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SPECTROSCOPIC ANALYSIS OF FRACTIONS OF *TABERNAEMONTANA DIVARICATA* (LINN) R.Br LEAF

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ABSTRACT

Tabernaemontana divaricata a common garden plant in tropical countries has been used as a traditional medicine. It has wide-ranging biological activities especially due to the alkaloidal and non-alkaloidal and phytosteroidal constituents. The beneficial properties of *Tabernaemontana divaricata* are antioxidant, anti-infection, anti-tumour action, analgesia, anti-inflammatory, antifertility and the enhancement of cholinergic activity in both peripheral and central nervous systems. The augmentation of cholinergic function may be of therapeutic benefit for many neurodegenerative diseases, particularly myasthenia gravis and Alzheimer's disease. During the study leave of the plant are shade dried and converted into coarse powder which further subjected to extraction by soxhlation process using petroleum ether and ethanol. The obtained crude extract further subjected to fractionation and the fractionated extracted was further analysed by different spectroscopy methods.

KEYWORDS: *Tabernaemontana divaricata*, Phytochemical study, Spectroscopy study, Alkaloids, non-alkaloids.

1. INTRODUCTION

Plants are well known as a major source of modern medicines. From ancient times, humans have utilized plants for the treatment or prevention of diseases, leading to the dawn of traditional medicine. *Tabernaemontana* is one of the genera that is used in Chinese, Ayurvedic and Thai traditional medicine for the treatment of fever, pain and dysentery[1,2]. Tabernaemontana plants are widely distributed in Thailand. Species found in Thailand are T. *bufalina, T. crispa, Tabernaemontana divaricata, T. pandacaqui, T. pauciflora* and T. rostrata[3,4]. One of the most interesting species is *Tabernaemontana divaricata* (L.) R. Br. Ex Roem. & Schult. (Synonym: *Ervatamia coronaria, Ervatamia microphylla, Ervatamia*

divaricata, T. coronaria). Growing evidence suggests that this plant has medicinal benefits and its extracts and fractions could possibly be used as pharmacological interventions in various diseases. Already several scientific papers have been published on *Tabernaemontana divaricata* (Linn) R.Br. and used traditionally in folk medicine as thermogenic, anodyne, astringent, vermifuge, anti-inflammatory[5], anthelmintic, emmenagogue, aphrodisiac, tonic to the brain, liver, spleen[6]and advocated for family planning[7]. *Tabernaemontana divaricata* (Linn) R.Br. reported to contain phytochemical constituents such as, Flavonoids, Steroids, Alkaloids, Tannins and others.[8] The present study is to determine detailed phytochemical constituents in the leaf of *Tabernaemontana divaricata* (Linn) R.Br by spectroscopic analysis.

1.1 About Plant:*Tabernaemontana divaricata* (Linn) R. Br.

- **Synonyms**: Ervatamia coronaria (Jacq)
    - Ervatamia divaricata (Linn)
    - Tabernaemontana coronaria (Jacq)
- **Family:** Apocynaceae.
- **Distribution and Habitat**
    - It is found in Tropical Asia, Australia, and Polynesia. In India it is found at Upper Gangetic Plain, Garhwal, E. Bengal, Khasia Hills, Assam, Burma, N. Circars, hills of Visakapatnam. It is cultivated as an ornamental plant grows wild in hedges and shady forests[6, ,9].
- **Description**
    - A glabrous, evergreen shrub 1.8-2.4 m in height with silvery grey bark and milky latex; leaves are simple, opposite, elliptic or elliptic-lanceolate, smooth, glossy green, acuminate and wavy margins; flowers are white, sweetly fragrant in 1-8 flowered cymes at the bifurcations of the branches, lobes of corolla overlapping to right in the bud; fruits follicles are 2.5-7.5 cm long, ribbed and curved, orange or bright red within narrowed into a slender curved beak; seeds are dull brown, minutely pitted, irregular, enclosed in a red puply aril[5].
- **Common names**[5]
    - Eng : East Indian rosebay
    - Hin : Chandni
    - Kan : Kottubale, Nandibatlu
    - Mal : Nantyarvattam
    - San : Nandivrksha
    - Tam : Nantiyavattam
    - Tel : nandivardhanamu
- **Chemical constituents present in different parts of the plant[10,11,12]**
- **Seeds: Citric**, oleic, palmitic acids and coronaridine.
- **Latex:** unidentified amino acids, milk-clotting and proteolytic enzymes, two proteins, bacteriolytic enzyme, galactose and glucose.
- **Flowers:** Dregamine, 20-epiervatamine, tabernaemontanine, vobasine, voacangine, voacamine, flavonoid aglycones, flavonol glycosides; isovoacristine, voaphylline-hydroxyindolenine, janetine (tetrahydrolivadine), N-methyl-voaphylline (hecubine). Kaempferol and apparicine, tabersonine, 3,4: 4,19-tetrahydroolivacine.
- **Twig:** Unidentified amino acids, milk-clotting and proteolytic enzymes, galactose and glucose.
- **Leaves :** Dregamine, 20-epiervatamine, tabernaemontanine, vobasine, voacangine, voacamine, flavonoid aglycones, flavonol glycosides, isovoacristine, α-amyrin, lupeol

and their acetates, β-sitosterol, coronaridine, appericine, ervaticine (2-acyl indole derivative), ervatinine, hyderabadine, lochnoricine, mehranine, stapfinine, voacristine, voharine and a dimeric alkaloid, conophylline and aspidosperma alkaloids, taberhanine, voafinine, N-methylvoafinine, voafinidine, voalenine, conophyllinine, conofoline[13] voaphylline, N-methylevoaphylline, kaempferol, salicylic, P-hydroxybenzoic, protocatechuic, vanillic , syringic and sinapic acids, quercetin.

- **Stem:** Alkaloids: coronaridine, heyneanine, voacristine, voacamine, descarbomethaoxyvoacamine, bisindole alkaloid, 19,20-dihydroervahanine A and Phenolic acids: vanillic, gentisic, syringic, 4-hydroxybenzoic and salicylic acid and dregamine, tabernamontanine, vobasine, (-) – ibogamine, voacangine, isovoacagine[11]
- **Stem bark:** α-amyrin, lupeol and their acetates, β-sitosterol, Ibogamine, isovoacangine, voacangine, 19-epi-voacangine, 11-methoxy-N-Me-dihydroperi cyclivine and an isomer of voacamine.
- **Roots:** Tabernamontanine, vobasine, D-mannitol.
- **Root bark:** Amino acids, a bacteriolytic enzyme, proteins, galactose (latex), coronaridine-hydroxyindole-nine, 3-oxo, 5-oxo-, 6-oxo, 5-hydroxy-6-oxo- and (±) –19-hydroxy-coronaridines, pseudovobparicine (dimeric indole alkaloid); aurantiamide acetate, benzoic acid, campesterol, cycloartenol, ibogamine and (+) – heyneanine, (-) – heyneanine, voacamine, α-amyrine and lupeol and their acetates, β-sitosterol, palmitic, oleic and linoleic acids.
- **Plant:** Olivacine, heyneanine, 19S-heyneanine- hydroxyindolenine, 3-oxo-, 19-oxo- and 19-(2- ketopropyl)-coronaridines, 3-oxo-vaacangine, voacangine-hydroxyindolenine, voacristine-hydroxyindolenine; caoutchouc, glycoflavones, leucoanthocyanins, gentisic and sinapic acids; resin, sugars and cycloart-23-ene-3β25-diol, 3- - hydroxycycloart – 25 – ene – 24 – one, cycloeucalenol, β-amyrin acetate, 3,8-ervatamine[14]

2. MATERIAL AND METHOD:

2.1 Material

Leaves of *Tabernaemontana divaricata* (Linn) R. Br collected from the surrounding of Bhainsa. The authentication done by Botanist and Department of Pharmacognosy. **Voucher specimen A75** deposited at the museum of college. Chemicals used during study were purchased from sigma Aldrich Mumbai, India.

2.2 Methods

2.2.2 Preparation of Extracts:

The leaves of *Tabernaemontana divaricata* (Linn) R.Br. collected and shade dried. The dried leaves coarse powdered and the powder packed into Soxhlet column and extracted successively with pet ether (60-80 C) and ethanol (60 C) to get sufficient crude extracts and stored in air tight container at 10 C. Preliminary phytochemical screening done.[15,16]

2.2.2.1 Fractionation of Ethanolic extract by Column chromatography: The crude ethanolic extract of the fresh leaves of the plant subjected to careful column chromatography on silica gel. Elution carried out with increasing polarities of petroleum-ether (40-60), chloroform and ethanol. The fraction obtained on elution with chloroform and 95% chloroform-ethanol afforded a mixture of alkaloids which were further purified by preparative TLC.[17]

2.2.2.2 Fractionation of Petroleum ether extract by Column chromatography: This fractionation was conducted by using wet column chromatography. Mixture of n-hexane: ethyl acetate (4:1) was used as mobile phase (eluent) while gel silica 60 was used as

stationary phase. The bottom of the column was filled with glass wool/cotton and eluent. Diameter of column was 1.5 cm and flow velocity for elution was 1.5ml/min.

2.2.2.3 Preparation of stationary phase: 10 g of gel silica was activated at 110 C for 2hr, then cooled for 15 min in desiccator. Gel silica was added eluent and homogenized to form slurry. Gel silica slurry was put in treated column for 24h. Ethyl acetate fraction (0.1g) was diluted in 1 ml of eluent. The sample was load in the treated column for elution process. Every 2ml of eluate was collected in a vial as a fraction.

UV, FTIR, and GCMS studies carried out for Ethanolic and Pet ether fractions in association with University College of Technology Osmania university Hyderabad.

2.2.2.4 Monitoring of separation by TLC: The separated fractions were monitored using TLC. A mixture of n-hexane: ethyl acetate (4:1) of eluent was used for the monitoring. A 10x10 cm gel silica was activated for 30 min and the eluent was saturated in a container for 1 h.

Each fraction was spotted to the activated plate, afterward the plate was eluted. The stains in the plate were observed under UV at 254 and 366 nm after spraying with Leiberman-burchard reagent. The Rf value was calculated and the isolates' contained steroids were gathered and evaporated it.[18]

3. RESULTS

3.1 Preliminary phytochemical screening

Preliminary phytochemical screening was carried out for the presence of carbohydrate, proteins, amino acids, steroids, saponins, flavonoids, alkaloids, tannins and glycosides for petroleum ether and ethanolic extracts of leaves of *Tabernaemontana divaricata* (Linn) R.Br. Results are shown in Table.

Table 1: Preliminary phytochemical screening of*Tabernaemontana divaricata* (Linn) R.Br. leaves

Phytochemical constituents	Ethanolic Extract	Petroleum ether extract
Carbohydrates	+	--
Proteins	--	--
Amino acids	+	--
Steroids	**+**	**+**
Glycosides	+	--
Flavonoids	+	--
Alkaloids	+	--
Tannins	+	--

-- absent + present

Table 2: Percentage yield of crude extracts of *Tabernaemontana divaricata* (Linn) R.Br. leaves.

Sl. No.	Solvent	Colour and Consistency	Percentage yield
1	Petroleum Ether (60-80°C)	Blackish and sticky	7%
2	Ethanol	Blackish green and pasty	23%

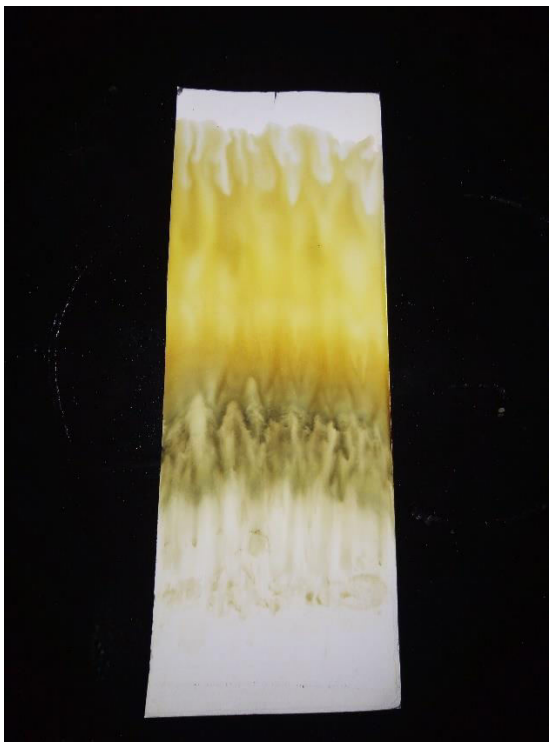

Figure 1: pTLC upper fraction shows Isolation ethanolic fraction.

Steroids: Steroids are active compound bind naturally polar group of glycosides. The polar group of glycoside makes steroids easy to extract with methanol. Hydrolysis is one of the steps in extraction to break glycoside bonds between steroids and glycosides compounds. This process was conducted by addition of HCL to disrupt the bonds and neutralization with sodium bicarbonate to stop hydrolysis reaction. The hydrolysed product was partitioned by petroleum ether solvent. Greenish black concentrated product was obtained[18].

3.2 SPECTROSCOPIC RESULTS

3.2.1 Petroleum Ether Fraction: The petroleum ether fraction obtained on continuous Soxhlet extraction wit 60% Petroleum ether for 24 hours which was subjected to spectroscopic studies. The steroids are predominant compounds in the extract and can be seen in the UV spectrum showing absorption in the sequence of the peaks ranging from 1 to 86. The serial number (1) in the spectrum corresponds to 1067.60nm lambda max with absorbance 0.007 and minimum 191.90nm with absorbance -0.779are the values in the UV spectrum.

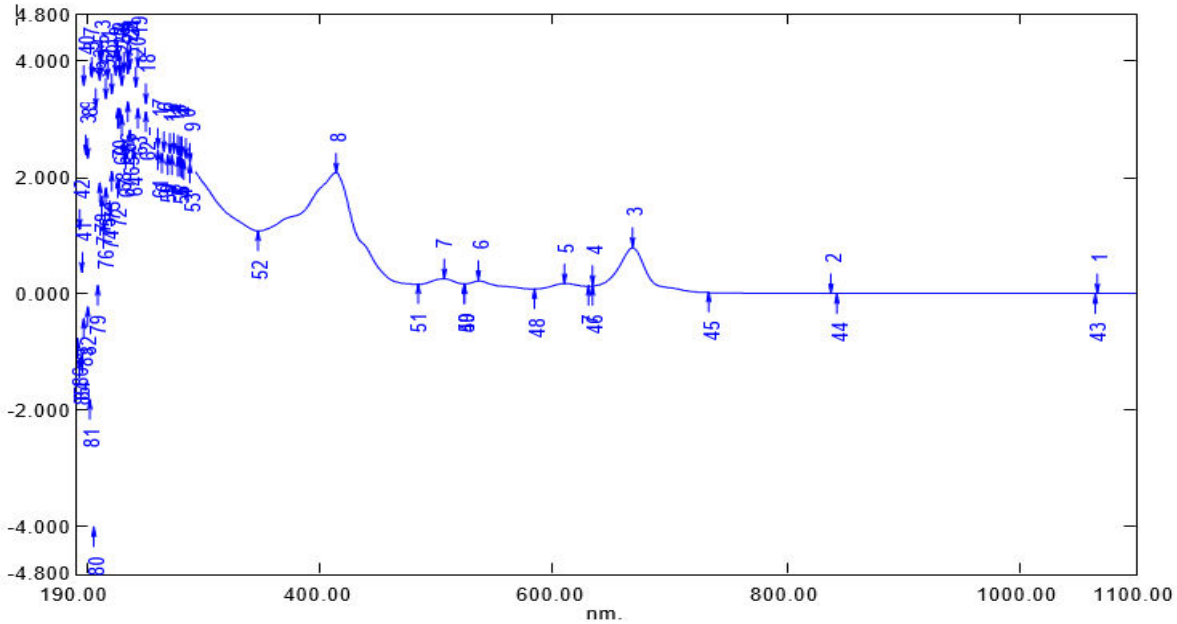

Fig. 2: Petroleum ether fraction UV spectroscopy result

3.2.2 Ethanolic Fraction: The ethanolic fraction obtained on elution with chloroform and 95% chloroform-methanol afforded a mixture which was subjected to spectroscopic studies. The indolic alkaloid is predominant alkaloid in ethanolic fraction and can be seen in the UV spectrum showing absorption maxima at 284nm in the sequence of the peaks ranging from 1 to 233. The serial number 99 in the spectrum corresponds to 284nm lamda max and absorbance 1.661. But the 1094.50nm maximum and 193.50nm minimumare the values in the UV spectrum. The ethanolic fraction obtained on continuous Soxhlet extraction with 90% ethanol for 24 hours which was subjected to spectroscopic studies.

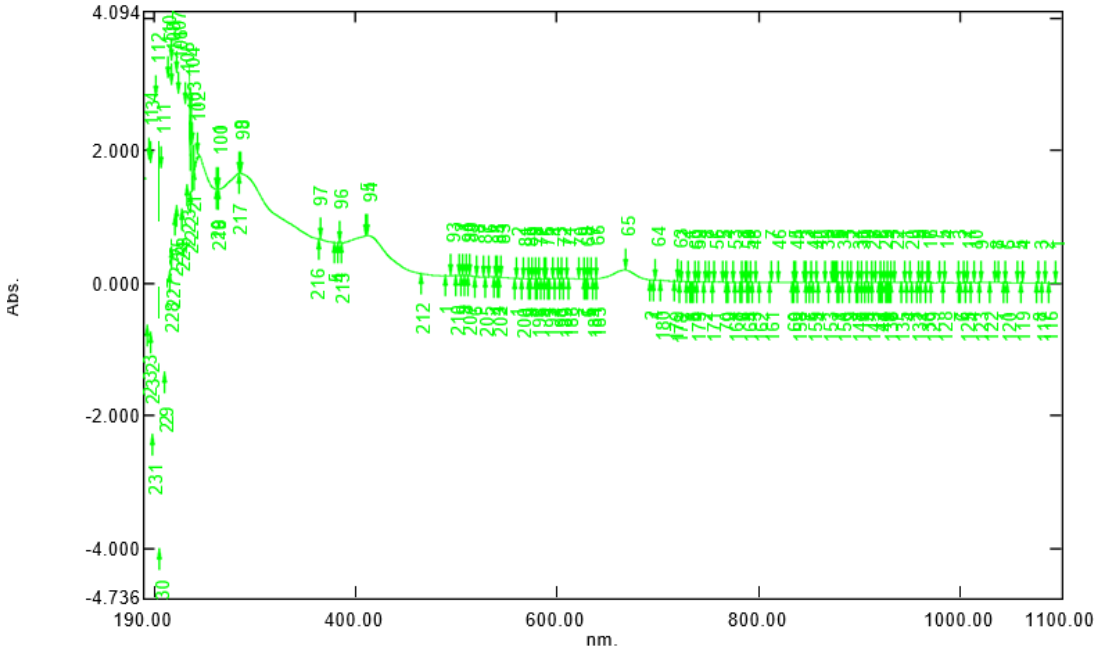

Fig.3: Ethanolic fraction UV spectroscopy result

3.2.3 Petroleum Ether Fraction FTIR: The FTIR spectrum was used to identify the functional groups of the active components present in the fraction based on the peak values in the finger print region of IR radiation[19,20]. When the extract was passed into the FTIR, the

functional groups of the components were separated based on its peak's ratio. The results of FTIR peak values and functional groups representedintable.

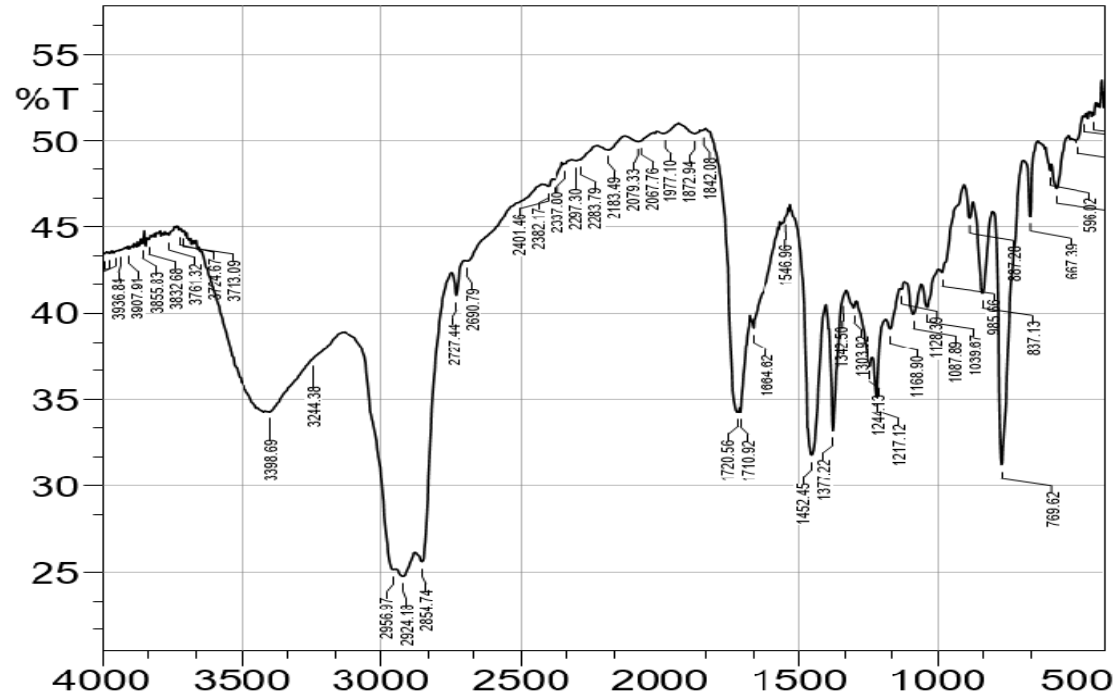

Fig-4: FTIR spectra of Petroleum ether fraction

- afforded the peaks in the range of 2800-3000 cm-^{1} with strong broad appearance suggesting N-H stretching vibration belong to the compound class amine salt.
- 1710 cm-^{1}: C=O stretching with strong appearance carboxylic/conjugated acid
- 1450 and 1375/80 range cm-^{1}: C-H bending with medium appearance alkane/aldehyde.
- 770 cm-^{1} C-Hbending with strong appearance, monosubstituted.

The functional groups ranging from 495 to 3936 peak values are Halo compounds, Alkyl groups, Alkanes, Alkenes, Sulphonamides, Aliphatic primary amines, Alcohols carboxylic acids etc.

Table- 3: Petroleum ether fraction FTIR data

Fraction	Peak Value	Functional group	Functional group name	Vibrations
FractionA	495.72			
	574.81	C-Br	Halo compound	Stretching strong
	596.02	C-I		
	667.39			
	700-900cm^{-1}		Alkyl group	Bending strong
	769.62	C-H		1,2 disubstituted
	650-1000			
	667.39		Alkyl group	Bending strong
	837.13	C-H		
	887.28	C=C		
	985.66	C=C	Alkene	Bending strong
	1000-1400			
	1039.67	S=O	Sulfoxide	Stretching strong
	1087.89		Secondary/tertiary	Stretching strong
	1128.39	C-O	alcohol	
	1168.90	C-O	Ester	Stretching strong
	1217.12			
	1244.13	C-O	Alkylaryl ether	Stretching strong
	1303.92			
	1342.50	S=O	Sulfonamide/	Stretching strong
	1377.22		Sulfonate	
	1300-1600			
	1452.45	C-H	Alkane	Bending medium
	1546.96	N-O	Nitro	Stretching strong
	1664.62	C=C	Alkene	Stretching weak
	1600-1670			
	1650-2000			
	1710.92	C=O	Conjugated/	Stretching strong
	1720.56		Carboxylic acid	
	2000-2400	N=C=S	Isothiocyanate	Stretching strong
	2067.76			
	2401.46	O=C=O	Carbondioxide	Stretching strong
	2500-3000			
	2690.79	C-H	Alkane	Stretching
	2727.44			mediuim
	2854.74	N-H	Amine salt	Stretching strong

	2924.18 2956.97 **3000-4000** 3244.38 3398.69 3713.09 3936.84	 N-H O-H	 Aliphatic primary amine Secondary amine Alcohol	broad Stretching medium Stretching medium sharp

3.2.4 Ethanolic Fraction FTIR: The FTIR afforded peaks 2920, 2850 (C-H stretching with medium appearance belongs to compound class alkane) cm^{-1}. The different functional groups ranging from 516 to 3936 peak values are alkanes, alkenes, alkynes, halo compounds, alcohols, vinyl ether, aromatic compounds, nitro groups, carboxylic acid, etc.

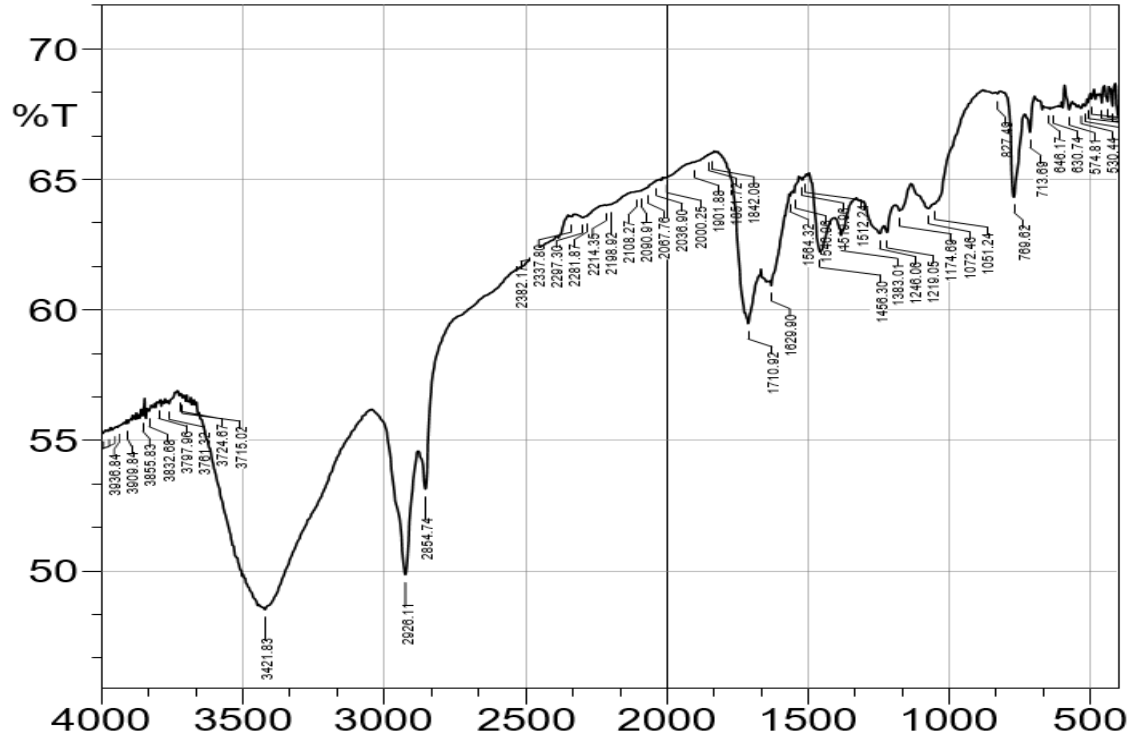

Fig-5: Ethanolic fraction FTIR result

Table- 4: Ethanolic fraction FTIR data

Fraction	Peak Value	Functional group	Functional group Name	Vibration
Fraction B	516.94 630.74 646.17 **700-900** 769.62 **650-1000** 827.49	C-I C=C C=C	Halo compound Alkene Alkene	Stretching strong Bending strong Bending medium

	1000-1400			
	1051.24 1072.46	C-O	Primary alcohol	Stretching strong
	1219.05	C-O	Vinyl ether	Stretching strong
	1383.01	O-H	Phenol	Bending medium
	1456.30	C-H	Alkane	Bending medium
	1300-1600			
	1512.24 1519.96 1546.96 1564.32	N-O	Nitro compound	Stretching strong
	1650-2000			
	1629.90	C=C	Alkene	Stretching medium
	1710.92	C=O	Carboxylic acid	Stretching strong
	1842.08 1851.72	C-H	Aromatic comp	Bending weak
	1901.88	C=C=C	Allene	Stretching medium
	2000-2400			
	2108.27 2198.92	C≡C	Alkyne	Stretching weak
	2214.35	C≡N	Nitrile	
	2281.87 2297.30 2337.80	N=C=O	Isocyanate	Stretching weak Stretching strong
	2382.17	O=C=O	Carbon dioxide	Stretching strong
	2500-3000			
	2854.74 2926.11	C-H	Alkane	Stretching medium
	3000-4000			
	3421.83 3724.47 3797.96 3855.68 3936.84	O-H	Alcohol	Stretching strong broad

3.2.5 GCMS study of Petroleum ether fraction

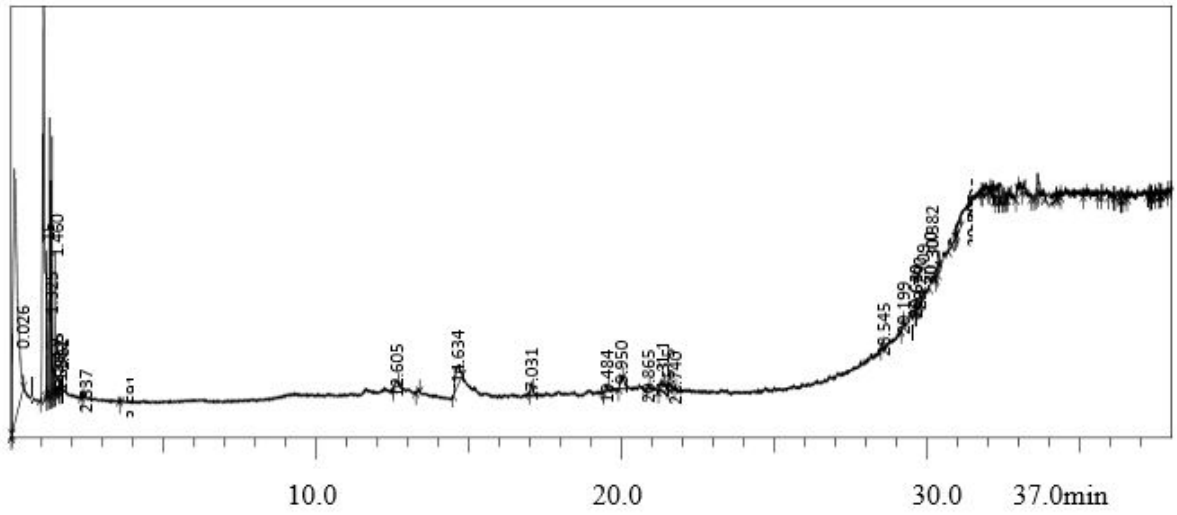

Fig-6: GCMS study of Petroleum ether fraction

Table- 5: GCMS data of Petroleum ether fraction

GCMS study of the Petroleum ether fraction shows the presence of many phytochemical constituents among them the prominent are 80 compounds shown below in table

Peak	R.Time	Area	Area %	Height	A/H	Base m/z	Name
1	0.026	37109569	1.23	40417631	0.92	44.00	1H-Pyrrole,2,5-dihydro-1-nitroso-
2	0.115	940900915	31.12	102139808	9.21	55.05	3-Trifluoroacetoxydodecane
3	1.034	652618811	21.58	105708945	6.17	46.75	(S)-(-)-2-Chloropropionicacid
4	1.150	118419265	3.92	58961668	2.01	58.05	3-Buten-1-amine, N,N-dimethyl-
5	1.264	198136492	6.55	111290793	1.78	42.05	1-Butanol,3-methyl-
6	1.301	77751026	2.57	85852229	0.91	56.05	Pentane,2,3-dimethyl-
7	1.325	37252794	1.23	36430802	1.02	57.10	Hexane
8	1.349	113354845	3.75	103317531	1.10	41.80	Butanal,3-methyl-
9	1.435	31567817	1.04	14826560	2.13	56.10	1-Heptene,2-methyl-
10	1.460	56305467	1.86	56956021	0.99	55.95	1-Pentene,2-methyl-
11	1.562	36015685	1.19	11741092	3.07	105.00	Silane,dimethoxydimethyl-
12	1.590	7923039	0.26	7649337	1.04	73.05	Propane,2,2-dimethoxy-
13	1.615	14495464	0.48	15219174	0.95	56.05	Cyclohexane

14	2.337	1450779	0.05	1929894	0.75	43.05	2,2-Dimethoxybutane
15	3.591	2197675	0.07	1858749	1.18	149.00	1-Buten-3-one,1-(2-carboxyl-4,4-dimethylcyclobutenyl)-
16	12.605	16742578	0.55	3085356	5.43	71.10	Docosane
17	13.344	3918250	0.13	1578547	2.48	57.10	Nonahexacontanoic acid
18	14.634	84914975	2.81	8058363	10.54	149.00	DiethylPhthalate
19	17.031	11284030	0.37	3802326	2.97	57.05	Nonane,3-methyl-5-propyl-
20	19.484	13404509	0.44	2890540	4.64	73.05	.alpha.-d-Riboside,1-O-dodecyl-
21	19.950	11119278	0.37	3527238	3.15	149.00	1,2-Benzenedicarboxylicacid,bis(2-methylpropyl)ester
22	20.865	5172920	0.17	2123438	2.44	57.10	Methyl3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate
23	21.311	22107337	0.73	3441238	6.42	148.95	1,2-Benzenedicarboxylic acid, butyloctylester
24	21.535	6210996	0.21	1915729	3.24	73.00	Heptasiloxane,hexadecamethyl-
25	21.740	2632481	0.09	1491708	1.76	135.00	1,2-Diphenyltetramethyldisilane
26	28.545	6560451	0.22	2757433	2.38	206.90	d-Mannitol,1-decylsulfonyl-
27	29.199	3750476	0.12	2171517	1.73	43.05	Nonane,5-(1-methylpropyl)-
28	29.630	2543407	0.08	2370199	1.07	44.00	1,2-Bis(trimethylsilyl)benzene
29	29.640	8361511	0.28	1759967	4.75	280.95	1H-Indole-2,3-dione,1-(tert-butyldimethylsilyl)-5-chloro-,3-(O-ethyloxime)
30	29.790	2377773	0.08	1320587	1.80	206.90	Silicicacid, diethylbis(trimethylsilyl)ester
31	30.090	3019573	0.10	2641292	1.14	280.90	Benzoicacid,4-methyl-2-trimethylsilyloxy-,trimethylsilylester
32	30.300	3408013	0.11	1331685	2.56	280.95	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
33	30.382	14198149	0.47	7587039	1.87	218.10	Lup-20(29)-en-3-ol,acetate,(3.beta.)-
34	30.781	16128098	0.53	3619892	4.46	280.90	Haloxazolam
35	30.805	3178092	0.11	2729842	1.16	281.90	Dimethylchrysin
36	30.83	224034	0.74	381342	5.8	280.9	3-Ethoxy-1,1,1,5,5,5-

	0	04		5	7	0	hexamethyl-3-(trimethylsiloxy)trisiloxane
37	30.945	8001518	0.26	4783472	1.67	280.85	Cyclotetrasiloxane, octamethyl-
38	31.071	28702925	0.95	5440216	5.28	280.90	Cyclotetrasiloxane,(iodomethyl)heptamethyl-
39	31.785	2584825	0.09	1931630	1.34	208.90	Sarpagan-17-ol, 16-[(acetyloxy)methyl]-,acetate(ester)
40	31.890	16170309	0.53	5107328	3.17	208.85	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
41	32.005	6501928	0.22	2000456	3.25	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
42	32.075	4657873	0.15	1395795	3.34	252.85	1-Methoxy-4-nitro-2,3,5,6-tetramethylbenzene
43	32.165	4125296	0.14	2503952	1.65	280.90	Pentasiloxane,dodecamethyl-
44	32.190	3443531	0.11	2802434	1.23	73.05	11-Methyl-13-tetradecen-1-olacetate
45	32.225	37972802	1.26	5860175	6.48	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
46	32.346	7156583	0.24	6571367	1.09	207.75	Benzeneaceticacid,4-methoxy-.alpha.-[(trimethylsilyl)oxy]-,methyl ester
47	32.393	25822741	0.85	6771969	3.81	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
48	32.490	3193590	0.11	1183880	2.70	96.00	Sarpagan-17-ol, 16-[(acetyloxy)methyl]-,acetate(ester)
49	32.515	4974038	0.16	2704576	1.84	96.05	Benzenepropanoicacid,4-[(2,4-dinitrophenyl)azo]-,1-methylethylester
50	32.609	5261666	0.17	3131282	1.68	190.80	1-(2,4-Dinitrophenyl)imidazole
51	32.709	14025570	0.46	4246267	3.30	280.90	2-Monooleoylglyceroltrimethylsilyl ether
52	32.961	11237732	0.37	3053362	3.68	281.90	Bicyclo[2.2.2]oct-2-ene-2,3-dicarboxylicacid, 1-hydroxy-8,8-dimethyl-5-oxo-, dimethyleste
53	33.195	20502307	0.68	5121873	4.00	281.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
54	33.410	5806997	0.19	840838	6.91	207.90	Benzene,1,2,4-trimethoxy-5-(1-propenyl)-,(Z)-
55	33.510	2463694	0.08	3036318	0.81	281.90	Sarpagan-17-ol, 16-[(acetyloxy)methyl]-,acetate(ester)
56	33.61	307418	1.02	925529	3.3	218.0	Aceticacid,3-hydroxy-7-

	6	07		0	2	5	isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-
57	33.680	3035424	0.10	2688603	1.13	207.90	N,N,N',N'-Tetracyclohexyloxamide
58	33.845	2948245	0.10	2633510	1.12	96.00	Benzoicacid, 2-benzoyl-,trimethylsilylester
59	33.880	4267284	0.14	5474068	0.78	190.90	Ethanethioicacid,S-[8-(diethylphosphono)octyl]ester
60	34.145	14398325	0.48	3818836	3.77	280.95	Tartronicacid,4-(dimethylethylsilyl)phenyl-, dimethylester
61	34.214	6759549	0.22	3307693	2.04	280.90	Cyclotetrasiloxane, octamethyl-
62	34.236	2563453	0.08	3548531	0.72	192.90	1,2-Selenagermolane,2,2-dibutyl-
63	34.300	10310845	0.34	2299975	4.48	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
64	34.385	4134465	0.14	2779958	1.49	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
65	35.110	10971762	0.36	1891366	5.80	207.90	Cyclotetrasiloxane,(iodomethyl)heptamethyl-
66	35.590	9068851	0.30	712057	12.74	207.75	m-Hemipicanhydride
67	35.723	2615514	0.09	2469817	1.06	73.00	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
68	35.980	10443466	0.35	1931986	5.41	281.90	1,2,3,4-Tetrahydroisoquinolin,2-acetyl-6,7-dimethoxy-1-phenmethylene-
69	36.130	3237194	0.11	1797449	1.80	280.90	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
70	36.297	18411167	0.61	3662243	5.03	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
71	36.336	3378402	0.11	3779544	0.89	40.00	Nickel,pentamethylcyclopentadienyl-(N,N,N'-trimethyl)-o-phenylenediamine-N'-o-
72	36.419	10777127	0.36	3551387	3.03	280.90	Cyclotetrasiloxane, octamethyl-
73	36.525	14954399	0.49	3254229	4.60	280.90	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
74	36.600	6365787	0.21	2824810	2.25	252.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
75	37.225	2700620	0.09	1190722	2.27	252.90	trans-3,4,5-Trimethoxy-b-methyl-b-nitrostyrene
76	37.298	4047246	0.13	3154988	1.28	280.95	Tartronicacid,4-(dimethylethylsilyl)phenyl-, dimethylester
77	37.34	289307	0.10	191356	1.5	280.9	Cyclotetrasiloxane, octamethyl-

	5	7		6	1	0	
78	37.390	6541817	0.22	2214211	2.95	280.90	Bis[2-(2,4-dinitrobenzoyloxy)-1-naphthyl]methane
79	37.461	10907581	0.36	3570551	3.05	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
80	37.625	3373592	0.11	2300518	1.47	280.90	Tartronicacid,4-(dimethylethylsilyl)phenyl-, dimethylester

3.2.6 GCMS data of Ethanolic fraction

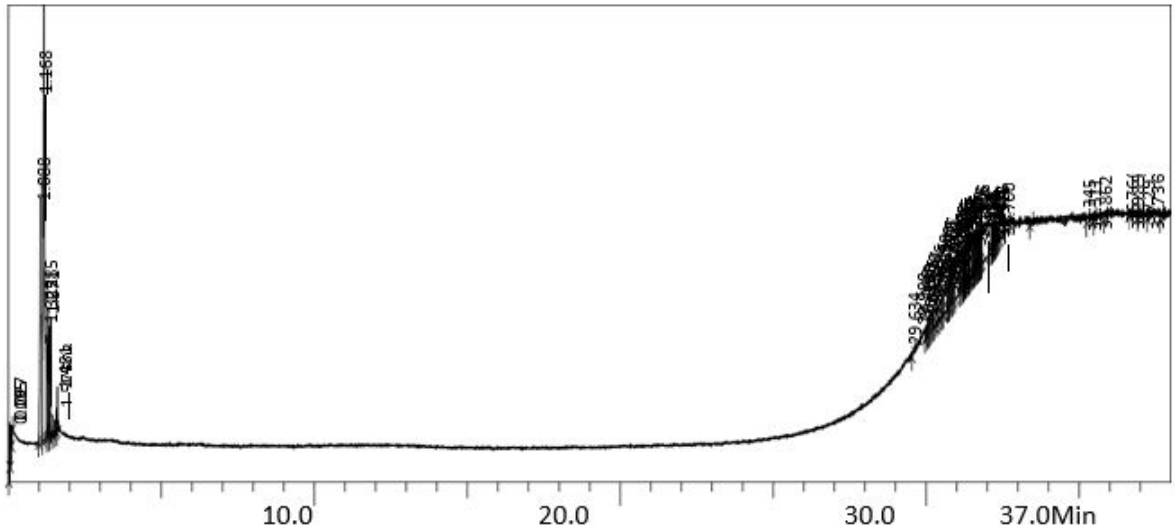

Fig-7: GCMS study of Ethanolic fraction

Table- 6: GCMS data of Ethanolic fraction

GCMS study of Ethanolic fraction also shows the presence of many phytochemical constituents among them the prominent are 70 compounds shown below in table

Peak	R.Time	Area	Area%	Height	A/H	Basem/z	Name
1	0.027	33048326	1.13	20474373	1.61	44.00	Bicyclo[2.2.1]heptane-5-(ethyl-1-amine)
2	0.095	32272158	1.10	8631992	3.74	41.10	1,4-Methano-1H-indene,octahydro-4-methyl-8-methylene-7-(1-methylethyl)-,[1S-(1.alpha.,3
3	0.115	8900852	0.30	5200979	1.71	41.05	N-[1-(4-Butoxyanilino)-2,2,2-trifluoro-1-(trifluoromethyl)ethyl]-2,4-dichlorobenzamide
4	1.088	376547029	12.85	97547325	3.86	62.85	Cyclohexanol,ethynyl-,carbamate
5	1.168	498822860	17.03	118645045	4.20	60.20	Propane,1,2-dimethoxy-
6	1.285	115867260	3.96	49094990	2.36	41.95	1,3-Dioxolane,4-ethyl-
7	1.325	37507258	1.28	41505342	0.90	57.05	Pentane,3-methyl-
8	1.371	93286544	3.18	44521148	2.10	57.05	Hexane
9	1.481	37725293	1.29	16592727	2.27	56.05	Cyclopentane,methyl-
10	1.575	23157585	0.79	9080660	2.55	105.00	Silane,dimethoxydimethyl-
11	1.603	16723084	0.57	16700904	1.00	73.05	Propane,2,2-dimethoxy-
12	29.634	10063319	0.34	2738849	3.67	206.90	2,4,6-Cycloheptatrien-1-one,3,5-bis-

							trimethylsilyl-
13	29.988	13255169	0.45	4595330	2.88	280.90	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
14	30.040	9356342	0.32	4048208	2.31	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
15	30.119	11886515	0.41	4713150	2.52	207.85	Ethanol,2-[4-vinyl-2-methoxy-6-methyl]phenoxy-
16	30.140	13994830	0.48	4772602	2.93	73.05	(t-Butyl-dimethylsilyl)[2-methyl-2-(4-methyl-pent-3-enyl)-cyclopropyl]-methanol
17	30.225	20178805	0.69	4985868	4.05	280.90	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
18	30.270	8573254	0.29	6505280	1.32	280.90	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
19	30.330	19135993	0.65	6731j803	2.84	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
20	30.426	26552491	0.91	7371623	3.60	44.00	1-Pentene,1,3-diphenyl-1-(trimethylsilyloxy)-
21	30.515	41926620	1.43	6804991	6.16	264.85	Ethyl4-chloro-1-methyl-2(1H)-oxo-3-quinolinecarboxylate
22	30.580	19390148	0.66	8660671	2.24	280.90	Tartronicacid,4-(dimethylethylsilyl)phenyl-, dimethylester
23	30.610	57615004	1.97	8437067	6.83	190.85	Tartronicacid,4-(dimethylethylsilyl)phenyl-, dimethylester
24	30.710	19849071	0.68	9731710	2.04	280.90	1,2-Dihydroanthra[1,2-d]thiazole-2,6,11-trione
25	30.740	14100211	0.48	9991413	1.41	208.90	3,5-Ethanoquinolin-10-ol,decahydro-1,7-dimethyl-,[3R-(3.alpha.,4a.beta.,5.alpha.,7.beta.,8a
26	30.770	17816228	0.61	10100681	1.76	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
27	30.805	42105752	1.44	10515249	4.00	280.90	Cyclotetrasiloxane, octamethyl-
28	30.870	12637905	0.43	11091740	1.14	252.85	3-Trifluoromethyl-7-phenothiazone
29	30.945	37651708	1.29	12069173	3.12	44.00	1H-Indole-2,3-dione,5-bromo-1-(tert-butyldimethylsilyl)-
30	30.980	121913110	4.16	13878512	8.78	280.90	Cyclotetrasiloxane, octamethyl-
31	31.146	48040128	1.64	15701069	3.06	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
32	31.180	22431560	0.77	15470341	1.45	281.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
33	31.200	17801092	0.61	14765759	1.21	280.90	4-Methyldodecane,3-(methylsulfonyloxy)-1-(t-butyldimethylsilyloxy)-
34	31.230	31784269	1.09	15772101	2.02	252.85	12-Crown-4,phenyl-
35	31.275	41603698	1.42	16526584	2.52	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
36	31.300	18119509	0.62	14961887	1.21	280.90	1,3,5,7-Tetraethylbicyclo[3.3.1]tetrasiloxane
37	31.346	47100190	1.61	16761352	2.81	280.90	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester

38	31.385	36694627	1.25	15864330	2.31	96.00	Tricyclo[4.3.1.1(2,5)]undec-3-en-10-one
39	31.415	27756233	0.95	17205161	1.61	133.05	Benzene,1,1'-ethenylidenebis-[4-methyl-
40	31.493	60084683	2.05	15821177	3.80	207.90	5-Methyl-2-N-methylaminobenzophenonesemicarbazone
41	31.520	45500921	1.55	16430098	2.77	252.85	1H-Indole-2,3-dione,1-(tert-butyldimethylsilyl)-5-chloro-,3-(O-ethyloxime)
42	31.566	32267721	1.10	16717122	1.93	208.90	1,8-Dimethyl-3,6-diazahomoadamantan-9-spiro-2'-thiirane
43	31.611	39673900	1.35	15754949	2.52	252.85	N-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)malonamicacid
44	31.635	22343006	0.76	15858747	1.41	96.90	Benzenepropanoicacid,4-benzoyl-,methylester
45	31.670	31745763	1.08	16744212	1.90	280.90	1,2-Cinnolinedicarboxylicacid,1,2,3,5,6,7,8,8a-octahydro-4-trimethylsilyloxy-,diethylester
46	31.700	39393101	1.34	15438069	2.55	280.90	1H-Indole-2,3-dione,1-(tert-butyldimethylsilyl)-5-chloro-,3-(O-ethyloxime)
47	31.750	25362757	0.87	15157657	1.67	280.85	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
48	31.780	16550559	0.57	14501892	1.14	280.90	Cyclotetrasiloxane, octamethyl-
49	31.816	29965304	1.02	16140572	1.86	280.90	1,3,5,7-Tetraethylbicyclo[3.3.1]tetrasiloxane
50	31.906	97220161	3.32	15542567	6.26	280.90	2H-1,3,4-Benzotriazepine-2-thione,5-benzyl-1,3-dihydro-3-methyl-
51	32.031	123433396	4.21	12491840	9.88	280.90	1-Pentene,1,3-diphenyl-1-(trimethylsilyloxy)-
52	32.125	14320191	0.49	10854496	1.32	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
53	32.162	20199756	0.69	10454337	1.93	75.00	Methyl2R,3s(2s,3R)-2-bromo-2,3-dichlorobutyrate
54	32.197	24310944	0.83	10506369	2.31	190.90	(p-Tolyl)-acetonyl-dimethylsilane
55	32.230	19134255	0.65	9001128	2.13	281.85	Octadecanoicacid,16-oxo-,methylester
56	32.270	14519085	0.50	9202635	1.58	207.75	4-Methylbenzylidene-4-methylaniline
57	32.315	14011407	0.48	8477956	1.65	73.05	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
58	32.360	19734087	0.67	7639068	2.58	73.05	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
59	32.401	14917180	0.51	6719318	2.22	207.75	Benzaldehyde,2-nitro-4-trimethylsilyl-
60	32.472	26505001	0.90	6318592	4.19	280.90	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
61	32.515	10515683	0.36	5818821	1.81	44.00	N-(2-Hydroxy-3,5-dimethylbenzyl)-.beta.-aminobutanoic acid
62	32.700	11200093	0.38	3754383	2.98	280.85	1,3,5-Triethyl-1-(ethylbutoxysiloxy)cyclotrisiloxane
63	33.415	10173216	0.35	2541318	4.00	280.90	Cyclotetrasiloxane, octamethyl-
64	35.345	12356571	0.42	2833987	4.36	248.80	3beta,17beta-Diacetoxy-17-isopregn-5-

							en-20-one
65	35.511	11204462	0.38	3104365	3.61	280.90	Pentasiloxane,1,1,3,3,5,5,7,7,9,9-decamethyl-
66	35.862	13832176	0.47	3499811	3.95	280.90	Benzoicacid,3-methyl-2-trimethylsilyloxy-,trimethylsilylester
67	36.760	10459401	0.36	3166702	3.30	280.85	Cyclotetrasiloxane, octamethyl-
68	36.985	10079762	0.34	2815705	3.58	280.90	Tartronicacid,4-(dimethylethylsilyl)phenyl-, dimethylester
69	37.291	12002162	0.41	4047103	2.97	191.80	2-(4-Hydroxy-3-methoxyphenyl)-2-ethoxyethanol, di-TMS
70	37.736	13090091	0.45	3627694	3.61	207.75	5,7a-Didehydroindicinepertrimethylsilylether

4. DISCUSSION

Tabernaemontana divaricata, which is used in Chinese, Ayurvedic and Thai traditional medicine, has been reported to exhibit diverse medicinal properties,[21,22]. In the current investigation, Petroleum ether fraction and Ethanolic fraction of *Tabernaemontana divaricata* leaves was screened to detect phytochemical constituents using UV, FTIR, GCMS studies. The results obtained from the studies shows the presence of diverse Phytochemical constituents belonging to the Alkaloids, non-Alkaloids, Steroids, Tannins etc.

UV spectroscopy shows presence of Dienes, trienes and tetraenes in compounds with progression of conjugation till 550-600nm. More than 20 double bonds in conjugation give polyenes with yellow colour. λmax increases with addition of each substitution such as: Acyclic and heteroannular dienes 215nm and homoannular dienes 253nm acyclic trienes 245nm. Addition of substituents R- alkyl, OR- alkoxy, SR- thioether, CL, Br, OCOR- acyloxy and CH=CH- also increases λmax. This empirical relationship is called Woodward rule.The intensity of λm increases within conjugation and substitution in case of polyene and polyeneyne[23]. The present UV spectroscopy study suggests presence of benzene 239nm 268nm, pyridine 264nm and others in thefractions.

FTIR study of the Petroleum ether fraction and Ethanolic fraction shown the presence of various functional groups present in phytochemical constituents and these functional groups might have numerous pharmacological activities as reported by many research papers. The functional groups in Petroleum ether fraction ranging from 495 to 3936 peak values are Halo compounds, Alkyl groups, Alkanes, Alkenes, Sulphonamides, Aliphatic primary amines, Alcohols carboxylic acids etc.Few of the functional groups present in the Petroleum ether fractionafforded the peaks in the range of 2800-3000 cm^{-1} with strong broad appearance suggesting N-H stretching vibration belong to the compound class amine salt.

- 1710 cm^{-1}: C=O stretching with strong appearance carboxylic/conjugated acid
- 1450 and 1375/80 range cm^{-1}: C-H bending with medium appearance alkane/aldehyde.
- 770 cm^{-1} C-Hbending with strong appearance, monosubstituted.

The FTIR Ethanolic fractionafforded peaks to 3300, 2926, 2854 to (C-H stretching with medium appearance belongs to compound class alkane cm^{-1}. The different functional groups ranging from 516 to 3936 peak values are alkanes, alkenes, alkynes, halo compounds, alcohols, vinyl ether, aromatic compounds, nitro groups, carboxylic acid, etc. The results revealed the presence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpenes due to C-H group[24]. The FTIR afforded peaks (indole N-H), 2920, 2850 (C-H) cm^{-1} but did not show peaks in the carbonyl region. The details of above

functional groups in Petroleum ether and Ethanolic fractions can be seen in Table 3 and Table 4 respectively.

GCMS study of the Petroleum ether fraction shows the presence of many phytochemical constituents among them the prominent are 80 compounds shown in the Table-5. The other important phytochemical constituents seen in data are below.**Indoles:** Pyroles, Ergoline 8-methanol (Indole quinoline derivative) and Sarpagan.**Steroids:**B- sitosterol, Lupeol-20 acetate, Unsaturated fatty acidsand Cholest-8(14)ene.**Miscellaneous**: Gibberellic acid. Mannitol, Milbemycin B,α-Glucopyranoside, D-Glucopyranosidesand others such as Benzoic acid, β-amyrene are present.

GCMS study of Ethanolic fraction also shows the presence of many phytochemical constituents among them the prominent are 70 compounds shown in the Table-6. Important phytochemical constituents of different class are : **Alakloids:Indoles** (pyroles, pyredoles), Quinaxazolidone, triazole, triazines, thiadiazoles, benzazepines, **Quinolones**: Benzothiazoles, Diethylamine carbazoles, Phenothiazones, Phenanthrene-done, Benzophenone hydrazone, Benzotriazepine, Benzopropionic acid, Dihydropyramidine and Sarpagan.**Alkanes:** Cyclo alkanes, Dimethylchrysin and Shikimic acid.**Benzene:** Benzodiazepine, Haloxazolam,Phenanthrene, Anthracene, Benzoic acid are present.**Steroids:**Stigmasta-5,22-dien-30l acetate, Isopregna5-en20-.**Miscellaneous:** Dextroamphetamine, Iodohistidine, Adnosine, 12 crown-4phenyl, Pseudogem (E), Germane, D-glucopyranosyl, Tosyl, Pseudoheptulose, Cinnoline are present.

5. CONCLUSION

The current work is assessment ofthe major phytoconstituents present within the *Tabernaemontana divaricata* which has reported to posses' various pharmacological potential. The various secondary metabolites derived from *Tabernaemontana divaricata* such as terpenes, lactones, steroids, phenols, flavonoids, and alkaloids are often utilized in ethnobotany for their curative effects. Furthermore, these bioactive components have displayed numerous biological activities including antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, anti-neurodegenerative, anticancer, antidiabetic, antivenom, larvicidal, antihypertensive action, wound healing and analgesic effects. However, despite the presence of biologically active phytochemical compounds many species in the genus *Tabernaemontana* lack chemical and biological evaluation. Thus, the further research is crucial to gain insight about the bioactive compounds and relative pharmacological activities of this genus. So, the attempt has made in the present work to establish the biologically active phytochemical compounds in the *Tabernaemontana divaricata* leaf using spectroscopic analysis of Petroleum ether fraction and Ethanolic fraction and this led to the determination of various compounds and further study of these compounds will help in establishing various pharmacological activities.

CONFLICT OF INTEREST: There is no conflict of interest.

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