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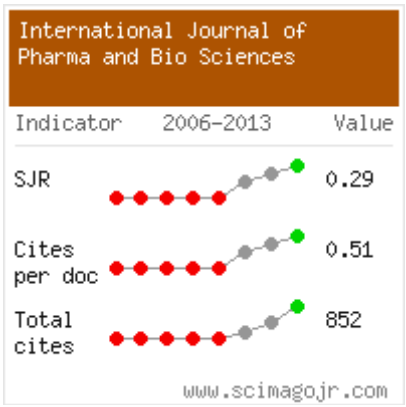

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ISOLATION AND STRUCTURE ELUCIDATION OF NOVEL COMPOUND FROM LEAVES OF *DERRIS TRIFOLIATE*

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ABSTRACT

In the present work, leaves of mangrove plant, *Derris trifoliata* was successively extracted with methanol and for characterization, the leaf extract was subjected to thin layer chromatography and high performance thin layer chromatography. Different compounds were isolated and the structure of one particular compound was elucidated by NMR, IR, UV and GC-MS spectra.

KEYWORDS: **Derris trifoliata, Methanol extract, Characterization, Phytochemical analysis, Structure elucidation**

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INTRODUCTION

Biological organisms produce two distinctly different types of chemical products. The first type, primary metabolites, consists of compounds such as sugars and proteins that are common to most organisms and are essential for functional metabolism. Secondary metabolites, on the other hand, are chemicals unique to a single species or related group of organisms. Not until the 1990s would scientists fully realize that these secondary metabolites are more than mere leftovers from an organism.s metabolic processes; they actually serve in a wide variety of important roles[1]. The biological activity of these chemicals is beneficial to the organism that produces it, but it is often harmful to other species, including humans[1]. This toxicity can adversely affect the functions of the entire human body or only a specific biological process, such as the growth of cancer cells. In this way, certain foreign, naturally produced chemicals can act as powerful drugs when administered at the proper concentration. Natural products have been used by native cultures as a source of remedies for thousands of years, dating back to ancient empires in Mesopotamia, Egypt, China, Greece, and Rome[2]. Now scientists in the modern industrial world are turning to plants, microbes, and marine organisms as a potential storehouse of medicines waiting to be discovered[3]. Today, natural products are responsible for about half of the approved drugs that are currently available[4]. The percentage is even higher for treatment of infection or cancer, as natural products for those illnesses account for approximately 60% of the drugs either in use or awaiting FDA approval between 1989 and 1995[5]. For example, 18 of the 42 new drugs discovered in 1992 are either natural products or synthetic analogs of natural products[6]. *Derris trifoliata* is one of the most commonly used plant sources to yield a wide range of compounds. The leaves contain the chemical compound rotenone, a poison that kills a wide range of creatures from insects to earthworms and fish[7,8]. This poison is used in catching the fishes by local fisher folks. The leaves of *D. trifoliata* were proved to have many therapeutic uses including stimulant, antispasmodic, counter-irritant, rheumatism, chronic paralysis, and dysmenorrhea. The decoction of roots is used against fever and internally against sores. The roots or stem are used as laxative, carminative and anti-arthritis agent [9]. Henceforth an attempt was made to investigate the compounds present in the leaves of D. trifoliata.

MATERIALS AND METHODS

Preparation of the Derris trifoliata leaf extract

Derris trifoliata leaves were randomly collected from Rameshwaram Mangrove forests (Tamilnadu), India. The leaves were dried in the shade for 7 days at room temperature (28 ±2°C) and ground to a fine powder using Grinder IKA®-WERKE, IKA MF10 Machine and sieved through a 0.25 µm mesh. The powdered samples were kept at room temperature in a covered glass containers to protect them from humidity and light prior to extraction. 50g of dried powder leaves were exhaustively extracted by maceration in 2.0 L methanol solvent for 2 days at room temperature (28±2°C). The solvent-containing extract was then decanted and filtered by vacuum filtration (GAST, DOA-P504-BN, USA) and further extracted twice with methanol (1.5 L each time). The filtrate from each extraction was combined and the excess solvent was evaporated under reduced pressure at 40°C using a rotary evaporator (Heidolph-instruments, Rotavapor, Germany) to give concentrated crude methanolic extracts, dried in oven at 50°C to give dark green extract.

Separation of compounds

Solvent-solvent partitioning (Fig I) was done using the protocol designed by Kupchan and Tsou[10,11] for the separation of compounds. The crude extract (5 gm) was triturated with 90% methanol and then fractionated successively using solvents of increasing polarity, such as hexane and ethyl acetate. The fractions were evaporated using rotary evaporator at low

temperature and kept in air tight containers for further analysis. The hexane- soluble and ethyl acetate soluble mixtures were fractionated using column chromatography. 30 gm of crude was mixed with 60 gms of Silica gel (60-120 mesh) to make the adhesive mixture. A column of diameter 2.4 cm and column bed height of 20 cm was packed with the adhesive mixture mixed with hexane. The column was eluted with increasing solvent polarity from hexane to ethyl acetate. Extracts obtained as above were concentrated to 1ml and 20µl was loaded on TLC plates (Silica gel G 0.2 ml) and developed by the solvents: Hexane: Ethyl acetate (1:1). The developed spots were observed on the TLC plates and *Rf* value was calculated by using the following formula: *Rf* = distance traveled by center of component /distance traveled by solvent front

Characterization of Purified compound

From the TLC, the compound was eluted for characterization. The TLC eluted compound was subjected to UV – visible spectrophotometer in the region 200-800 nm to check the purity of the compound. Further FTIR spectra were recorded for the purified compound in the mid IR – region 4000 – 400 cm^{-1} at resolution 4 cm^{-1}. Gas Chromatography Mass Spectrum (GCMS) was analyzed using Gas Chromatography (Shimadzu QP2010) equipped with a VF – 5 ms column (diameter 0.25 nm length 30.0 m, film thickness 0.25 µm) mass spectrometer (ion source 200˚C; EI-7000), programmed at temperature 40-650 ˚C with a rate of 4 ˚C/min. Injector flow rate was 240 ˚C; carrier gas was He 99.9995% purity, column flow rate 1.5 ml/min, injection mode – split (1:10). 1D NMR (^{1}H, ^{13}C) spectra of pure compound as a purity check was recorded.

RESULTS AND DISCUSSION

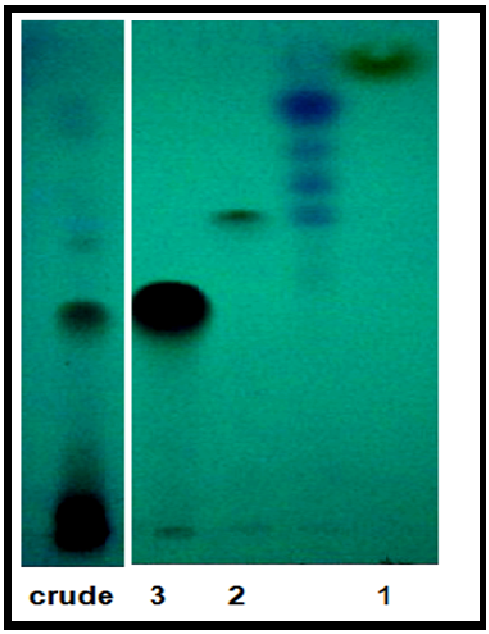
Chemical characterization of mangrove extract

Semi quantitative estimation and identification of active principles of the crude leaf extract of Derris trifoliata were performed by TLC method (Table I). In the present study TLC separation of methanolic extract of the plant material present three different compounds as revealed by fluorescent spots when visualized under UV light (Fig I). Three different compounds were isolated. The compound 1 Yellow Gel was collected from the fractions 21-26. Compound 2 Brown powder was collected from the fractions 41-51 and the compound 3 White powder was collected from the fractions 42-50 (Table I). The R_f value of the three different spots were determined as 2.296, 1.566 and 1.185 respectively. Further studies were carried out for the compound 2.

Table I
Isolated of compounds from Derris trifoliata by column chromatography

S.No	Number of Fractions	% of Solvent	Volume of Solvent (ml)	TLC Spot	Mobile phase
1	1-20	100% Hexane	500		H:Etoac(7:3)
2	21-26	97%Hex: 3% Etoac	200	Yellow gel liquid	H:Etoac(7:3)
3	27-36	95%Hex :5% Etoac	250		H:Etoac(7:3)
4	36-41	90%Hex:10% Etoac	300		H:Etoac(7:3)
5	41-51	87%Hex:13% Etoac	500	Brown powder	H:Etoac(6:4)
5	42-50	85%Hex:15% Etoac	500	White powder	H:Etoac(6:4)
6	51-56	80%Hex :20%Etoac	250		
7	57-61	70%Hex:30% Etoac	250		
8	62-70	100% Etoac	100		

Figure I
Photograph of TLC plate under UV light



UV and GC – MS SPECTRA

The UVspectrum gave a single peak at 206.0 λmax. The GC-MS of compound 2 gave a Parent ion peak at 413.41 ppm and a base peak 391.24 ppm. (Fig II & III)

Figure II
UV Spectrum of Compound 2

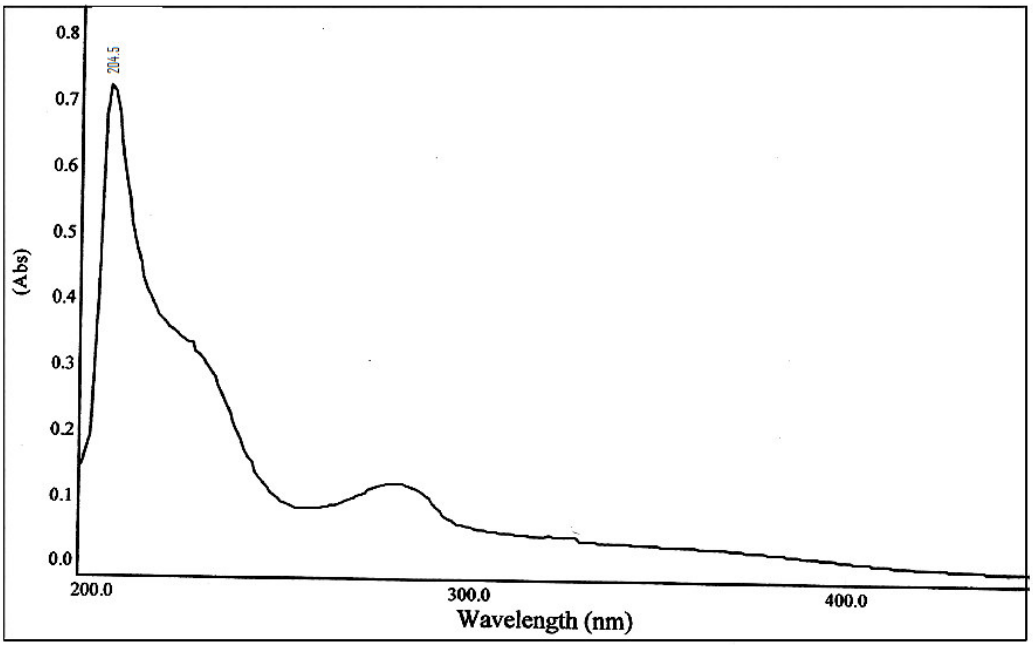
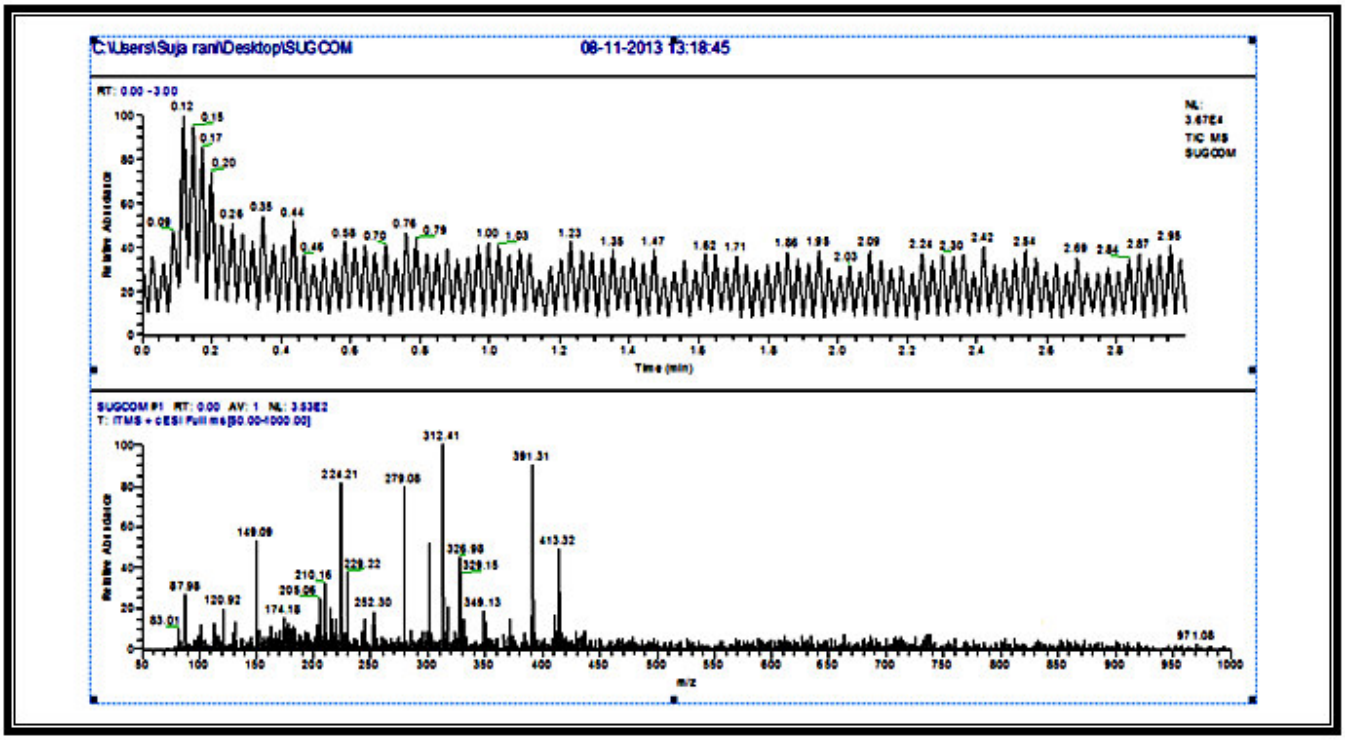


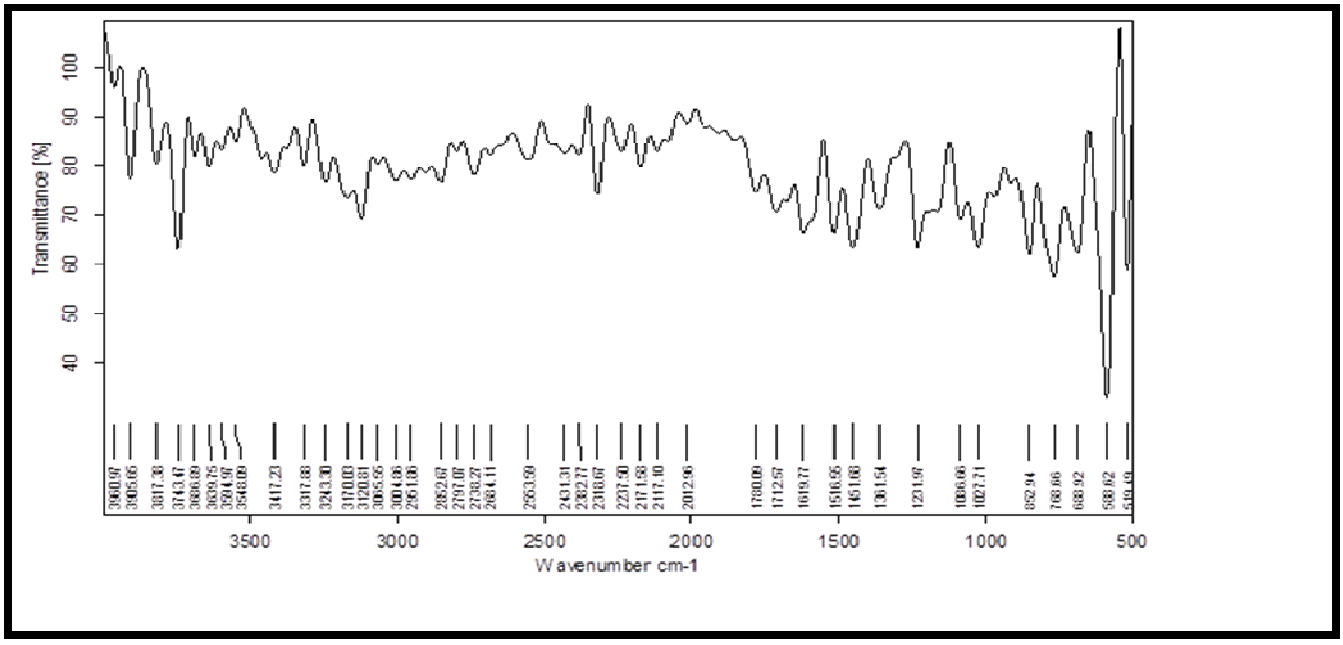
Figure III
GCMS of Compound 2



FTIR SPECTRA

The IR spectrum gave a peak at 3900 cm-1 (OH stretching), and at 3719 & 3594 (CH2OH).

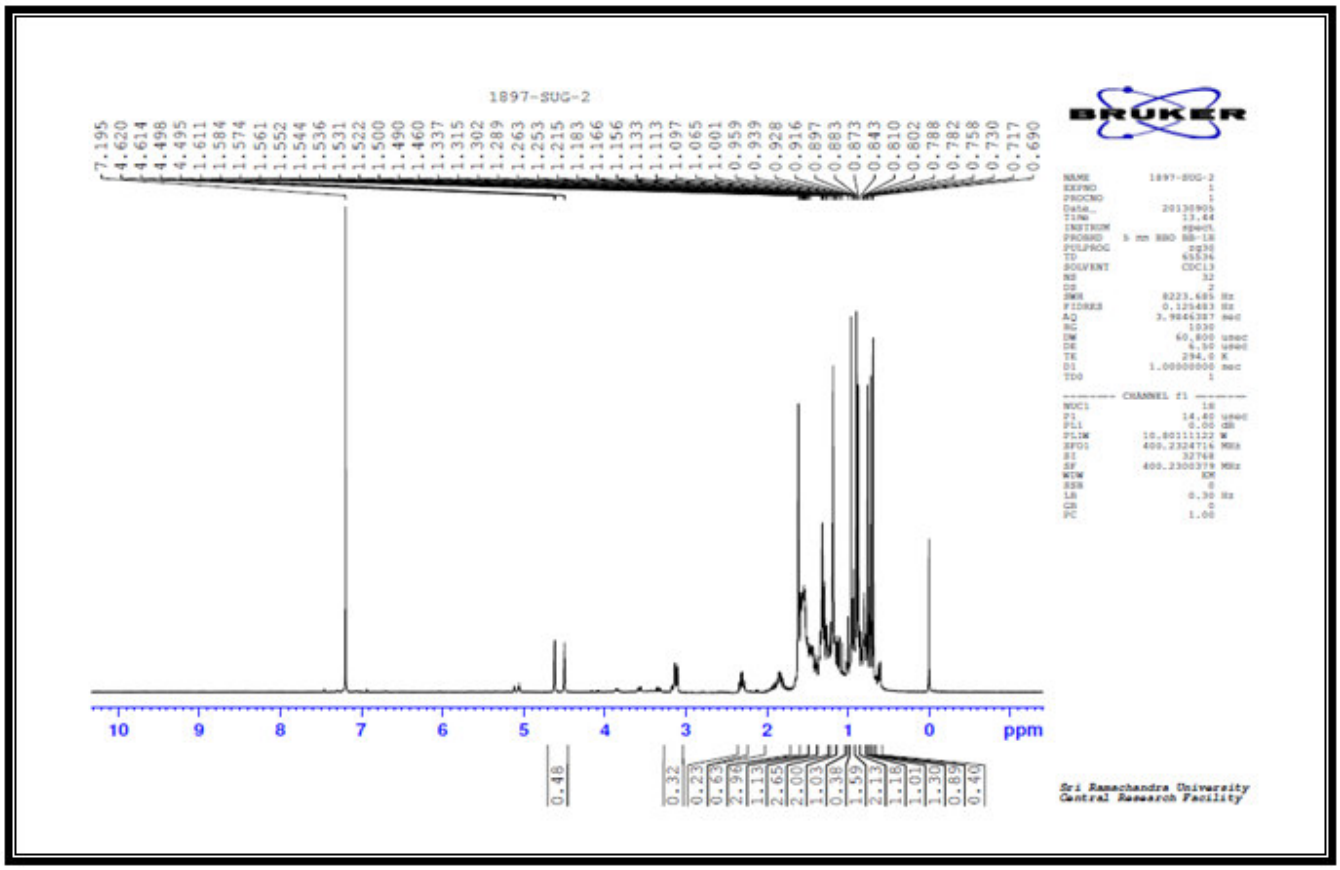
Figure IV
IR Spectrum of Compound 2



[1]H NMR SPECTRA

For [1]H NMR, the compound 2 yielded peaks between 0.690 and 1.065 ppm that indicated the position of terminal free methyls; gave peaks from 1.097 ppm to 1.611 ppm indicating the position of cyclic CH2; a peak at 2.200 ppm indicating the position of proton beside the OH; two peaks at 3.25 ppm and 7.195 ppm indicating the position of the functional group OH and the position of unsaturation respectively (Fig V).

Figure V
H NMR Spectrum of Compound 2



[13]CNMR SPECTRA

For [13]C NMR, the compound 2 gave a peak at 25.126 ppm which indicated the position of terminal free methyl groups; peaks at 27.395-34.269c 38.041-55.285 ppm indicated the position of quarternary carbon; a peak at 79.012 ppm where carbon is bonded to the hydroxyl group; a peak at 151.031 ppm indicating carbon - carbon unsaturation (Fig VI).

Figure VI
^{13}CNMR Spectrum of Compound 2

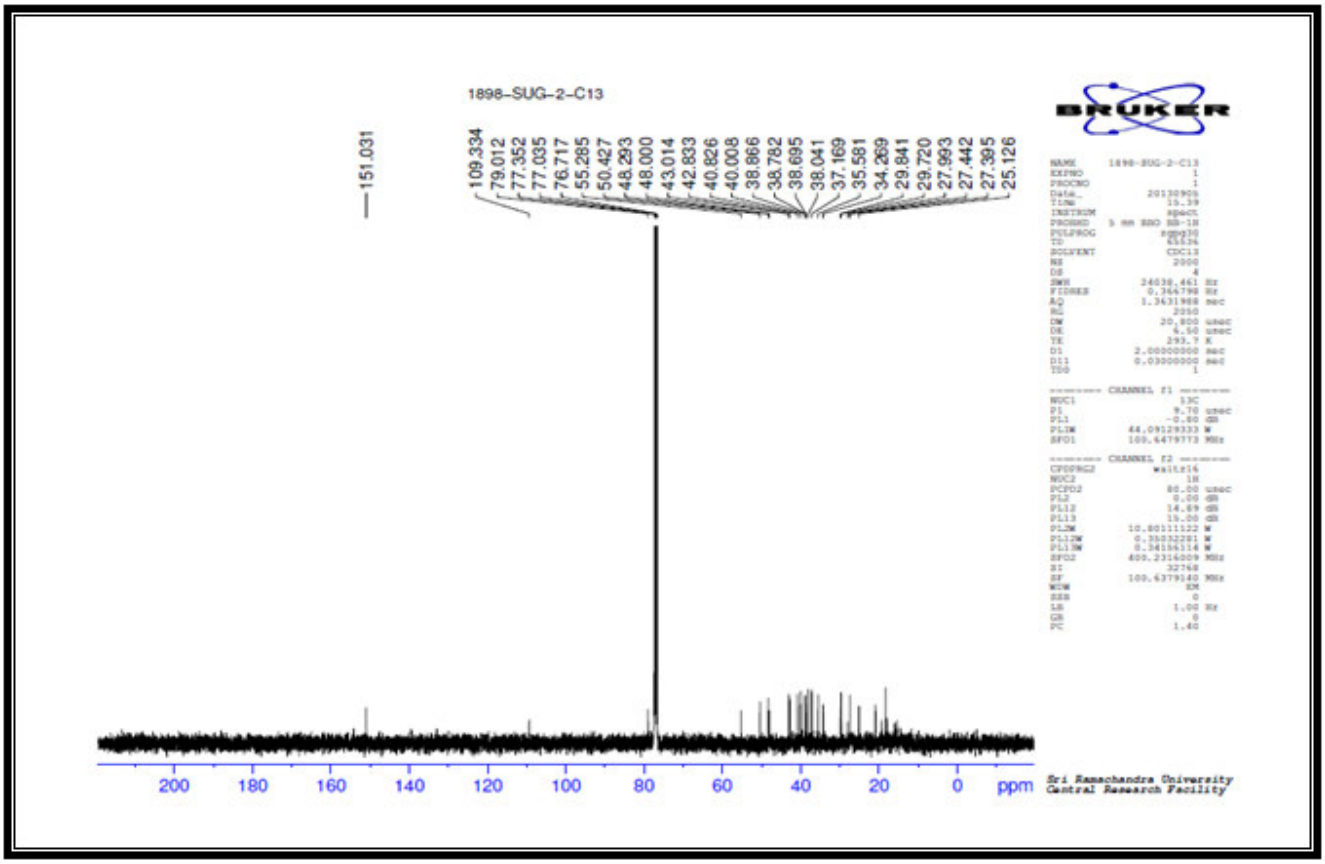
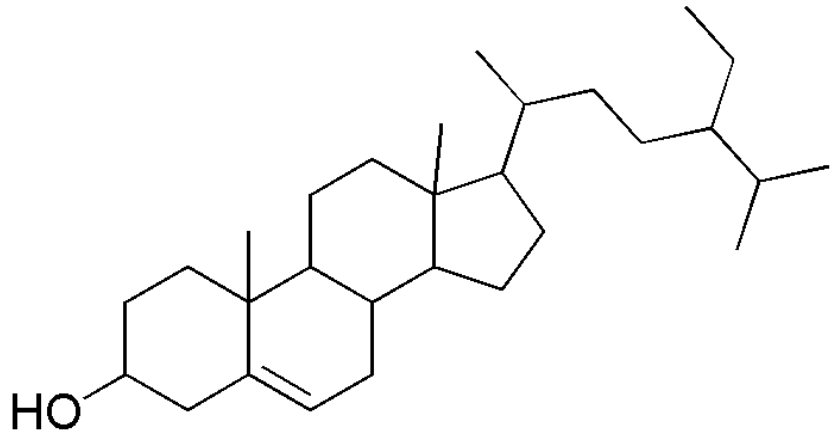


Figure VII
Structure Elucidation of Compound 2 – Phytosterol derivative
Probable Structure: Phytosterol derivative



17-(4-Ethyl-1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-ol

CONCLUSION

The goal of ethnopharmacology is to preserve the cultural heritage by documenting information on medicinal plants and their isolates compounds[13]. Medicinal plants used in the folk medicine may be an interesting and largely unexplored source for the development of potential new compounds[14]. But it is necessary to isolate the active principles and characterized their constituents for the beneficial of human being. In the present study, three compounds were isolated and characterized from the medicinal plant Derris trifoliata. The plant kingdom offers a way of hope because of its enormous chemical diversity[15]. Several known anticancer drugs have been derived from medicinal plants and some of these include vincristine, vinblastine and taxol[16]. These compounds were isolated for the first time from this plant and the literature review revealed that leaf extract of Derris trifoliata has much biological activities including stimulant, antispasmodic, counter-irritant, rheumatism, chronic paralysis, and dysmenorrheal, laxative, carminative and anti-arthritis agent [6]. This attempt of phytochemical investigation from *Derris trifoliata*, further isolation and purification of other fractions of this plant is recommended which could yield some novel and bioactive compounds.

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