 4.5 μm silica gel with an inert

HPTLC REPORT



Cumulative graphs of HPTLC



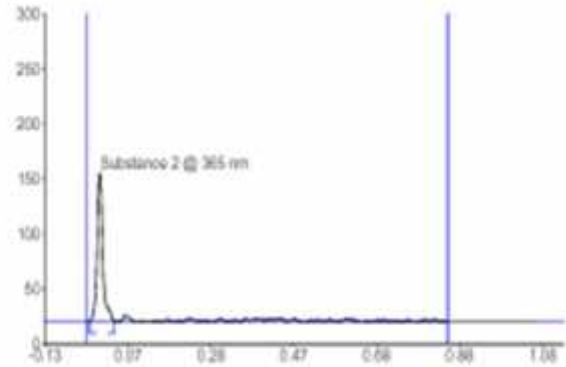
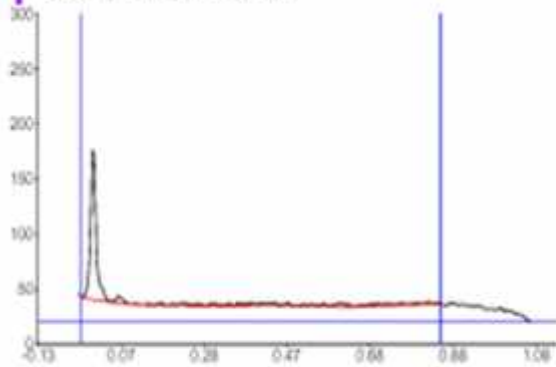
binder to form a 200µm layer. Plates exposed to high humidity are kept out to make them activated by placing in an oven at 110-120°C for 30 min prior to sample spotting⁸. Benzene:chloroform in the ratio of 7:3 .Sample are solubilized with specified solvent of the extract. The sample volume normally applied on to the plate is around 0.2 µl. Streaking the sample on the plate results in better separation than spotting. In Ascending development the optimum separation distance is 20-25 mm with separation time of about 4 min. Detection of coloured substances or colourless substances absorbing in longer wave UV region (365 nm) or substances with intrinsic fluorescence can be easily detected. Quantization was performed with

photometric measurement of absorbed light or emitted fluorescence. In absorption densitometry, the spots in HPTLC plates are scanned by a beam of monochromatic light.

RESULTS AND DISCUSSION

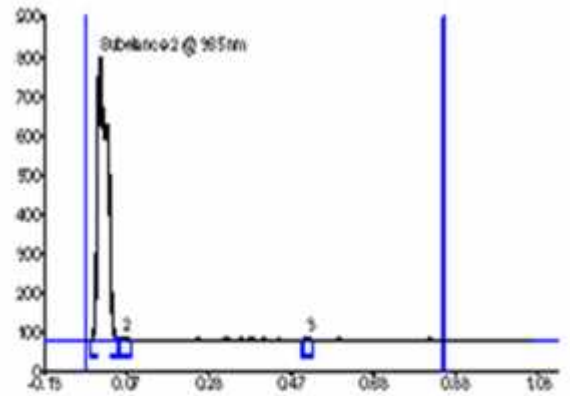
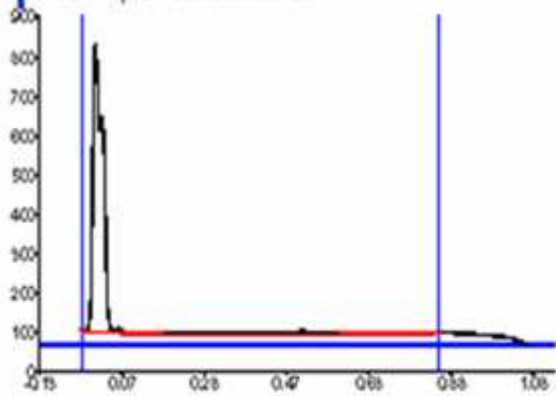
- Colour : upper-Dark green, lower-Light green
- Odour : Odourless
- Taste : Bitter
- Texture : upper-Rough, lower-Smooth
- Margin : Waxy
- Lamina : Deltoid
- Nature of leaf : Simple

Track 3, ID: Pet. ether ext



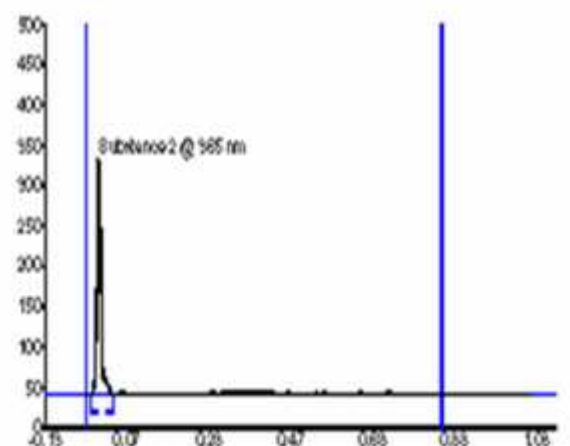
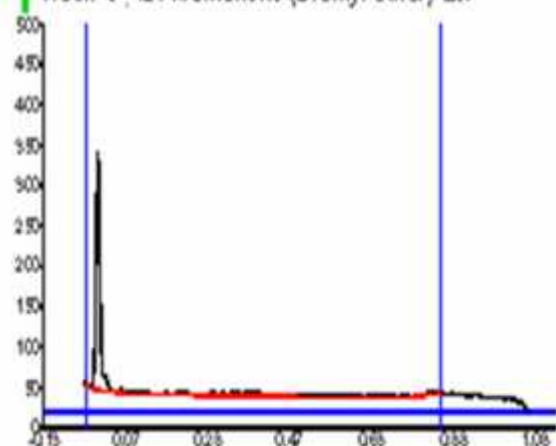
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.02	1.8	0.01	135.7	100.00	0.04	1.4	1737.2	100.00	Substance 2

Track 4, ID: Chloroform ext

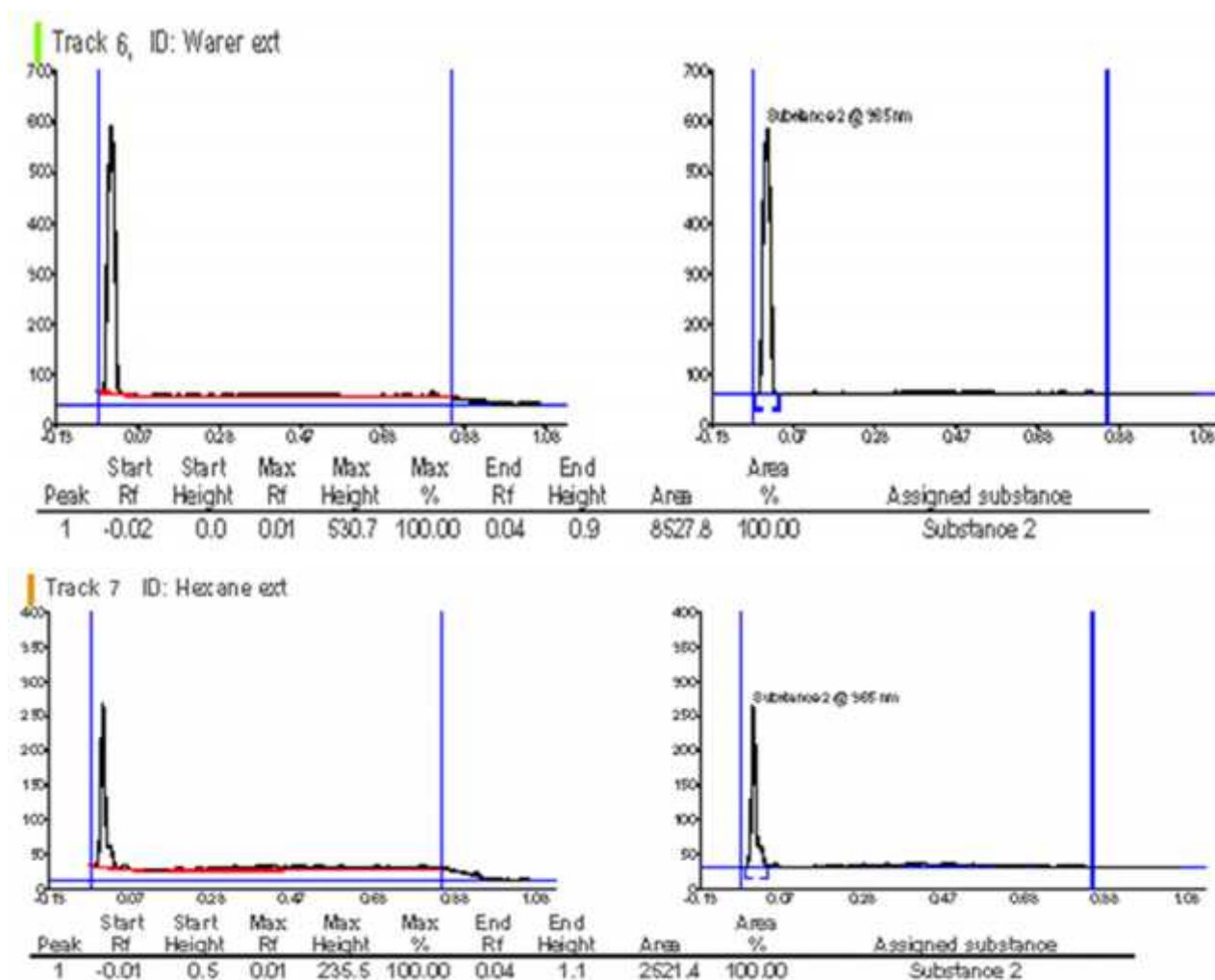


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.6	0.01	726.6	96.84	0.06	7.4	16071.4	98.38	Substance 2
2	0.06	7.0	0.07	12.1	1.62	0.09	0.2	156.2	0.96	unknown *
3	0.50	0.9	0.51	11.5	1.54	0.53	0.8	109.0	0.67	unknown *

Track 5, ID: Methanolic (Diethyl ether) ext



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.01	0.0	0.01	292.0	100.00	0.04	0.3	2753.6	100.00	Substance 2



Apex : Accuminate
 Base : Codate
 Venation : Reticulate,unicostate
 Petiole : Petiolated

Physiochemical Evaluation: The different ash values and different physiochemical parameters were screened and were reported in the tables.

RESULTS AND DISCUSSION

Ficus religiosa of family Moraceae is found through out tropical and subtropical region in India. The Various parts of plant have claimed to have several traditional medicinal properties. *Ficus religiosa* leaf is used to cure some of the diseases like Asthma, Diabetes, Epilepsy, Gastric problems, Inflammatory disorders etc., Literature survey said that review was made in this plant and also few pharmacological work like microbiological assay and anti inflammatory activity, anti convulsant activity was performed. There is no research work on the standardisation, isolation of the active constituent in this plant. So Present investigation an attempt was made to study its pharmacognostical features like Macroscopy (colour, odour, taste, fracture etc.,) and phytochemical evaluation.

The different ash values and different physiochemical parameters were screened. Extraction was performed by

using soxhlet apparatus. The qualitative analysis of the extracts shows the presence of Alkaloids, flavanoids, steroids, triterpenoids etc. The extracts were spotted on the TLC plates and runned by using Benzene: chloroform 7:3. The plate shows blue fluorescence at 365nm in chloroform extract, ethyl acetate extract and methanolic extract so the plant contains flavonoids.

The same extracts were screened through HPTLC. The methanolic extracts, pet.ether extracts, hexane and water extracts shows 100% constituents. But blue fluorescence was observed on methanolic extract. So this is a correct extract for the isolation of the active constituents. The highest percentage of flavonoids containing extracts were selected for the isolation studies to develop a new drug molecule in future. It is more useful and helpful to cure some of the diseases like Asthma, Diabetes, Epilepsy, Gastric problems, Inflammatory disorders etc., with less side effects.

CONCLUSION

It was concluded from the present investigation that the selected species of *Ficus religiosa* contains various active phytoconstituents, which was confirmed by preliminary phytochemical screening. Hence detailed screening need to isolate the active moiety. The special investigation was performed by using TLC and HPTLC procedure. By the

HPTLC report, the percentage of active constituents can be determined. The highest percentage of flavonoids containing extracts were selected for the isolation studies to develop a new drug molecule in future. It is more useful and helpful to cure some of the diseases like Asthma, Diabetes, Epilepsy, Gastric problems, Inflammatory disorders etc., with less side effects.

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