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ISOLATION AND CHARACTERIZATION OF β -SITOSTEROL FROM *TEPHROSIA VILLOSA* PERS

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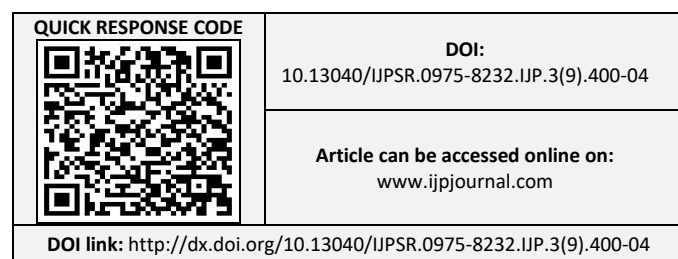
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ABSTRACT: Natural products and herbal remedies used in traditional folklore medicine have been the source of many medically beneficial drugs because they elicit fewer side effects, relatively cheap, affordable and claimed to be effective. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and to understand their mechanism of action. *Tephrosia villosa* Pers. (Fabaceae) is a medicinal plant widely used as a folk medicine in India. The present study deals with the isolation and partial purification of bioactive compounds from the crude methanol extracts of the leaves of *Tephrosia villosa* Pers. The quantification and the identification of compounds in the crude extract and active bands isolated by preparative TLC were accomplished using spectroscopic analysis. The most important compounds β -sitosterol identified in the crude extract appreciable amounts may account for its various biological activities.

INTRODUCTION: In many of the developing countries the use of plants drugs is increasing because modern life – saving drugs are beyond the reach of three quarters of the third world population, although many such countries spend 40 – 50% of their total health budget on drugs because of natural origin and lesser side effect or dissatisfaction with result of synthetic drugs¹. Standardization of plant-based medicine is a difficult task; because plants synthesize not only single compounds but it may vary even up to hundreds of compounds may be present in the plant. Hence, it is difficult to standardize herbal medicines as compared to other medicines.

Correct identification and quality assurance of the starting material is, therefore, an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy^{2,3}. The quality and quantity of safety and efficacy information on traditional medicines are not sufficient to meet the criteria to support its use worldwide. The reason behind the lack of research data is not only due to health policies but also due to the lack of methodologies for the evaluation of herbal medicines. The plants possess many active therapeutically active chemical constituents associated with many inert substances such as cellulose, lignin and coloring agents, *etc.* The active constituents are extracted from plants and purified for their pharmacological utility.

So, the quality control of herbal drugs is important for their active chemical constituents in the modern system of medicine. To meet the new thrust of inquisitiveness, standardization of herbal drug is mandatory⁴⁻⁸. *Tephrosia villosa* Pers. has many



medicinally active compounds in it hence, the focus of this paper is on the analytical methodologies, which include the extraction, isolation, and characterization of active ingredients in leaves of *Tephrosia villosa* Plant. β -sitosterol is reported to exhibit a spectrum of pharmacological activities against various disease conditions. These include conditions such as inflammation, arthritis, diabetes, cardiovascular ailments, renal disorder, hepatic toxicity, microbial infections and cancer⁹. The available literature suggests that β -Sitosterol is a nontoxic agent and does not cause any systemic toxicity in animals at doses ranging from 30 to 2000 mg/kg¹⁰.

MATERIALS AND METHODS:

Plant Material: The plant specimens for the proposed study were collected from the Salem Dist. (T.N.) and authenticated by Dr. Marimuthu, Arts, science college Attur, Tamil Nadu (India). After drying 2kg leaves and stems of *Tephrosia villosa* milled and pass from sieve # 40. The milled powder material kept in airtight container up to further use. The collected, cleaned and powdered leaves of *Tephrosia villosa* Pers. was used for the extraction purpose. 200 gm of powdered material was evenly packed in the Soxhlet apparatus. It was then extracted successively with various solvents of increasing polarity¹¹⁻¹⁴.

Qualitative Estimations: Preliminary phytochemical screening of extracts¹⁵⁻¹⁶. The above extracts obtained from the leaves were subjected for the various chemical test for the identification of active phytoconstituents groups by following standard procedure.

Thin Layer Chromatography and Preparative TLC: For Thin Layer Chromatography and preparative TLC analysis, the method used was taken from quality standards of Indian medicinal plants ICMR (volume 5, 2008) for β -sitosterol. Improvements were made to the sample preparation and standard preparation.

Identification of Separated Compound: Pinch of the sample was added in a clean and dry test tube and dissolved in chloroform. Acetic anhydride (1 ml) was added in the test tube. Few drops of sulphuric acid solution were added from the wall of the test tube; solution shows violet color indicates the presence of triterpenoids.

RESULTS AND DISCUSSION: For thin layer chromatography, improvements were made to the sample preparation 0.25 gm of *Tephrosia villosa* methanolic extract was diluted with 10ml methanol and standard preparation dissolve 10 mg of β -sitosterol (available from total herb solution) in 10 ml of methanol. TLC plates developed with toluene: ethyl acetate (80: 20) showed a violet spot at an R_f value of 0.57 **Fig. 1** when sprayed with 1% anisaldehyde-sulfuric acid reagent, heat to 105°C for 15 min.

The spot coinciding with the standard was marked and then scraped from the plate and scraped silica was sonicated in methanol for 15 min then the solution was filtered and β -Sitosterol was obtained by evaporating methanol. Further, analysis was done for the isolated compound. By repetitive preparative TLC, the β -sitosterol separated was about 25 mg¹⁷.

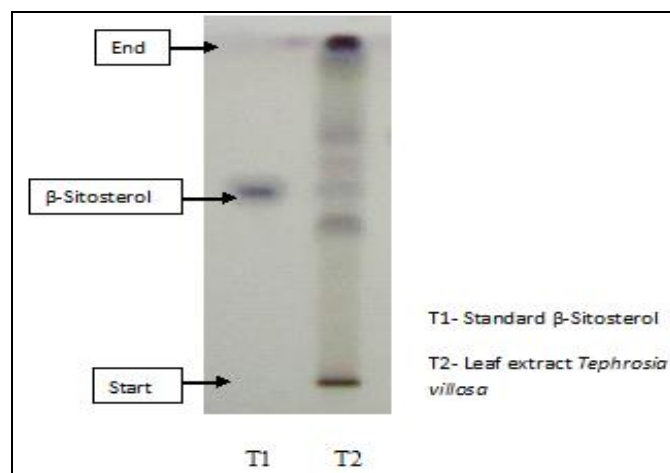


FIG. 1: TLC OF EXTRACT WITH STANDARD β -SITOSTEROL

Isolation of β -sitosterol was done using Preparative Thin Layer Chromatography (Prep TLC): Prep TLC is an alternative technique to column chromatography much faster than classic Column Chromatography. For sample preparation for preparative TLC methanolic extract, 25 g was partitioned with 50 ml portions of petroleum ether to separate the nonpolar terpenoids.

The petroleum ether extract was concentrated and used for isolating β -sitosterol by preparative thin layer chromatography. Silica gel 60F₂₅₄ pre-coated TLC plates (Merck) 20 × 20 cm developed with toluene: ethyl acetate (80: 20) showed a violet spot at an R_f value of 0.57 **Fig. 2** when sprayed with

1% anisaldehyde-sulfuric acid reagent, heat to 105 °C for 15 min.

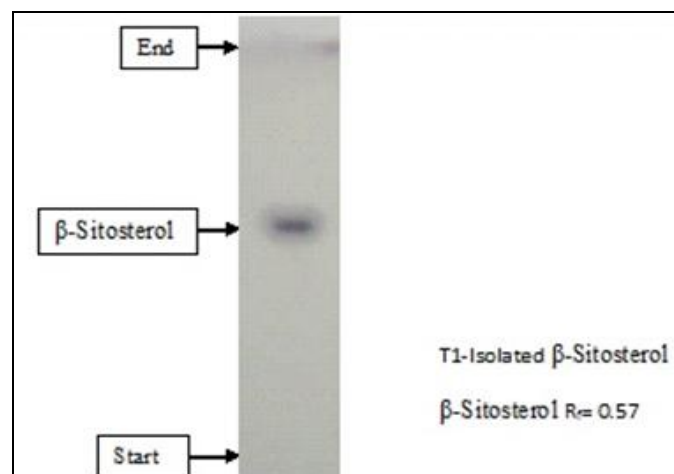


FIG. 2: TLC OF STANDARD β -SITOSTEROL

IR spectrum was taken as KBr pellets on Perkin-Elmer IR spectrometer. 3426.3 (stretching, O-H), 2936.9, 2864.2 (stretching, C-H), 1648, 1636 (stretching, C=C) results were showed in Fig. 3 and Table 1.

TABLE 1: FUNCTIONAL GROUP RANGES FOR IR SPECTRUM OF COMPOUND

S. No.	Functional group	Range	
		Actual	Observed
1	OH	3200-3600	3426.3
2	C-H Strech	2850-2970	2936.9, 2864.2
3	C=C Strech	1500-1680	1648, 1636
4	C-H Bend	1340-1470	1461.8
5	C-O	1050-1300	1056.1

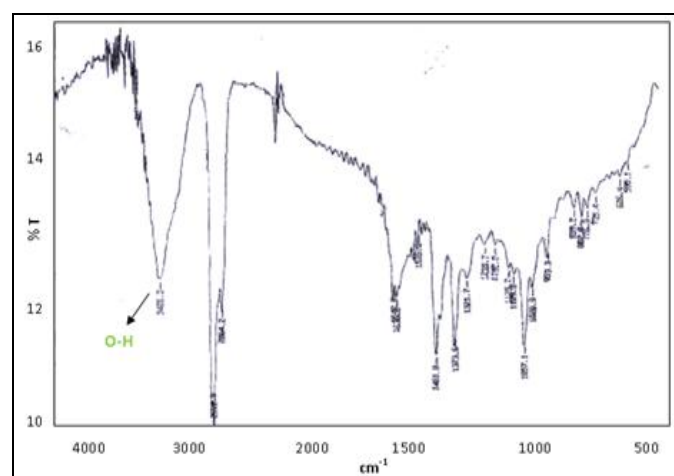


FIG. 3: IR SPECTRUM OF COMPOUND

^1H NMR-spectra of Compound ^1H NMR (CDCl_3 , 400 MHz): H- 3-3.52 (1H, m), H-6-5.34 (1H, m), H-18-1.14 (3H,s), H-19-1.26 (3H, s), H-21-0.91 (3H, s), H-26-1.01(3H, s), H-27-0.97 (3H,s), H-29-0.93 (3H, s) Fig. 4 and 5 and Table 2.

High-Performance Liquid Chromatography (HPLC) for β -sitosterol: The data obtained from the chemical test, physical tests and spectral studies of the isolated compound is matching well with that of reported for β -sitosterol and by which the isolated compound is characterized as β -sitosterol Fig. 6.

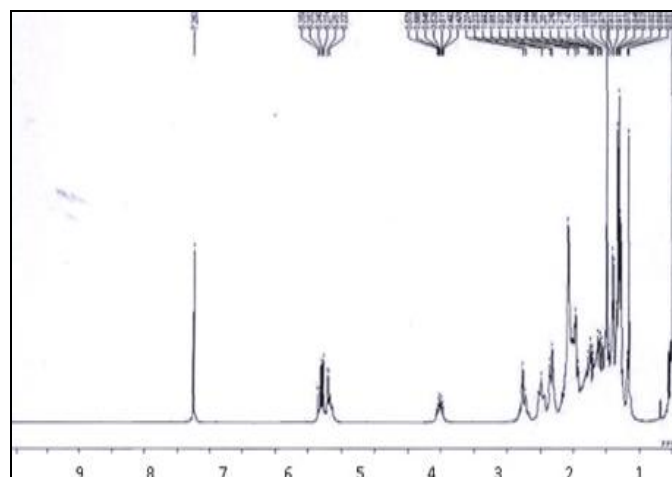


FIG. 4: ^1H NMR SPECTRA OF COMPOUND RECORDED IN CDCl_3 , 400 MHz

TABLE 2: ^1H NMR AND ^{13}C NMR SPECTRA FOR COMPOUND

Position	δ_{H} NMR	δ_{C} NMR
1		31.9
2		36.1
3	3.52 (1H,m)	71.8
4		42.2
5		140.7
6	5.34 (1H,m)	121.6
7		31.9
8		28.2
9		42.3
10		39.7
11		21.0
12		31.8
13		42.3
14		45.8
15		20.2
16		21.0
17		50.1
18	1.14 (1H,s)	20.2
19	1.26 (3H,s)	19.4
20		30.2
21	0.91 (3H,s)	18.9
22		33.9
23		28.9
24		45.8
25		29.1
26	1.01 (3H,s)	19.8
27	0.97 (3H,s)	19.8
28		23.0
29	0.93 (3H,s)	11.9

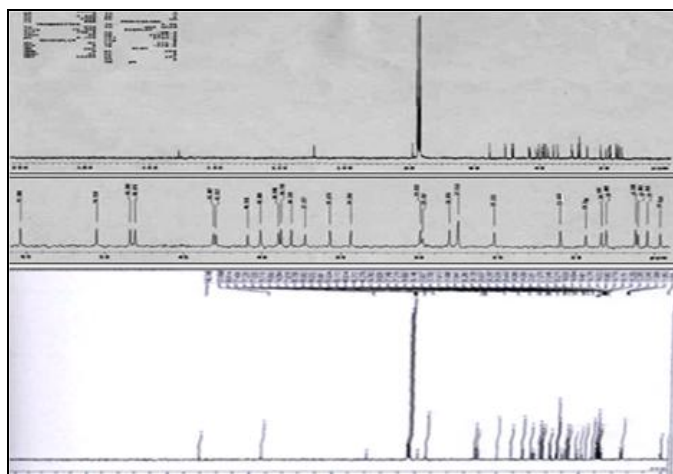


FIG. 5: C^{13} NMR SPECTRA OF COMPOUND RECORDED IN $CDCl_3$, 400 MHz

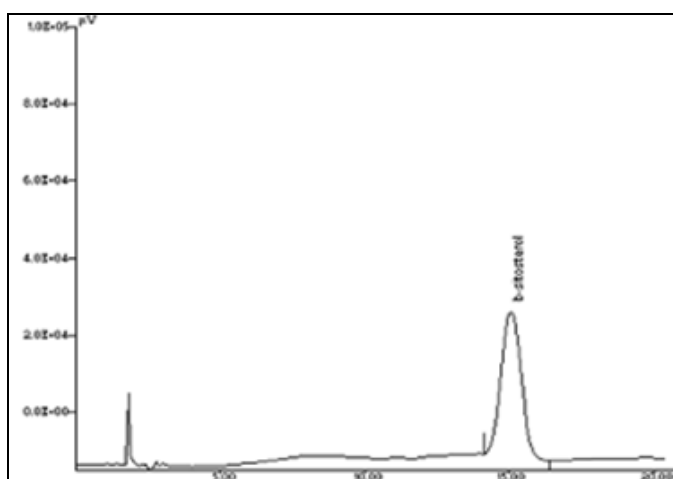


FIG. 6: HPLC FOR β -SITOSTEROL

CONCLUSION: Isolation of β -sitosterol was done using preparative Thin Layer Chromatography, the chemical formula for β -sitosterol $C_{29}H_{50}O$, synonym 22, 23-Dihydrostigmasterol, Stigmast-5-en-3-ol, β -sitosterin, description colourless crystals, solubility Freely Soluble in chloroform, petroleum ether and solubilized only upon sonication and heating in methanol, melting point $147-146^\circ C$, purity 90.36%. β -sitosterol IR (KBr) 3426.3 (stretching, O-H), 2936.9, 2864.2 (stretching, C-H), 1648, 1636 (stretching, C=C) results were showed in **Fig. 3** and **Table 1**. Mass (m/z): 415.13 **Fig. 7** and **Table 3**. H^1 NMR ($CDCl_3$, 400 MHz) H- 3-3.52 (1H, m), H-6-5.34 (1H, m), H-18-1.14 (3H, s), H-19-1.26 (3H, s), H-21-0.91 (3H, s), H-26-1.01 (3H, s), H-27-0.97 (3H, s), H-29-0.93 (3H, s). HPLC puro sphere RP 18 column, mobile phase Methanol: water: Acetic acid (70:30:1), flow rate 1ml/min, detector UV/VIS, λ_{max} 205nm and retention time 15 min. Estimation of β -sitosterol in *Tephrosia villosa* Pers. leaves chromatographic

technique column Microsorb-MV 100-5 C18 250 X 4.6 mm, Mobile Phase acetonitrile: methanol: glacial acetic acid (80: 20:0.01%), flow rate 1ml/min, detection 205 nm, injection volume 20 μL and run time 3.5 min. The percentage of β -sitosterol was found in *Tephrosia villosa* Pers. leaves 2.08 %.

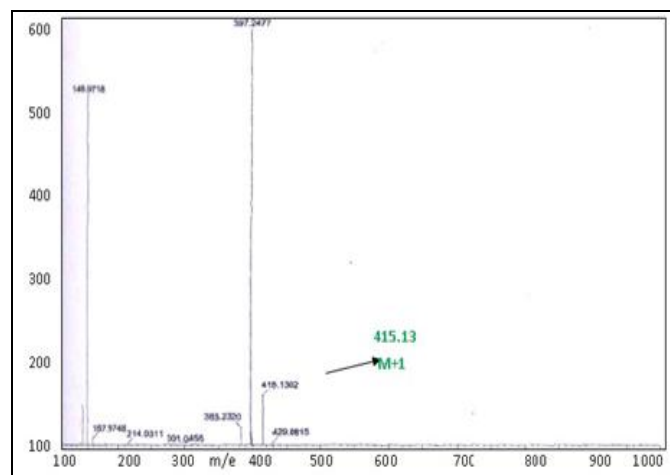


FIG. 7: MASS SPECTRA OF COMPOUND

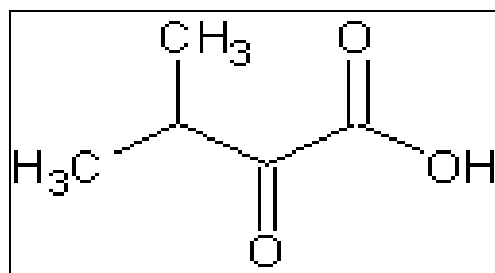


FIG. 8: CHEMICAL STRUCTURE OF β -SITOSTEROL

TABLE 3: MOLECULAR WEIGHT OF COMPOUND

S. no.	Molecular weight	
	Actual	Observed
1	414.70	415.13

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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