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Research Article

Isolation of Active Compound in *Ficus Religiosa Linn*

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ABSTRACT :
Herbs have always been the principal form of medicine in India. *Ficus religiosa* (L.), commonly known as pepal belonging to the family Moraceae, is used traditionally as antiulcer, antibacterial, antidiabetic, in the treatment of gonorrhea and skin diseases. The plant was subjected to extraction and also fractionated with two various solvent like diethyl ether and n-butanol. The Phytochemical test, TLC and HPTLC reports shows presence of compound only in n- butanol fraction.So this fraction subjected to structural elucidation. The identified compound was stigmasterol.

Keywords : Ficus religiosa leaf powder ,extraction, elucidation.

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 β-sitosteryl-D-glucoside, The seeds contain phytosterolin, β-sitosterol, and its glycoside, albuminoids. The fruit of *F. religiosa* contained appreciable amounts of total phenolic contents, total flavonoid[1].

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 authentificated by Dr.K.Madhava chetty, prof, dept.of botony,S.V.University, Thirupathi.
Chemicals: Methano,10% acetic acid ,Diethyl ether ,n-butanol
Instruments :Weighing balance ,Hot air oven ,Heating mantle ,HPTLC, IR spectrophotometer ,NMRspectrometer ,Mass spectrometer

EXPERIMENTAL INVESTIGATION

Procedure for extraction: Weighed accurately 50gms of fine leaves powder and 500ml of methanol was added. The crude drug was extracted through soxhlet apparatus for 72 hrs. Then filtered the solution and collected, to the residue of leaf powder again added 200ml of methanol, allowed the leaf powder to further maceration for 2days. Collected the filtrate and evaporated by using heating mantle until to get the dried powder.
Fractionation of active constituent: Weighed 3gms of extract powder and dissolved with 10% of acetic acid and allowed to reflux for 1hr.The content was transferred to separating funnel. Then equal quantity of diethyl ether was added. Repeat the procedure for 2times then collect the bottom layer, it contains the extract. Added equal quantity of n-butanol, shaken it well and kept aside for 15min. Then collected the n-butanol fraction and evaporated to dryness. The residue was taken for further evaluation[2].
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 substance. Silicagel G used as adsorbent and n-butanol: acetic acid : water in the ratio of 4:0.5:5 used as a mobile phase. Plate was prepared by pouring silica gel on glass plate and activated by heating at110^0c for 30 min. Take the n-butanol fraction in methanol which was washed by dietylether , spotted on TLC. The spots are detected under long uv at 365 nm and Rf values are calculated[3].
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 binder to form a 200µm layer.Plates exposed to high humidity are kept out to make them activated by placing in an oven at 110-120^0 C for 30 min prior to sample spotting. n-butanol :acetic acid :water in the ratio of 4:0.5:5 .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 length 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

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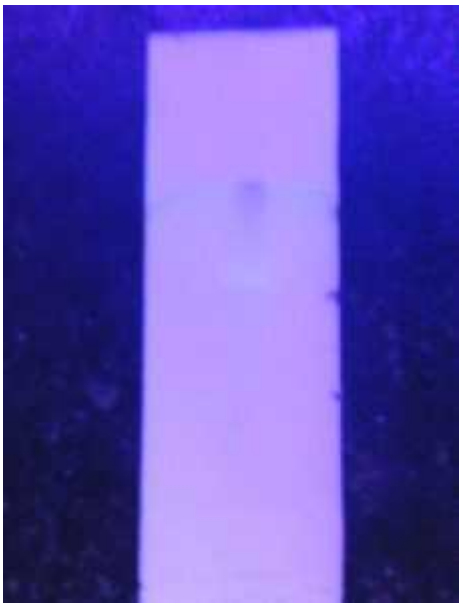
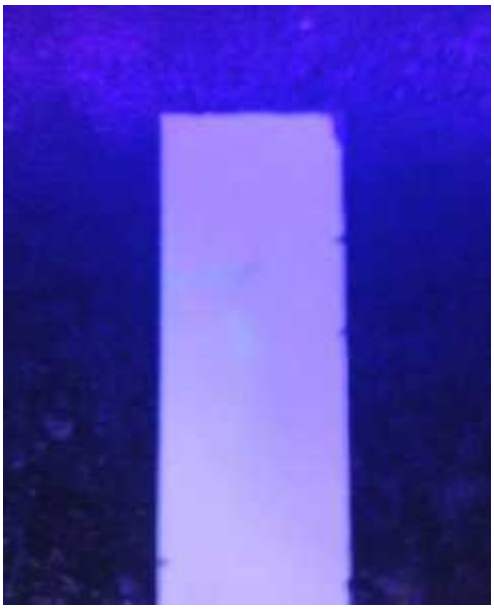
TLC:

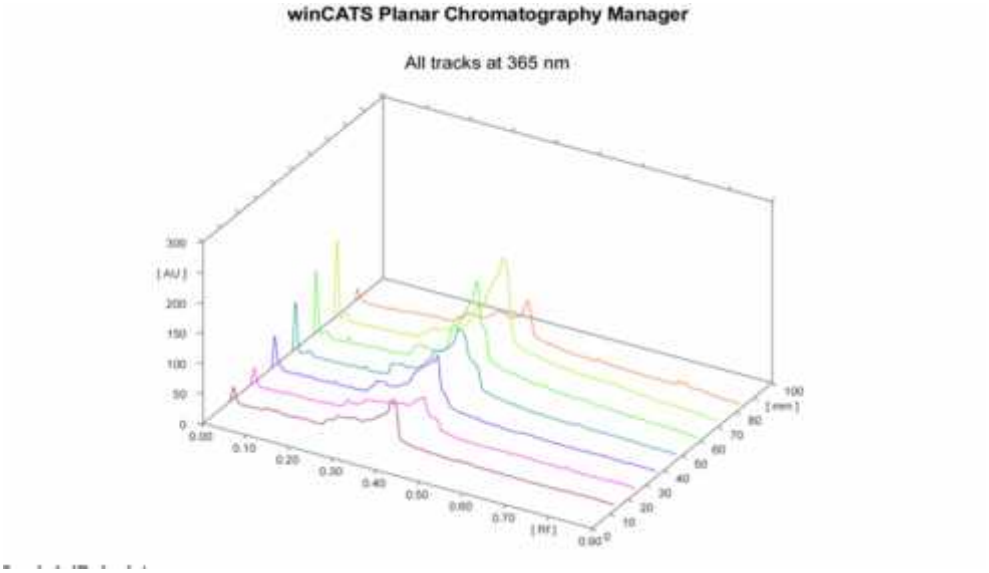




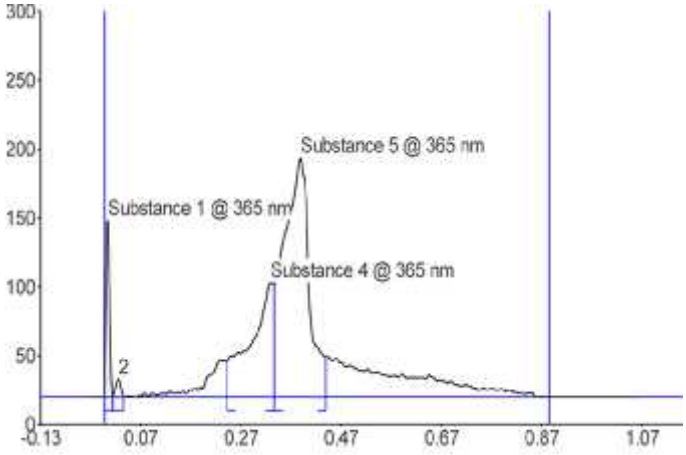
n-butanol fraction

diethyl ether fraction

HPTLC:



Cumulative graphs of HPTLC



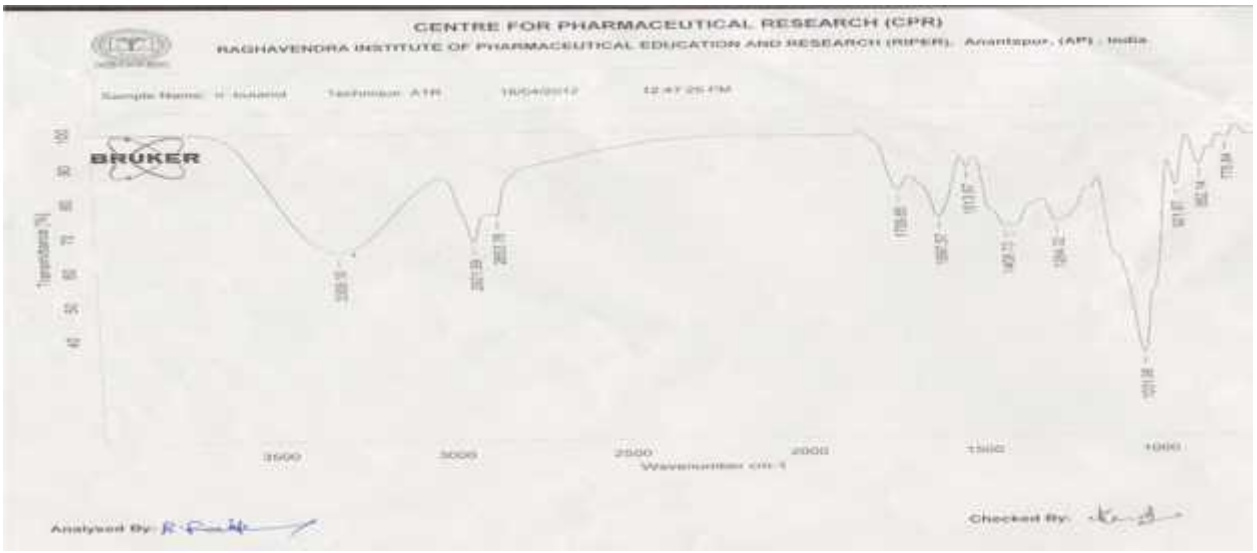
RF Values By TLC

Sl.No	Fraction	R_f values
1	n-butanol	0.70
2	Diethyl ether	0.489

HPTLC report for n-butanol fraction

SL.NO	R_f VALUES	AREA %	ASSIGNED SUBSTANCE
1	0.39	65.17	Substance 5

IR SPECTRUM :



IR-spectrum for n-butanol fraction

Report for IR spectrum

Test	standard	functional group
3309.10	2800-3400	Carboxy group
	3200-3500	aromaticOH
2921.99	2920-2930	alkanes(aliphatic straight)CH stretching
		alkanes(aliphatic straight)C-H stretching
2853.76	2920-2930	carboxylic C=O stretching
1709.85	1700-1715	aromatic C-C stretching C-O stretching
1597.57	1585-1600	aromatic C-C stretching C-O stretching
		aromatic C-C stretching C-O stretching
1513.67	1585-1600	aliphatic C-H bending
		may be ester or carboxylic group
1408.73	1585-1600	carboxylic O-H bending
1264.32	1200-1400	aromatic C-H bending
1031.98	1000-1300	aromatic C-H bending
921.67	910-950	
852.14	810-865	
775.84	750-810	

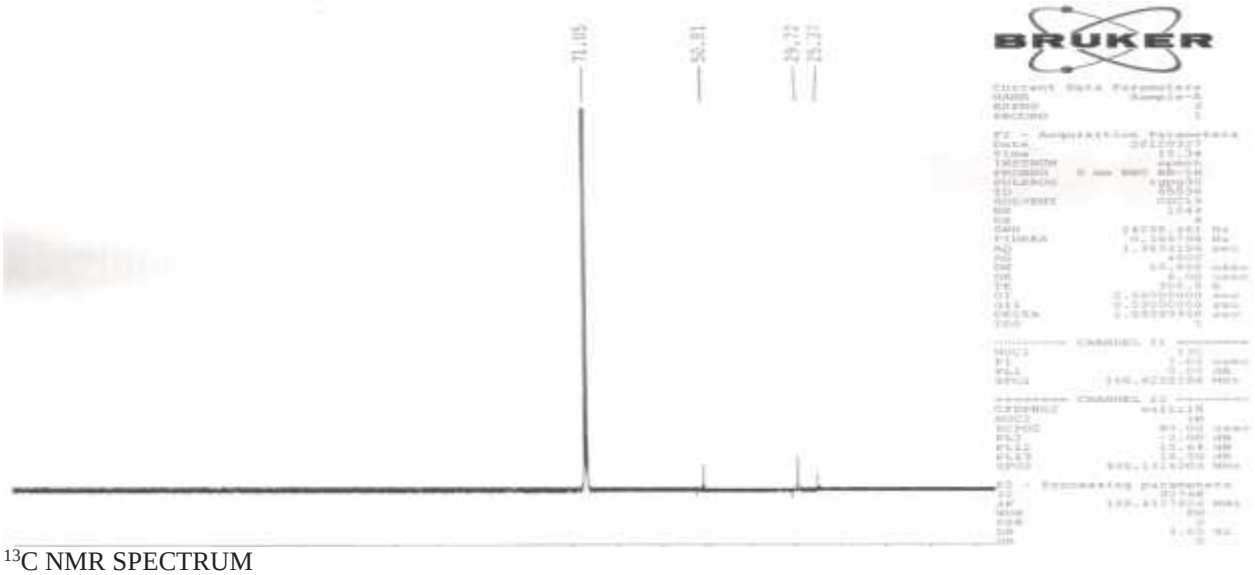
HPTLC plates are scanned by a beam of monochromatic light[3,4].

Structural elucidation: IR- spectroscopy: Instrument: Attenuated Total Reflectance Spectroscopy: IR spectroscopy is performed by using pressed pellet technique and it is useful in determining the important functional groups of the compound as a part of its structural identification. Then the n-butanol fraction was concentrated and recrystallized by methanol and sample packed in glass container. Examined by IR spectrophotometer and collected the spectra.

^{13}C,^{1}H NMR : Instrument: NMR Spectrometer ,Solvent : $CDCl_3$,Standard : TMS

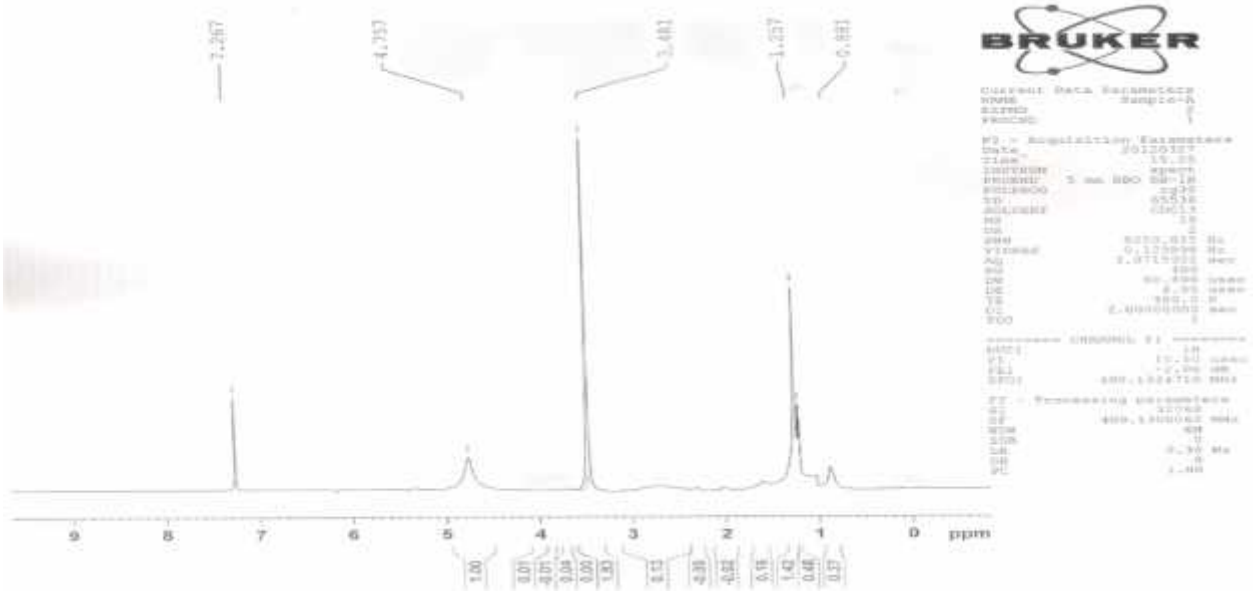
Extract was taken and that targeted to isolate by fractional method by using n-butanol. Then the n-butanol fraction

C^{13} NMR



^{13}C NMR SPECTRUM

^{1}H NMR spectrum



^{1}H NMR Interpretation		
^{1}H NMR test	Standard	Functional group
7.267	4-12	Aromatic hydrogen
4.757	4-12&	Phenolic OH
	4.6-5.8	Vinylic
3.481	3.4- 4	Alcohols
1.257	1- 5.5	Hydroxy
0.881	0.9	Primary OH

was concentrated and recrystallized by methanol and sample packed in glass container .Examined by using ^{13}C and ^{1}H NMR and collected the spectras[4,5,6].

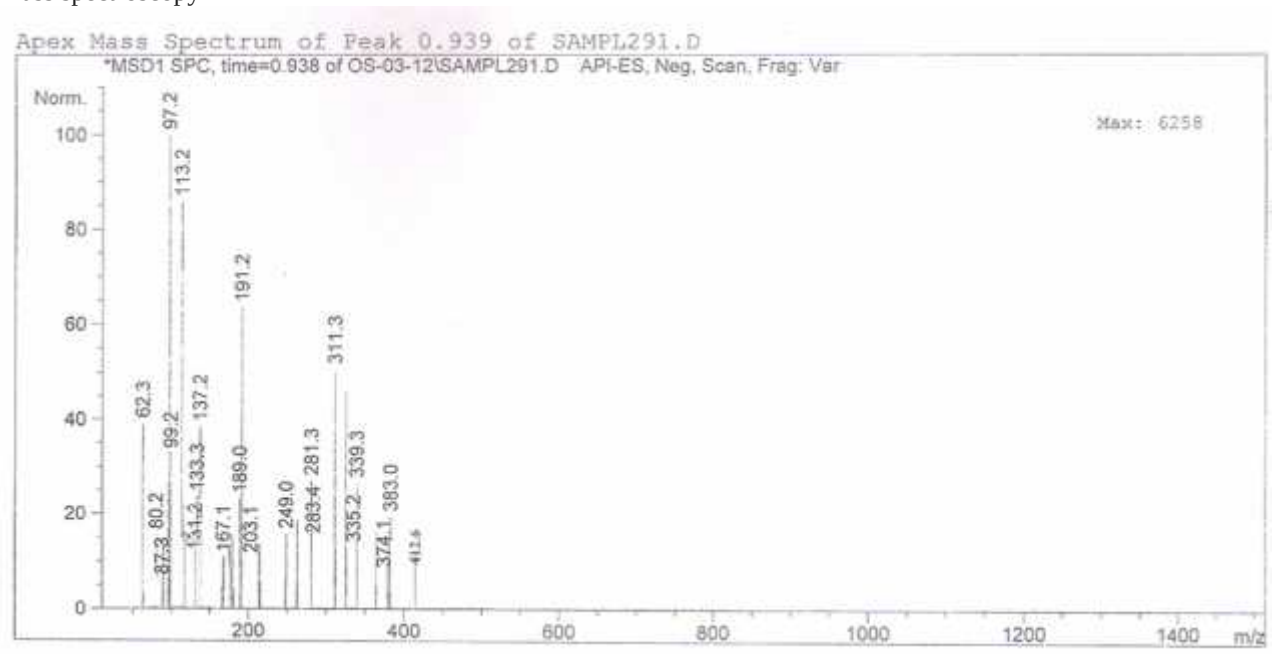
Mass spectoscopy:

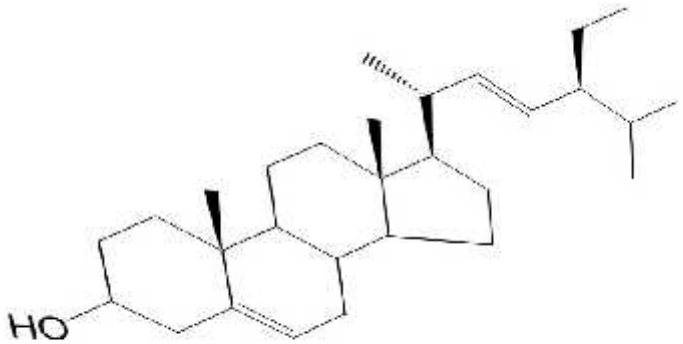
Instrument: Mass Spectrometer: Extract was taken and that targeted to isolate by fractional method by using diethyl ether and n-butanol. Then the n-butanol fraction was concentrated and recrystallized by methanol and sample packed in glass container .Examined by mass spectroscopy[6].

RESULTS AND DISCUSSION

Structural elucidation is a major part of analytical work to confirm pharmaceutical drugs. In this present study deals with isolation of active molecule in *Ficus religiosa* leaf.

Mass spectroscopy





Stigmasterol
$C_{29}H_{48}O$
Mol. Wt.: 412.69

Structure of Stigmasterol

The extraction was done by using methanol because methanol was solubilized all the active constituents in the plant or drug. The extract targeted to isolate by fractional method using diethyl ether, n-butanol. Then the fraction was concenterated , the residue was tested by TLC and n-butanol: glacial acetic acid: water in the ratio of 4:0.5:5.

The compound was screened through chemical tests. It shows the presence of steroids. The percentage yield of the composition screened by HPTLC. It shows high percentage of composition and also single peak. The structure was confirmed through IR, NMR and mass spectroscopy. The structure as follows.

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