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Spectral Studies of *Indigofera trita* L.F.

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Abstract

The present investigation was carried out to analyse the chemical nature of *Indigofera trita* using UV-VIS spectroscopy, Fourier Transform Infrared Spectroscopy, Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR), High Performance Liquid Chromatography and Gas Chromatography Mass Spectroscopy. The results of FT-IR studies confirmed the presence of primary and secondary amine and amides, alcohol, phenols, carboxylic acid, alkanes, alkyne, oximes and aldehyde. GC-MS analysis provides 9 compounds of this four are therapeutically potent. The major identified compounds are Octadecanoic acid (43.67%), 8-Carbethoxy-1,4,5,6,7,8-hexahydropyrrolo[2,3-b]azepin-4-one-3-carboxylic acid (30.5%), 4H-1-Benzopyran-4-one,3,5,7-trihydroxy-2-phenyl (18.6%). HPLC chromatogram of the isolated amorphous compound showed the presence of alkaloid (Choline) with single peak in specific retention time (5.134). The results of the present study indicated *I. trita* contains medicinally important compounds which are traditionally used for various diseases.

Keywords: GC-MS, HPLC, *Indigofera trita*, Leguminosae, Octadecanoic Acid, Phytoconstituents

1. Introduction

India has rich wealth of medicinal plants. Usually medicinal plants are widely used by all community either raw as folk medicines or different forms of medicine. Traditional information has been explained as a total body of knowledge, belief and practice, evolving by adaptive methods and transferred through generation to generation by traditional fest transmission[1]. Traditional system of medicine is used all over the globe as it mostly dependent on locally available plant sources[2].

As per WHO, nearly 65-80% of the world's populations in almost all part of the world, lack of knowledge and poverty about modern system of medicine, they depend lot for their preliminary healthcare need[3]. Plants synthesize a variety of phytochemicals and accumulate in their cells such as alkaloids, flavonoids, tannins, phenols and saponins[4]. These plant products occupy an important place in drug production in the pharmaceutical industries.

Indigofera trita is most important medicinal herb of Leguminosae. This family comprising 700 species of annual herbaceous or perennial shrubs and are widely distributed in tropics and subtropical areas of India, North Australia, Ceylon and South Africa. In Tamil language this is known as Kattuavuri and Punalmurukai. The plant seeds are used as nutritive tonic[5]. The plant also possesses strong antioxidant[6,7], hepatoprotective activity[8], anti-inflammatory and analgesic[9]. The whole plant is traditionally taken as different ailments it includes liver disorders and tumours[10]. The whole plant used for the treatment of diarrhoea, chest and body pains[11,12].

The main aim of the present investigation through spectral studies is a progress stone to undertake considerable work at a large scale in this direction.

The main objectives of the studies are scientific standardization of phytoconstituents through extraction, fractionation, separation and identification of novel components from *Indigofera trita*. Also to screen the

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bioactive nature of identified novel compounds which may be recommended to pharma industries.

2. Materials and Methods

2.1 Plant Collection

The study plant *Indigofera trita*, is collected from Viruthachalam, Cuddalore District, Tamil Nadu. The study material was taxonomically identified with the help of floras[13,14] and BSI, Southern region, Coimbatore, Tamil Nadu. The Herbarium number provided by BSI is "BSI/SRC/5/23/2016/Tech/1566". The specimen is maintained in the Herbarium of Vellalar College for Women (Autonomous), Erode 638 012, Tamil Nadu, South India.

2.2 Isolation

The shade dried entire plant powder (1000 gm) was extracted using ethanol 80% through Soxhlet apparatus. The obtained extract was diluted and then concentrated by *vaccuo* to dryness at 35 °C, with this (90 g) was partitioned then using H_2O and ethyl acetate, in which water portion was basified using ammonium hydroxide (pH 9) and again extracted repeatedly using chloroform. Then with the help of *vaccuo* the combined chloroform layer was concentrated to dryness. Then obtained concentrate (32 g) was subjected to column chromatography in four fractions of hexane:chloroform (95:05) increasing the polarity gradient elution (90:10, 85:15, 80:20), from this hexane:chloroform (80:20) fraction was allowed to concentrate in *vaccuo* at 35°C and subjected to TLC[15]. The plates of thin layer chromatograpy were prepared by using silica gel-G with the mesh size of 60 as absorbent. 15 grams silica gel-G was dissolved in 30 ml of distilled water (1:2) to makes slurry. The prepared slurry was poured immediately into TLC plates. Then the plates were allowed to dry for one hour and the plates were fixed by drying at 110°C for 1½ hours. About 10 µml of extracts were loaded gradually using a micropipette, over the plates and allowed to air dried. The entire setup was developed with the solvent system as (hexane/chloroform (80:20). Then the plates were allowed to dry and exposed to iodine balls. The Rf value of the developed system was calculated by using the formula (Rf:Distance travelled by solute/Distance travelled by solvent) and was documented. Single spot at Rf 0.62 in TLC finger print from hexane:chloroform fraction (amorphous substance) was collected (4 g) and was used for present spectral studies as UV-VIS spectroscopy, Fourier Transform Infrared Spectroscopy, Nuclear Magnetic Resonance (^1H and ^{13}C NMR), High Performance Liquid Chromatography and Gas Chromatography Mass Spectroscopy.

2.3 UV-VIS Spectroscopic Studies

The UV-VIS spectrum of identified compound was analysed by using Elico SL-159 Double beam UV-VIS spectrophotometer[16]. UV-VIS spectra were recorded in the region 200-1100 nm. The UV light region is scanned normally over the range 200-400 (nm) and the visible range is from 400-800 nm. During a short period, the spectrophometer scansed automatically all the components of wavelengths and the peak values were recorded.

2.4 FT-IR Spectroscopic Analysis

Fourier Transform-Infrared Spectroscopy (FT-IR) analysis was done using Perkin Elmer, Germany Vertex 70 infrared spectrometer was used to analyse the peaks obtained and functional groups. A pinch of amorphous substance was mixed with dry potassium bromide (KBr). Later it was thoroughly mixed with mortar and pressed for 2 min at a pressure of bars to form a KBr thin disc. Then the pellet was placed in a sample holder of a diffuse reflectance accessory for scanning of sample from 400 to 4000 cm^{-1} and was recorded[16].

2.5 Nuclear Magnetic Resonance

^1H and ^{13}C NMR spectrum of isolated amorphous substance were in a NMR-400 and chemical values (shifts) were recorded as δ values. The obtained spectra were compared with the standard chart and available functional groups present in the sample were determined[17]. The amorphous purified sample was kept in inert solvent deutro chloroform (CD Cl_3) and the solution was placed in between the poles of the powerful magnets. The various chemical shifts of the protons based on molecule environment within the same molecule were analysed in the NMR equipment relative to a standard TMS (Tetra Methyl Silane). The chemical shifts were measured and expressed in ppm units.

2.6 HPLC

The High Performance Liquid Chromatography was performed by (Shimadzu, Japan; Pump: LC-20AT; Detector: SPD 20A) by using a Luna C18 (250 × 4.6 mm, particle size 5 µm, Phenomenax) column[18], acetonitrile (flow rate 20 µl/20 min) as mobile phase and peak detection at 220 nm confirmed their purity.

2.7 GC-MS studies

Gas Chromatography Mass Spectroscopy studies was performed in therma GC-Trace Ultra ver:5.0 and a GC interface to a Mass spectrometer equipped with a thermo MS DS Q II fused a DB35-MS capillary standard non-polar column (30 x 0.25 µm ID x 0.25 µm df). For detection, an impact mode with ionization energy of 70 ev. Helium was used as a carrier gas at a constant flow rate of 1 ml⁄min, and an injection volume of 1 µm was employed (a split ratio of 10:1). The temperature of the injector was maintained at 250^{0}C, the oven temperature was maintained from 150^{0}C for 2 min, then increased to 5^{0}C⁄min to 220^{0}C, then 10^{0}C⁄min to 260^{0}C, finally isothermal at 350^{0}C was provided at 20 min. At 70 ev the Mass Spectra were taken. The scan interval of 0.5 sec and fragments from 50 to 650 Da. The delay of solvent was 0 to 4 min and the GC-MS total running time was 37.50 min. The relative percentage and amount of each component was calculated by its average peak area and were compared to the total areas[19,20].

3. Results

3.1 UV-VIS and FT-IR Analysis

UV-VIS profile showed the wavelength at 344 and 269 with the absorption of 0.010 and 0.032 respectively (Table 1 and Figure 1). FT-IR peaks and functional groups are given in Table 2 and Figure 2. The major peak at 3545.32 cm^{-1}, 3409.33 cm^{-1} signifying the presence of N-H stretch, 2876.95 cm^{-1} indicating the presence of (O-H) carboxylic

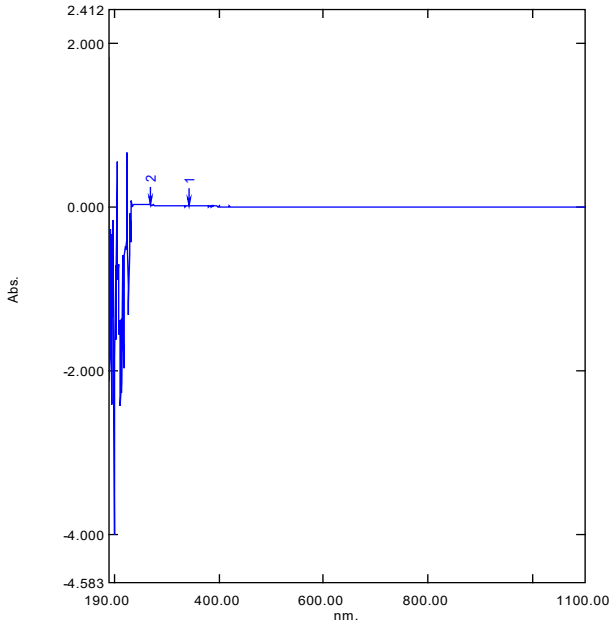

Figure 1. UV- Visible spectrum ofisolated amorphous substance.

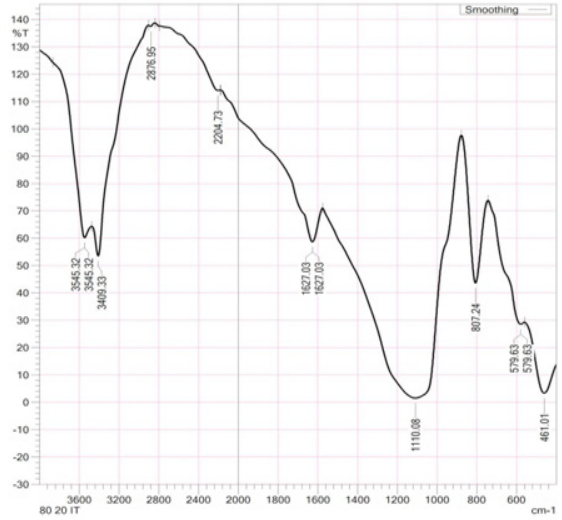

Figure 2. FT-IRspectrum of isolated amorphous substance.

Table 1. UV- VIS peak values ofisolated amorphous substance

S. No.	Wavelength (nm)	Absorption.
1	344.00	0.010
2	269.00	0.032

Table 2. FT-IR peak values and functional groups ofisolated amorphous substance

S.No.	Peak values (cm^{-1})	Functional groups	Bond	Wavelength	Intensity
1	3545.32	Primary and secondary Amine and Amides	N-H Stretch	m	60.18
		Alcohol, Phenols	O-H Stretch	s	
2	3409.33	Primary and secondary Amine and Amides	N-H Stretch	m	53.55
3	2876.95	Aldehyde	C-H Stretch	s	137.47
		Carboxylic acid	O-H	m	
4	2204.73	Alkyne	C≡C	m-w	114.01
5	1627.03	Alkene	C=C	m-w	58.63
		Primary and secondary Amines and Amides	N-H bend	m-s	
		Ketone	C=O Stretch		
6	1110.8	Alcohols, Ethers, Esters, Carboxylic acid, Anhydrides	C-O Stretch	s	1.52
		Amines	C-N	m-s	
		Fluoride	C-X	s	
7	807.24	Aromatics	C-H Bend out of plane	s	43.63
8	576.63	Chloride	C-X	s	28.52
9	461.01	Bromide, Iodide	C-X	s	3.36

acid group, 2204.73 cm^{-1} shows the presence of (C≡C) alkyne. The peak values at 1110.8 cm^{-1} was described the presence of esters, 804.24 cm^{-1} as (C-H bend) aromatics. The FT-IR results confirmed the presence of primary and secondary amine and amides, alcohol, phenols, carboxylic acid, alkanes, alkyne, ketone, ethers, esters, anhydrides, aromatics, bromide, iodide, fluoride and chloride (Table 2 and Figure 2).

3.2 ^{1}H and ^{13}C NMR

^{1}H NMR spectrum showed chemical shifts values at various signals and nature of proton are given in Table 3 and Figure 3. The range from 0.842 to 0.894 ppm indicates the occurrence of methyl proton ($-CH_3$) at shielded environment. The methylene proton R CH_2 R is identified at 1.246 to 1.275 ppm. The singlet at 1.587 ppm showed the allylic (R_3CH). The doublet signal at 2.047

Table 3. ^{1}H NMR spectral data of isolated amorphous substance

S.No	Chemical Shifts	Nature of Proton
1	0.842	RCH_3 (Methyl)
2	0.855	
3	0.867	
4	0.880	
5	0.894	
6	1.246	RCH_2R (Methylene)
7	1.255	
8	1.260	
9	1.275	
10	1.587	R_3CH (Allylic)
11	2.047	$R(C=O)CH_3$ (Ketone) (Glutamate)
12	2.174	
13	4.729	$R_2C=CH_2$ (Vinylic) (Malic acid)
14	7.263	ArH (Aromatic) (Alanine)

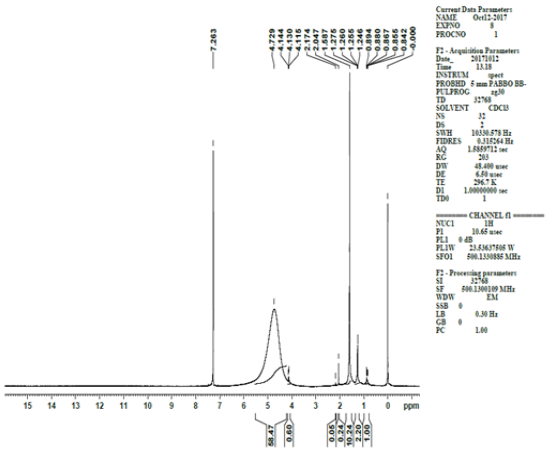

Figure 3. ^{1}H NMR spectrum of isolated amorphous substance.

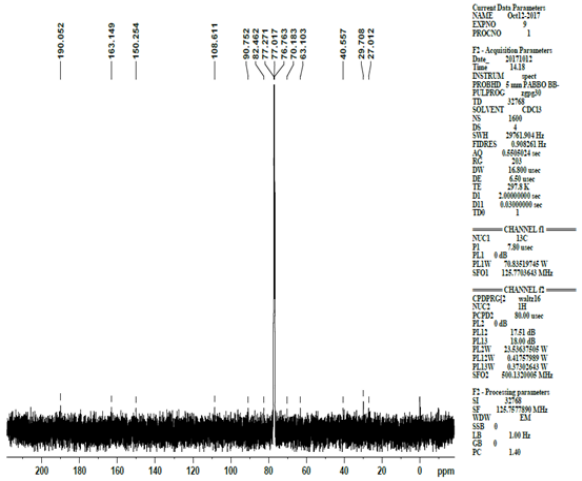

Figure 4. ^{13}C NMR spectrum of isolated amorphous substance.

ppm [R(C=O) CH_3] indicates the presence of ketone. The chemical shifts at 4.729 and 7.263 showed the vinylic nature of proton.

The assignment of ^{13}C NMR spectrum showed in Figure 4 and Table 4. The chemical shift signal at 27.012 to 40.557 indicates the presence of primary, secondary and

Table 4. ^{13}C NMR spectral data of isolated amorphous substance

S.No	Chemical shift	Types of Carbon
1	27.012	Primary, Secondary, Tertiary Alkyl
2	29.708	
3	40.557	
4	63.103	Alkyl halides or amines
5	70.183	Alcohol or ethers or alkynes
6	76.763	
7	77.017	
8	77.271	
9	82.462	
10	90.752	
11	108.611	Alkenes or aryls
12	150.254	Alkenes or aryls or amides
13	163.149	
14	190.052	Aldehydes, ketones

tertiary alkyl. The chemical shifts at 63.103 ppm indicate the presence of alkyl halides. The signals from 70.183 to 90.752 ppm indicate the presence of alcohol or ethers or alkynes. The occurrence of alkenes at 108.611 to 163.149 ppm. The singlet signal at 190.052 ppm represents the aldehydes and ketones.

3.3 HPLC

HPLC chromatogram of the isolated amorphous substance showed presence of alkaloid (Choline) with single peak at specific retention time 5.134 (Figures 5 and 6 and Table 5). Since, RT obtained for our sample matched with that of choline (based on the literature

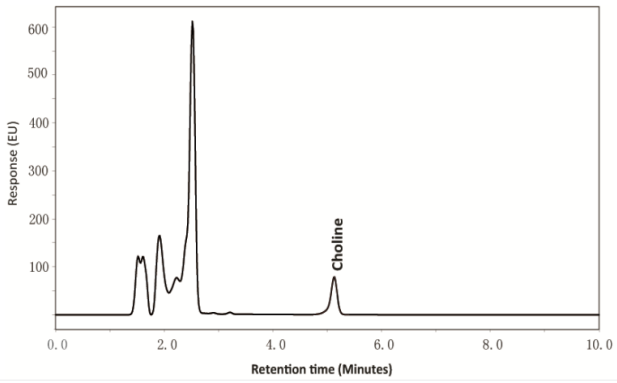

Figure 5. HPLC chromatogram of isolated amorphous substance.

Table 5. HPLC finger printing ofisolated amorphous substance

Peak	Ret.Time	Area	Height	Area %	Height %	Conc.	Units
1	5.134	12458445	1252587	100.000	100.000	0.000	mg/L
Total		12458445	1252587	100.000	100.000		

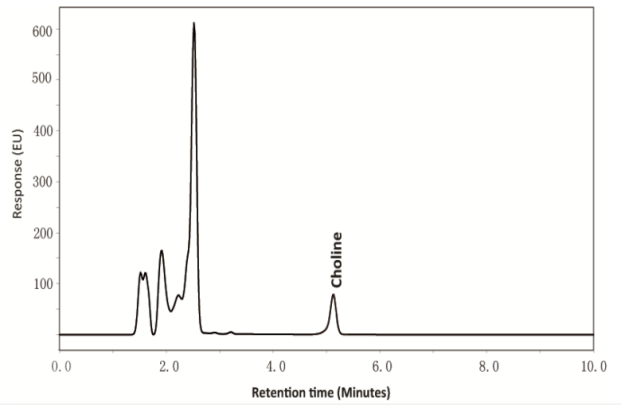

Figure 5a. HPLC chromatogram of Choline.

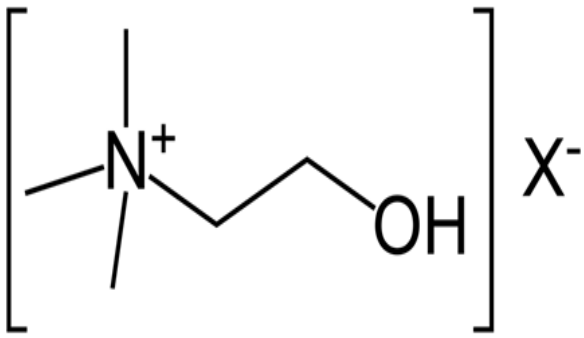

Figure 6. Structure of Choline.

Table 6. GC-MS analysisofisolated amorphous substance

S.No	Name of the compound	Retention Time	Molecular Formula	Molecular Weight	Area %
1	Octadecanoic acid	16.65	$C_{18}H_{36}O_2$	284.00	43.6
2	8-Carbethoxy-1,4,5,6,7,8-hexahydropyrrolo[2,3-b]azepin-4-one-3-carboxylic acid	18.27	Unknown	280.95	30.5
3	4H-1-Benzopyran-4-one,3,5,7-trihydroxy-2-phenyl	16.05	$C_{15}H_{10}O_5$	270.00	18.6
4	10-Octadecenoic acid, methyl ester	17.72	$C_{19}H_{36}O_2$	296.00	7.6
5	Dasycarpidan-1-methanol,acetate(ester)	19.1	$C_{20}H_{26}N_2O_2$	326.00	7.2
6	Ethanone,4-[2,4-dihydroxybenzylidenamino]phenyl	10.18	Unknown	254.89	6.3
7	Coumarine,3-[2,4-dinitrophenyl]	20.32	$C_{15}H_8N_2O_6$	312.00	5.4
8	2-Pyridinecarboxamide,N-[2-(3-methoxy-1-propynyl)phenyl]	13.12	Unknown	265.75	3.9
9	1,3-Benzodioxole,5,5′-(tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diyl)bis-,[1S-(1a,3aa,4a,6aa)]	21.78	$C_{20}H_{18}O_6$	354.00	3.6

survey[21]) we confirmed that our compound was choline. The HPLC chromatogram of choline is given in Figure 5a which matches the RT with the extract.

3.4 GC-MS Analysis

The results of the present studies are presented in Figure 7 and Table 6. Totally the fraction yielded 9

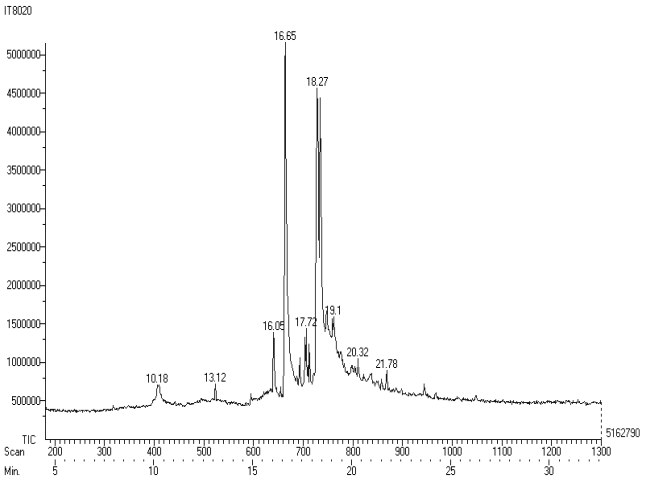

Figure 7. GC-MS analysis ofisolated amorphous substance.

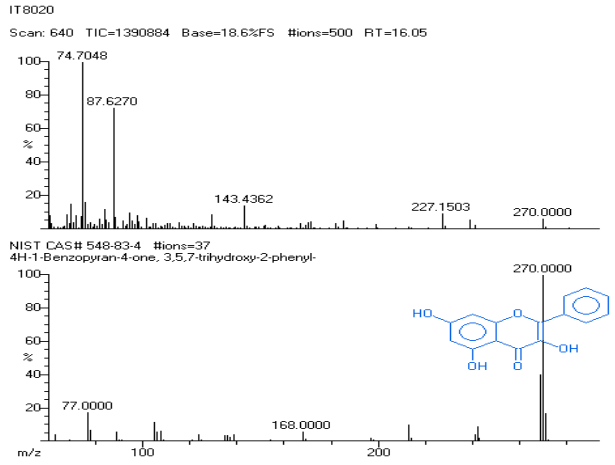

Figure 7a. Ma Octadecanoic acid.

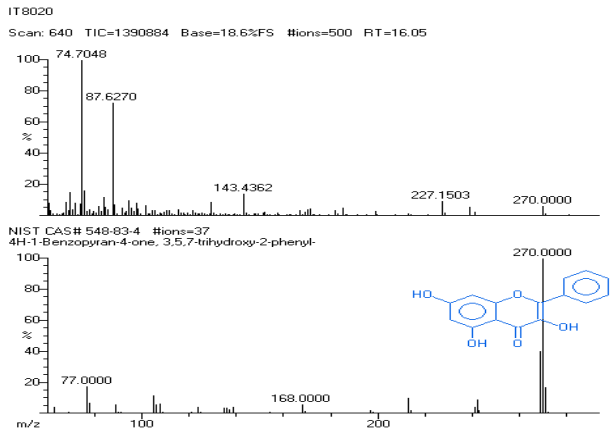

Figure 7b. Mass spectrum of 10-Octadecenoic acid, methyl ester.

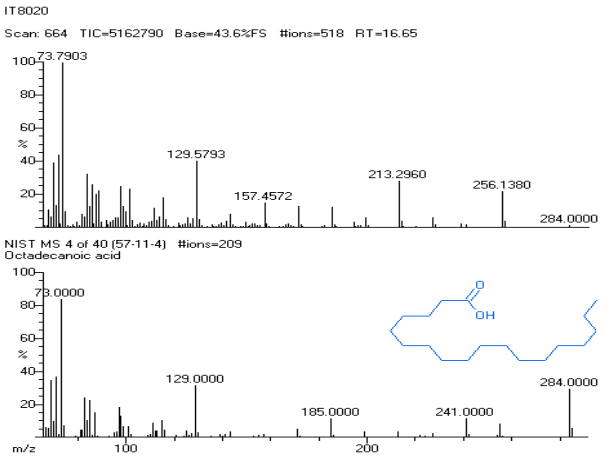

Figure 7c. Mass spectrum of 10-Octadecenoic acid, methyl ester.

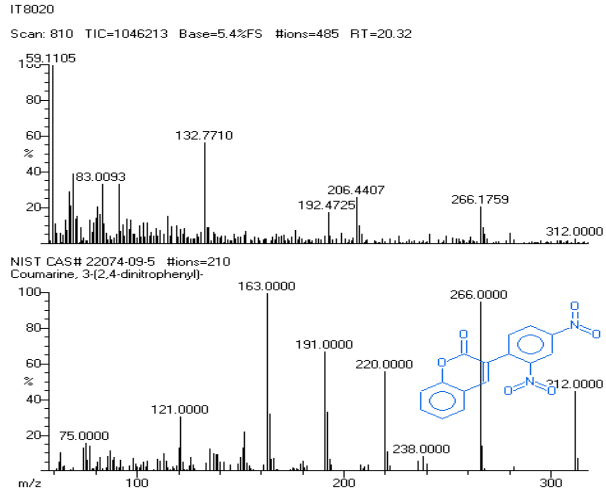

Figure 7d. Mass spectrum of Coumarine, 3-[2,4-dinitrophenyl]

chemical constituents. The major phytoconstituents are Octadecanoic acid; 8-Carbethoxy - 1,4,5,6,7,8 - hexahydropyrrolo [2,3-b] azepin -4-one-3-carboxylic acid; 10 - Octadecenoic acid, methyl ester; 4H-1-Benzopyran – 4 - one,3,5,7 – trihydroxy -2- phenyl; Dasycarpidan-1-methanol,acetate(ester); Ethanone,4-[2,4-dihydroxybenzylidenamino]phenyl; Coumarine, 3- [2,4- dinitrophenyl]; 2- Pyridinecarboxamide, N- [2-(3-methoxy -1- propynyl) phenyl]; 1,3- Benzodioxole, 5, 5′ - (tetrahydro -1H, 3H- furo [3,4-c] furan-1, 4-diyl) bis-, [1S-(1a,3aa,4a,6aa)]. The identified phytoconstituents are given in their Molecular Formula (MF), Molecular Weight (MW) Retention Time (RT) and concentration (%). The

spectrum of GC-MS biologically active compounds is given in Figure 7a-7d and Table 7.

4. Discussion

A lot of advanced techniques can be used to detect and analyse the presence of phytoconstituents. Chromatography is an important analytical technique concern with the separation of relatively allied components from a mixture[22]. The Infra Red spectrum of isolated amorphous compound possesses absorption bands due to carboxylic acid at 2876.95 cm^{-1} and 807.24 cm^{-1} showed aromatic nature[23]. The FT-IR result confirm the occurrence of peak at 3409.73 cm^{-1} reveals that the absorption bands are due to the presence of polyhydroxy compound and its derivatives in the *Bryonopsis laciniosa* fruit[24]. The chemical shift at 0.84 to 0.89 indicates the presence of methyl. The above obtained spectral features are closely resembled for β- sitosterol[25&26]. Alkaloids are a group of important naturally occurring organic compound that are basically contains nitrogen atoms. Alkaloids show a wide range of pharmacological properties. Many alkaloids are still used in medicine. Choline is a water soluble vitamin like essential nutrient[27]. Commonly Choline is used for liver disease including cirrhosis and chronic hepatitis. It is also used for depression and memory loss[28] which is reported in HPLC of the present study. This is also reported in *Canola*[29]. GC-MS result shows the occurrence of therapeutically important nine compounds (Table 5). The result is in agreement with[30], who reported that, the ethanol extract of *Phyllanthus vasukii* that the same compounds like Dasycarpidan-1-methanol, acetate (ester). The similar compounds 10, octadecenoic acid, methyl ester were studied on the active principles in the *Jatropha curcas*[31]. Previous studies on *Indigofera aspalathoides* and *Albizia adianthifolia* contains the octadecanoic acid which are concordance with the present spectral study[32&33]. Medicinal herbs used by the human beings are interesting and important and are mostly unexplored resource to develop potential new compounds. But it is very important to isolate and identify the active constituents. The present results of the spectral study will be useful in the identification of new compounds which are biologically important.

5. Acknowledgements

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