pISSN 2302-1616, eISSN 2580-2909
Vol 11, No. 1, June 2023, pp. 130-143
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v11i1.36824

GC-MS analysis of bioactive compounds present in leaves, stem, and roots extract of *Tylophora indica* (Burm.fil.) Merr.

Anamika Gupta[1], Deeksha Singh[1], Rajiv Ranjan[1*]
[1]Department of Botany, Dayalbagh Educational Institute
DayalBagh, Agra, Uttar Pradesh, 282005, India
*Email: rajivranjanbt@gmail.com

ABSTRACT. Plants serve as a boundless source of raw materials for pharmaceuticals and have been used as an important source of medicine for several years. Identifying natural compounds from medicinal plants is useful for the discovery of novel therapeutic agents. *Tylophora indica* is perennial climber which possess many medicinal properties. Although it also contains many bioactive compounds which have role in various biological activity such as anti-inflammatory activity, anti-oxidant activity, and anti-cancer activity. The present study aimed to identify the bioactive compounds from methanolic and hexane extract of the leaves, stems, and roots of *T. indica* using GC-MS analysis. GC-MS analysis was performed by using standard protocols. Willey and NIST libraries were used in the identification of components, and their retention indices were compared. The GC-MS analysis revealed the presence of various prevailing compounds like Levomenthol, 2-Butanone, 3,3-dimethyl-1-(methylsulfonyl)-,O[(methylamino)carbonyl] oxime, Squalene, 5–methyl–2–(1–methylethyl)–(1α,2β,5β)– cyclohexanol, Oleic acid and 2-propyloxirane etc. These identified compounds play a vital role in various biological activities which were confirmed by Dr. Duke's phytochemical and ethnobotanical databases (https://phytochem.nal.usda.gov/). The findings of this study demonstrate that *T. indica* is a rich source of numerous bioactive compounds that can be useful in halting the progression of several disorders.

Keywords: GC-MS analysis; medicinal plant; phytochemicals; secondary metabolites; *Tylophora indica*

Article History: Received 29 March 2023; Received in revised form 8 May 2023; Accepted 27 May 2023; Available online 30 June 2023.
How to Cite This Article: Gupta A, Singh D, Ranjan R. 2023. GC-MS analysis of bioactive compounds present in leaves, stem, and roots extract of *Tylophora indica* (Burm.fil.) Merr. *Biogenesis: Jurnal Ilmiah Biologi*. vol 11(1): 130–143. doi: https://doi.org/10.24252/bio.v11i1.36824.

INTRODUCTION

In India, plant based herbal medicines were utilized since ancient times, particularly in remote areas. Plants are the source of many powerful and potent medicines (Mamani & Alhaji, 2019). They contain several phytochemicals or primary and secondary metabolites. These compounds are the organic chemicals which play vital role in the metabolism of plants and perform various biological functions (Enegide & Okhale, 2023). Some primary metabolites are amino acids, nucleic acids, proteins etc. and secondary metabolites include alkaloids, terpenes, phenolics, fatty acids, etc. (Hussein & El-Anssary, 2019; Gururani *et al.*, 2020). Medicinal and pharmacological uses of many of these phytochemicals have beneficial effects.

The selected medicinal plant *Tylophora indica* belongs to the family Asclepiadaceae (Gupta *et al.*, 2020). It is a perennial climber which is commonly known as *Antmool/Antamul*. The other names are *Indian ipecac, Dambel, Dambuti* etc. (Gantait & Kundu, 2017; Prasad *et al.*, 2019; Bembde *et al.*, 2020; Sharma, 2022). Traditionally, this plant was used for the treatment of skin disorders, microbial infection, allergy, asthma, jaundice, and respiratory disorders (Gupta *et al.*, 2010; Kour & Gupta, 2015; Gupta *et al.*, 2020, Maheshwari & Vijayarengan, 2020, Vani *et al.*, 2021). The plant is widely harvested for its medicinal properties in the wild, and because the harvesting is uncontrolled and unmonitored, it is listed as endangered (Kaur *et al.*, 2014). Several policies and programs have been implemented by the Indian government to protect endangered plant species, including *T. indica.* The Wildlife Protection Act of 1972 (Government of India, 1972) prohibits the harvesting or trading of this plant without prior permission. To cultivate medicinal plants, an herbal garden was recently constructed at NRIUMSD, Hyderabad called Ibn-Al-Baitar Herbal Garden. A total of 162 Unani medicinal plants are maintained in this garden by the Central Council for Research in Unani Medicine, Ministry of Ayush, Government of India, and *T. indica* is one of them. *T. indica* has also been

traditionally used by local Indian communities for medicinal purposes (Rani *et al*., 2012; Nazar *et al*., 2020).

Apart from the medicinal properties, it also shows some pharmacological activities i.e., antitumor, antioxidant, anticonvulsant, antidiabetic, and hepatoprotective (Samaddar *et al.,* 2012; Maheshwari *et al.,* 2020). Aside from stimulating and emetic properties, roots act as expectorants, stomachic, diaphoretics, antifeedants, and antimicrobial (Raut *et al*., 2012; Vivean *et al.,* 2014). It has been reported that roots and leaves are suitable natural preservatives for food, and they are used to treat rheumatic and gouty pains (Ranemma *et al.,* 2017). This plant contains various important secondary metabolites which may have a role in various medicinal and pharmacological activities.

This study was conducted for the determination of the organic compounds present in the different plant part of *T. indica* by GC-MS technique, which may provide an insight in phytochemical compounds that are used for traditional medicine. It is the first time that GC-MS studies on *T. indica* have been conducted in a semi-arid region such as Agra. In literature very few works are reported on this plant, so it needs further investigation. Most of the phytochemical studies was reported on leaves and roots but there no such report on stem with methanol and hexane solvent. Based on the GC-MS analysis of the different parts of *T. indica* (leaves, stem, root), 39 bioactive compounds were identified with their pharmacological activities.

MATERIALS AND METHODS

Collection of plant sample. Healthy and disease-free plant samples were collected in the month of September from Herbal Garden, Dayalbagh Educational Institute, Agra. Leaves, stems, and roots of the plant were used for phytochemical analysis.

Preparation of plant extracts. Plant material, such as leaves, stems, and roots, was thoroughly washed with distilled water to remove impurities. All plant parts were shade dried at room temperature. Finely ground dried plant parts were stored in an airtight container. After mixing 1 g of fine powder with 10 ml of solvents (hexane and methanol), the mixture was shaken continuously for 30 min. Afterward, the mixture was centrifuged at 4500 rpm for 20 min and the upper layer was filtered with 0.22µ filter paper. An extract was collected for GC-MS analysis after being filtered (Azcue, 1996; Thakur *et al*., 2018).

Gas chromatography-mass spectrometry (GC–MS) analysis. For the identification of compounds, analysis was performed using an Agilent 7890A gas chromatography instrument coupled with an Agilent 5975C inert mass selective detector (single quadrupole mass spectrometer). HP-5MS column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) was used for separation which coated with a non-polar phase of 5% phenyl methyl silox. The initial temperature of the GC column oven was 50°C held for 2 min with a ramp of 8°C/min from 50°C to 250°C followed with a ramp of 10°C/min from 250 to 280°C and the final temperature was held for 28 min. The carrier gas was helium (99.999%) at a constant flow rate of 1 mL/min. About 2 µL of each extract was injected in split mode (split ratio 10:1) with an inlet temperature 260°C and MS interface temperature 280°C. The MS was run in scan mode from 40 to 650 amu, with the source and quadrupole set at 230°C and 150°C, respectively. The MS was operated in electron impact (EI) positive ion mode and the energy used was 70 eV (Craig *et al*., 1979).

Identification of bioactive compound. Peak interpretations were made on the basis of Wiley mass spectral library (W9N11) and databases of National Institute of Standard and Technology (NIST) libraries for identification of volatile compounds. The spectrums of unknown compound isolated during the GC-MS were compared with the spectrum of a known compound stored in these both libraries. The biological activities of the compounds are based on Dr. Duke's phytochemical and ethnobotanical databases (https://phytochem.nal.usda.gov/). Molecular weight, molecular formula and nature of identified compounds was confirmed by using pubchem (https://pubchem.ncbi.nlm.nih.gov/), and spectrabase (https://spectrabase.com/).

RESULTS AND DISCUSSION

In order to identify the bioactive compounds, present in the leaves, stems, and roots of *T. indica*, GC-MS analysis was carried out.

Table 1. Compounds identified in the methanol extract of *Tylophora indica* leaves

Peak#	RT	Compound name	Nature of compound	Area %	Molecular formula	Molecular Weight
1	4.110	3,3-dimethyl-1-(methylsulfonyl)-e	Ketones	6.18	$C_9H_{18}N_2O_4S$	250.32
2	6.089	Bicyclo [3.2.1] octane-2,4-dione	Bicyclo alkane	0.95	$C_8H_{10}O_2$	138.16
3	6.266	Bicyclo [3.2.1] octane-2,4-dione	Bicyclo alkane	1.06	$C_8H_{10}O_2$	138.16
4	17.405	Levomenthol	Menthol	35.79	$C_{10}H_{20}O$	156.26
5	23.329	4-Isopropenyl-1-methyl-1-Cyclohexene	Monoterpenes	14.47	$C_{10}H_{16}$	136.23
6	24.688	1-Tetradecanol	Fatty alcohol	1.88	$C_{14}H_{30}O$	214.39
7	30.879	(Cis) -2-nonadecene	Alkane hydrocarbon	1.65	$C_{19}H_{38}$	266.5
8	31.252	1,2-Benzenedicarboxylic acid	Diester	1.19	$C_{12}H_{14}O_4$	222.24
9	35.729	1-Hexadecanol	Fatty alcohol	2.11	$C_{16}H_{34}O$	242.44
10	36.016	1,3-dibenzyloxy-2-propyl acetate	Acetate ester	0.27	$C_{19}H_{22}O_4$	314.38
11	36.429	5-(hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidazol	-	0.41	$C_{12}H_{12}N_4O$	228.25
12	36.542	anti-(2RS,3RS)-7-(N-Phenylamido)-9-methyl-10-phenylthio-7-a	-	0.07	$C_{23}H_{28}N_2O_5$	380
13	36.662	Neophytadiene	Diterpene	14.00	$C_{20}H_{38}$	278.5
14	36.791	(4R,5R)-2-[(RS)-4,8-dimethylnonyl]-2-methyl-N,N'-di(prop-2-	Amide	0.48	$C_{23}H_{40}N_2O_4$	408.6
15	37.146	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Phytol	2.96	$C_{20}H_{40}O$	296.53
16	37.348	1,2-Benzenedicarboxylic acid,bis(2-methylpropyl) ester	Ester	0.59	$C_{16}H_{22}O$	278.34
17	37.489	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Phytol	4.36	$C_{20}H_{40}O$	296.53
18	39.096	2-[3-(3-Oxocyclohexyl)propyl]-2-phenyl-1,3-dioxolane	Ketals	0.29	$C_{18}H_{24}O_3$	288.39
19	39.486	Cyclohexadecane	Alkane hydrocarbon	0.58	$C_{16}H_{32}$	224.4
20	39.658	6-Methyl-2-(1'-propenyl) 8H-[1]benzopyran [7,8-d] oxazol-8-on	-	1.15	$C_{14}H_{11}NO_3$	241
21	41.502	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Phytol	9.56	$C_{20}H_{40}O$	296.53

GC-MS profiling of *Tylophora indica* methanol extract. The active compounds in *T. indica* were analyzed by measuring the peak area and retention time on the GC-MS chromatogram. A total of 68 compounds were identified from the GC-MS analysis of methanol extract of *T. indica* from which 21 were identify from leaves, 27 from stem and 20 from roots respectively which exhibiting various phytochemical activities. The main component fragmentation can be seen from the peaks in the mass spectrum as presented in Fig. 1-3. while the chemical constituents with their retention time

(RT), molecular formula, molecular weight (MW), area (%) along with the nature of compound are presented in Table 1-3.

Table 2. Compounds identified in the methanol extract of *Tylophora indica* stem

Peak#	RT	Compound name	Nature of compound	area %	Molecular formula	Molecular weight
1	3.184	2-[N-Benzyloxycarbonyl-(S)-phenylalanylamino]-4-methylpentan-1-ol	-	6.17	$C_{23}H_{30}N_2O_4$	398.5
2	4.103	2-Butanone, 3,3-dimethyl-1-(methylsulfonyl)-,O-[(methylamino)carbonyl) oxime	Thiocarbamate	10.18	$C_9H_{18}N_2O_4S$	250.31
3	5.581	1(Tetrahydropyranyloxy)hepta-2,6-diyn-4-ene	-	0.17	$C_{12}H_{14}O_2$	190.24
4	6.103	N-(2-Hydroxyethyl)-2-oximinoethanamide	-	0.90	$C_4H_8N_2O_3$	132
5	6.263	2,4-dimethoxybicyclo[3.2.1]octane	-	0.65	$C_{10}H_{18}O_2$	170
6	17.368	5-methyl-2-(1-methylethyl)-, (1α,2β,5β)- Cyclohexanol	Menthol	24.04	$C_{10}H_{20}O$	156.26
7	20.972	1-Phenylhex-5-en-2-one	Ketones	0.00	$C_{12}H_{14}O$	174.24
8	23.315	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-acetate	Tertiary alcohol	10.37	$C_{12}H_{20}O_2$	196.29
9	24.659	1-Tridecene	Acyclic olefin	0.83	$C_{13}H_{26}$	182.35
10	27.981	1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclo	-	1.11	$C_{15}H_{24}O$	220.35
11	29.879	1,3,3-trimethyl-cis, exotricyclo [3.3.0.0(2,4)]octan-6-one	-	1.58	$C_{11}H_{16}O$	164
12	30.842	1-Tetradecanol	Myristic alcohol	1.49	$C_{14}H_{30}O$	214.39
13	31.040	tert-Butyl 8-Methyl-10-azabicyclo[4.3.1]deca-3,7-diene-10-carboxylate	-	0.20	$C_{15}H_{23}NO_2$	249.35
14	31.216	1,2-Benzenedicarboxylic acid, diethyl ester	Diethyl phthalate	1.84	$C_{12}H_{14}O_4$	222.24
15	32.625	Naphthalene,1,2,3,5,6,7,8,8aoctahydro-1,8a-dimethyl-7-(1-Methylethenyl)	-	1.44	$C_{15}H_{24}$	204.36
16	32.753	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene	-	2.77	$C_{15}H_{24}$	204.36
17	35.721	Trichloroacetic acid, tridecyl ester	-	4.87	$C_{15}H_{27}Cl_3O_2$	345.733
18	36.467	6-Amino-3,4,7-triphenylpyrido[2',3':4,5]thieno[2,3-c]pyridazine	-	0.60	$C_{27}H_{18}N_4S$	430.53
19	36.657	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	-	1.52	$C_{20}H_{40}O$	296.5
20	37.346	3-Butyl-4-phenylfuran-2,5-dione	-	1.09	$C_{14}H_{14}O_3$	230.26
21	37.481	4-hydroxytetradec-2-ynal	-	0.16	$C_{14}H_{24}O_2$	224.34
22	38.457	1,11-Dodecadiene	-	0.32	$C_{12}H_{22}$	166.3
23	39.085	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	-	0.42	$C_{10}H_{10}O_3$	178.19
24	39.484	(cis)-2-nonadecene	-	0.50	$C_{19}H_{38}$	266.5
25	39.643	3-(Allylamino)-5-hydroxy-1H-indole-3-carbonitrile	-	0.22	$C_{12}H_{11}N_3O$	213.23
26	41.503	(2S,5R)-2-Isopropyl-5-methylhept-6-en-1-ol	-	0.38	$C_{11}H_{22}O$	170.3
27	43.774	Squalene	Triterpene	26.17	$C_{30}H_{50}$	410.7

As shown in Table 1, levomenthol (methanol) is the main active compounds present in methanol extract of *T. indica* leaves with an abundance of peak areas of 35.79%. Squalene (triterpene) is the main active compounds present in methanol extract of *T. indica* stem with an abundance of peak areas of 26.79% (Table 2). However, oleic acid (fatty acids) is the main active compounds present in methanol extract of *T. indica* roots with an abundance of peak areas of 45.25% as showed in Table 3.

Table 3. Compounds identified in the methanol extract of *Tylophora indica* roots

Peak#	RT	Compound name	Nature of compound	Area %	Molecular formula	Molecular weight
1	6.786	Nonanoic acid	Fatty acid	0.35	$C_9H_{18}O_2$	158.24
2	7.482	2-Undecenal	-	0.35	$C_{11}H_{20}O$	168.28
3	10.979	cis-9-Hexadecenal	Fatty aldehyde	0.61	$C_{16}H_{30}O$	238.41
4	12.769	9-Hexadecen-1-ol,(z)-	Fatty alcohol	0.28	$C_{16}H_{32}O$	240.42
5	14.512	Z,E-2,13-Octadecadien-1-ol	-	0.30	$C_{18}H_{34}O$	266.5
6	14.906	cis-11-Hexadecenal	Fatty aldehyde	0.83	$C_{16}H_{30}O$	238.41
7	15.565	Oleyl alcohol	-	0.36	$C_{22}H_{35}F_7O_2$	464.5
8	17.049	n-Hexadecanoic acid	Fatty acid	27.05	$C_{16}H_{32}O_2$	256.42
9	18.938	Oleic Acid	Fatty acid	45.25	$C_{18}H_{34}O_2$	282.5
10	19.099	cis- Vaccenic acid	Fatty acid	6.60	$C_{18}H_{34}O_2$	282.5
11	19.581	Benzenamine,4-methoxy-N-(triphenyl phosphoranylidene)-Eicosanoic acid	-	0.56	$C_{25}H_{22}NOP$	383.43
12	20.038	Benzenamine, 4-methoxy-N-(triphenyl phosphoranylidene)-	-	1.95	$C_{25}H_{22}NOP$	383.43
13	20.474	B(9a)-Homo-19-norpregna-9(11),9a-dien-20-one	-	0.47	$C_{26}H_{44}N_2O$	400.6
14	20.692	Benzenamine, 4-methoxy-N-(triphenylphosphoranylidene)-	-	0.76	$C_{11}H_{15}N_5O_3$	265.27
15	21.076	2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy] methyl]ethyl ester Benzenamine	Carboxylic acid	0.59	$C_{55}H_{106}O_6$	863.4
16	21.569	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy] methyl]ethyl ester Benzenamine	Carboxylic acid	4.95	$C_{55}H_{106}O_6$	863.4
17	22.119	Benzenamine	-	1.89	$C_{25}H_{22}NOP$	383.4
18	22.757	Benzaldehyde, 2-hydroxy-5-nitro-,2-iodophenylhydrazone	-	0.60	$C_{13}H_{10}IN_3O_3$	382.97
19	23.177	1,6-Dihydroxy-3-methylanthraquinone, O,O'-bis(trimethylsilyl)-Benzenamine	Anthraquinone	4.78	$C_{21}H_{26}O_4Si_2$	398.6
20	30.555	Iron, cyclopentadienyl-ethyl-1,2-di isopropyl phosphinoethane Benzenamine	-	1.47	$C_{20}H_{38}FeP_2$	396.3

GC-MS profiling of *Tylophora indica* **hexane extract.** The active compounds in *T. indica* were analyzed by measuring the peak area and retention time on the GC-MS chromatogram. A total of 46 compounds were identified from the GC-MS analysis of hexane extract of *T. indica* from which 10 were identify from leaves, 16 from stem and 20 from roots respectively, exhibiting various phytochemical activities. The main component fragmentation can be seen from the peaks in the mass spectrum as presented in Fig. 4-6 while the chemical constituents with their retention time (RT), molecular formula, molecular weight (MW), area (%) along with the nature of compound are presented in Table 4-6.

Table 4. Compounds identified in the Hexane extract of *Tylophora indica* leaves

Peak#	RT	Compound name	Nature of compound	Area %	Molecular formula	Molecular weight
1	4.202	2-Butanone, 3,3-dimethyl-1 (methylsulfonyl)-,O-[(methylamino)carbonyl] oxime	Ketones	79.10	$C_9H_{18}N_2O_4S$	250.32
2	36.670	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	7.22	$C_{20}H_{40}O$	296.5
3	37.158	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	Phytol	1.51	$C_{20}H_{40}O$	296.5
4	37.501	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	2.37	$C_{20}H_{40}O$	296.5
5	38.364	Tetracosanoic acid, methyl ester	Me-ester	0.36	$C_{25}H_{50}O_2$	382.66
6	41.195	2,2,4-Trimethyl-3,4-dihydro-2H-oxazolidine-3-oxide	-	0.27	$C_6H_{11}NO_2$	129
7	41.298	6(E),9(Z),13(E)-pendectriene	-	0.22	$C_{15}H_{26}$	206
8	41.510	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Diterpene alcohol	4.22	$C_{20}H_{40}O$	296.5
9	43.730	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl	Terpenoid Hydrocarbon	4.23	$C_{30}H_{50}$	410.7
10	44.239	7,9-(p-Methoxyphenylidenedioxy)-5-methoxy-2,4,6,8-tetraamethylnonan-1,3-diol	-	0.50	$C_{22}H_{36}O_6$	396.5

As shown in Table 4, among the compounds identified in the hexane extract of *T. indica* leaves, the most active compound is 2-butanone, 3, 3-dimethyl-1 (methylsulfonyl)-, O-[(methylamino)carbonyl] oxime. It had a peak area abundance of 79.10%. In contrast, 5– methyl–2–(1–methylethyl)–(1α,2β,5β)-cyclohexanol was identified as the main active compound present in the hexane extract of *T. indica* stem, belonging to the class of alcohols. It had an abundance of peak areas of 47.76% (Table 5).

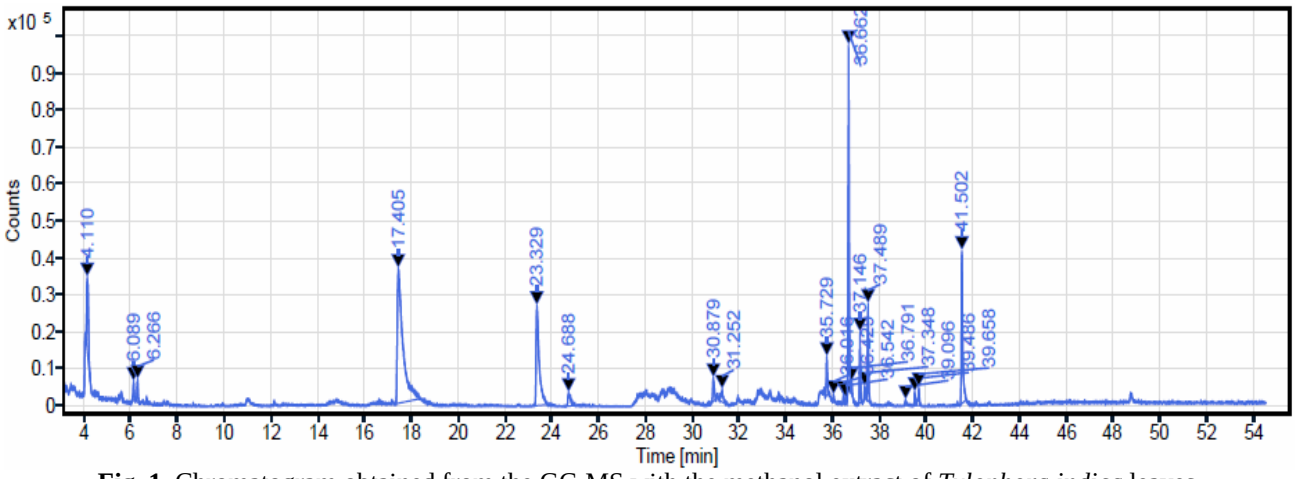

Fig. 1. Chromatogram obtained from the GC-MS with the methanol extract of *Tylophora indica* leaves

Lastly, 2-propyloxirane, belonging to the epoxide class, and with a peak area of 39.12%, was found to be the primary active compound present in the hexane extract of *T. indica* roots as showed in Table 6. The identified compounds showed various biological activities which was confirmed by Dr. Duke's phytochemical and ethnobotanical databases (https://phytochem.nal.usda.gov/) (Table 7).

Table 5. Compounds identified in the hexane extract of *Tylophora indica* stem

Peak#	RT	Compound name	Nature of compound	Area %	Molecular formula	Molecular Weight
1	3.429	1-Hexanol, 2-ethyl-(CAS)	Octyl alcohol	1.11	$C_8H_{18}O$	130.23
2	4.213	2-Butanone,3,3-dimethyl-1-(methylsulfonyl)-,O-[(methylami)]	-	15.39	$C_9H_{18}N_2O_4S$	250.32
3	6.518	7-methyl tetracyclo[4.1.0.0(2,4).(3,5) heptanes	-	1.71	C_8H_{10}	106.17
4	7.608	Nonane	Alkane hydrocarbon	1.73	C_9H_{20}	128.2
5	8.672	Undecane,2,6-dimethyl-	Alkanes	1.14	$C_{13}H_{28}$	184.36
6	8.902	Heptane,3-ethyl-2-methyl–	-	0.77	$C_{10}H_{22}$	142.29
7	11.000	Decane	Alkane hydrocarbon	1.46	$C_{10}H_{22}$	142.29
8	11.314	(1R,1”R,6S,9s,9”S)-dispiro (bicyclo[4.2.1]non-3-ene-9, 2’-o	-	0.86	$C_{18}H_{24}O$	256
9	12.076	Trifluoroacetyl-α-terpineol	Monoterpene	0.74	$C_{12}H_{17}F_3O_2$	250.26
10	17.339	Cyclohexanol,5-methyl-2- (1-methylethyl)-(1α,2β,5β)-	-	47.76	$C_{10}H_{20}O$	156.27
11	23.320	4-isopropenyl-1-methyl-1-cyclohexene	Monoterpene	14.03	$C_{10}H_{18}$	138.25
12	31.062	3-Octen-2-ol, (E)-	Aliphatic alcohol	0.06	$C_8H_{16}O$	128.21
13	31.327	4-Azido-2-methylbenzoic acid	-	0.20	$C_8H_7N_3O_2$	177.16
14	37.390	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Phthalate ester	1.00	$C_{16}H_{22}O_4$	278.34
15	49.293	Pentatriacontane	Alkanes	3.49	$C_{35}H_{72}$	492.9
16	50.761	γ-Tocopheryl methyl ether	Vitamin	8.55	$C_{29}H_{50}O_2$	430

In the present study, the GC-MS analysis of methanol and hexane extracts from leaves, stems, and roots of *T. indica* revealed the presence of various phytoconstituents, including ketones, alkanes, terpenes, fatty acid, fatty alcohol, aldehydes, carboxylic acid, anthraquinone (Harit & Sharma, 2017; Vanitha *et al.*, 2019). A variety of extracts of *T. indica* could be therapeutically effective because of these bioactive phytoconstituents. Different plant parts contained different active compounds. There are several factors that influence the composition of active compounds in plants, including genetic variation, environmental factors (such as temperature, light, soil nutrients, and water availability), developmental stages, herbivory, and harvesting time.

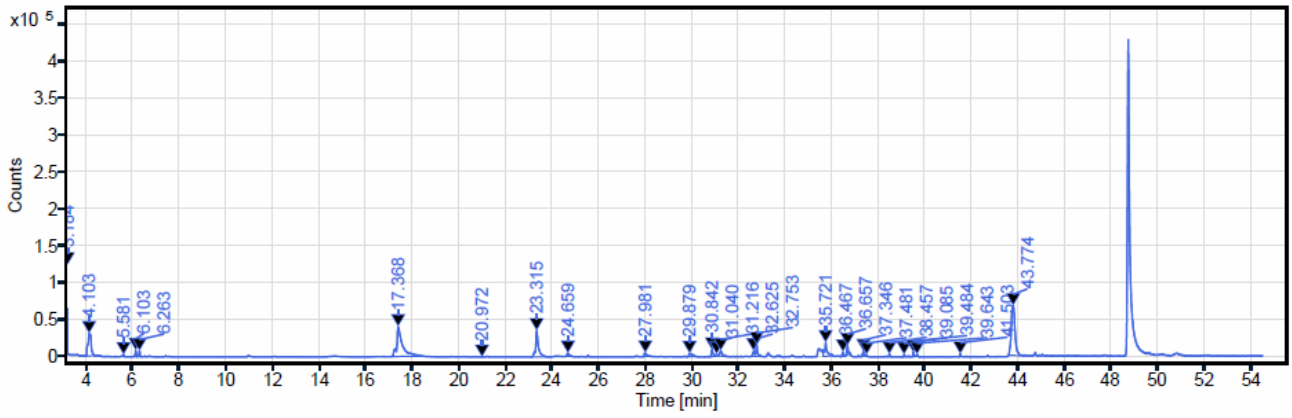

Fig. 2. Chromatogram obtained from the GC-MS with the methanol extract of *Tylophora indica* stem

Table 6. Compounds identified in the hexane extract of *Tylophora indica* roots

Peak#	RT	Compound name (Roots Hexane)	Nature of compound	Area %	Molecular formula	Molecular weight
1	1.909	propyl-Piperazine	Epoxides	39.12	$C_5H_{10}O$	86.13
2	2.049	methyl Cyclopentane	Cyclo alkane	0.32	C_6H_{12}	84.16
3	8.063	Oleic acid	Fatty acid	0.27	$C_{18}H_{34}O_2$	282.5
4	9.308	trans-13-octadecenoic acid	Fatty acid	0.55	$C_{21}H_{42}O_2Si$	354.7
5	9.951	cis-Vaccenic acid	Fatty acid	0.76	$C_{18}H_{34}O_2$	282.46
6	10.589	cis-Vaccenic acid	Fatty acid	0.45	$C_{18}H_{34}O_2$	282.46
7	11.015	cis-13-Octadecenoic acid	Fatty acid	0.77	$C_{18}H_{34}O_2$	282.46
8	11.622	cis-9-Hexadecenoic acid	Fatty acid	0.32	$C_{16}H_{30}O_2$	254.40
9	12.255	Oleic acid	Fatty acid	1.18	$C_{18}H_{34}O_2$	282.5
10	12.810	Hexadecanoic acid, 2-hydroxy-,methyl ester	-	0.25	$C_{17}H_{34}O_3$	286.5
11	13.179	9-Octadecenoic acid (Z)-, 2, 3- dihydroxy propyl ester	Elaidic acid	8.74	$C_{21}H_{40}O_4$	356.5
12	13.531	2,3-dihydroxypropyl elaidate	Elaidic acid	0.96	$C_{21}H_{40}O_4$	356.5
13	14.040	Oleyl alcohol, heptafluorobutyrate	Alkanes	22.26	$C_{22}H_{35}F_7O_2$	464.5
14	14.299	N'-(2-Bromobenzylidene)-2-(1-bromo-2-naphthyloxy) butyrohydrazide	-	7.13	$C_{21}H_{18}Br_2N_2O_2$	490.2
15	14.870	Acetic acid, 8-acetoxy-6-benzenesulfonyl-2-thia-6-aza-adamantan-4-yl ester	-	6.40	$C_{18}H_{21}NO_6S_2$	411.5
16	15.181	Quinoline, 6, 8- dichloro-4-bromoacetyl-2-(3-acetoxyphenyl)-	-	3.14	$C_{19}H_{12}BrCl_2NO_3$	453.1
17	15.794	Acetic acid, 8-acetoxy-6-benzenesulfonyl-2-thia-6-aza-adamantan-4-yl ester	-	1.42	$C_{18}H_{21}NO_6S_2$	411.5
18	20.951	9-Octadecenoic acid (z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Fatty acid	0.93	$C_{21}H_{40}O_4$	356.5
19	24.557	9-Octadecenoic acid (z), 2-hydroxy-1-(hydroxymethyl) ethyl ester	Fatty acid	1.37	$C_{22}H_{43}NO_3$	369.6
20	25.060	1,2-dihydro(4H) anthra(1,2-d)(1,3) oxazine-7,12-dione	Anthraquinone	3.67	$C_{16}H_{11}NO_3$	265.26

Based on the results of GC-MS analysis, 39 different phytochemical compounds have been discovered in the leaves, stems, and roots of *T. indica*, which may contribute to its medicinal properties. The major chemical compounds were identified in the methanolic and hexane leaves extract are :Bicyclo [3.2.1]octane-2,4-dione, Levomenthol, 4-isopropenyl-1-methyl-1-cyclohexene, 1-hexadecanol, 1,3-dibenzyloxy-2-propyl acetate, 5-(hydroxymethyl) -2-(1-methyl-2-imidazolyl) -1H-benzimidazol, anti-(2RS,3RS) -7-(N-Phenylamido) -9-methyl-10-phenylthio-7-a, Neophytadiene, (4R,5R)-2-[(RS)-4,8-dimethylnonyl]-2-methyl-N,N'-di(prop-2-, 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, 2-[3-(3-Oxocyclohexyl)propyl]-2-phenyl-1,3-dioxolane, Cyclohexadecane, 6-Methyl-2-(1'-propenyl) -8H-[1]benzopyran [7,8-d] oxazol-8-on, 2-Butanone, 3, 3-dimethyl-1 (methylsulfonyl)-,O-[(methylamino)carbonyl] oxime, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]], Tetracosanoic acid, methyl ester, 2,2,4-Trimethyl-3,4-dihydro-2H-oxazolidine-3-oxide, 6(E), 9(Z), 13(E)-pendectriene, 2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-Hexamethyl and 7,9-(p-Methoxyphenylidenedioxy)-5-methoxy-2,4,6,8-tetraamethylnonan-1,3-diol. The main active component of the methanolic extract of leaves is levomenthol, a well-known natural compound with a minty aroma and cooling sensation. A variety

of medicinal uses have been attributed to its use, including the relief of headaches, the treatment of nausea, and the reduction of inflammation (Kaur & Singh, 2012; Rani *et al.*, 2012; Wade *et al.*, 2019).

Table 7. Activity of all compounds identified in the *Tylophora indica*

S. No.	Compound name	Biological activity
1	2-Butanone,3,3-dimethyl-1-(methylsulfonyl)-,O-[methylamino)carbonyl] oxime	Anticancer; antidote
2	4-Isopropenyl-1-methyl-1-Cyclohexene	Methyl-guanidine-inhibitor
3	1, 2-Benzenedicarboxylic acid, diethyl ester	Acidifier
4	5-(Hydroxymethyl)-2-(1-methyl-2-imidazolyl)-1H-benzimidazol	Catechol-O-methyl-transferase-inhibitor
5	anti-(2RS,3RS)-7-(N-phenylamido)-9-methyl-10-phenylthio-7-a	Decrease norepinephrine production
6	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Provide oligosaccharide
7	1,2-Benzene di carboxylic acid, bis(2-methylpropyl) ester	Increase aromatic amino acid decarboxylase activity
8	Tetracosanoic acid, methyl ester	Catechol-O-methyl-transferase-inhibitor
9	Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate	Methyl-guanidine-inhibitor
10	2-Isopropyl-5methylhept-6-en-1-ol	Endocrine protective
11	Squalene	Squalene-Monooxygenase-Inhibitor
12	Heptane, 3-ethyl-2-methyl	Catechol-O-methyl-transferase-inhibitor
13	Cyclohexanol, 5-methyl-2-(1-methylethyl)-(1α, 2β, 5β)-	Methyl-guanidine-inhibitor
14	4-Isopropenyl-1-Methyl-1-Cyclohexene	Catechol-O-methyl-transferase-inhibitor
15	3-Octen-2-ol	Oligosaccharide provider
16	4-Azido-2-methylbenzoic acid	Increase aromatic amino acid decarboxylase activity
17	1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	Arachidonic-acid-inhibitor
18	γ-Tocopheryl methyl ether	Methyl-guanidine-inhibition
19	Nonanoic acid	Acidulant
20	9-Hexadecen-1-ol	Oligosaccharide provider
21	2,13-Octadecadien-1-ol	Provide oligosaccharides
22	Oleyl alcohol, heptafluorobutyrate	Alcohol-dehydrogenase-inhibitor
23	n-Hexadecanoic acid	Inhibit production of tumor necrosis factor
24	Oleic Acid	Inhibit production of uric acid
25	Vaccenic acid	Arachidonic acid-inhibitor
26	Benzenamine,4-methoxy-N-(triphenyl phosphoranylidene)	Arylamine-N-Acetyltransferase-Inhibitor
27	Purine-2,6-dione,1,3-di methyl-8-(morpholin-4-yl)-7-(2-oxo-2-phenyl ethyl)-3,7-dihydro-	Coronary-Dilator
28	Benzaldehyde, 2-hydroxy-5-nitro-,2-iodophenyl hydrazone	Aryl-hydrocarbon-hydroxylase-inhibitor
29	Iron, cyclopentadienyl-ethyl-1,2-di isopropyl phosphinoethane	Increase superoxide dismutase activity
30	Methyl Cyclopentane	Catechol-O-methyltransferase-inhibitor
31	Trans-13-octadecenoic acid	Increase aromatic amino acid decarboxylase activity
32	Hexadecanoic acid, 2-hydroxy-,methyl ester	17-beta-hydroxysteroid dehydrogenase-Inhibitor
33	9-Octadecenoic acid (z)-, 2, 3- dihydroxy propyl ester	Increase zinc bioavailability
34	Oleyl alcohol, heptafluorobutyrate	Detoxicant (Alcohol)
35	N'-(2-Bromobenzylidene)-2-(1-bromo-2-naphthyloxy) butyrohydrazide	Down regulation of nuclear and cytosol androgen
36	Acetic acid, 8-acetoxy-6-benzenesulfonyl-2-thia-6-aza-adamantan-4-yl ester	Thiamine-sparing
37	9-Octadecenoic acid (z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Acidifier
38	9-Octadecenoic acid (z), 2-hydroxyethyl ester	Provide zinc
39	1,2-dihydro(4H) anthra(1,2-d)(1,3) oxazine-7,12-dione	Hypothalmic-depressant

Source: Dr. Duke's phytochemical and ethnobotanical databases (https://phytochem.nal.usda.gov/).

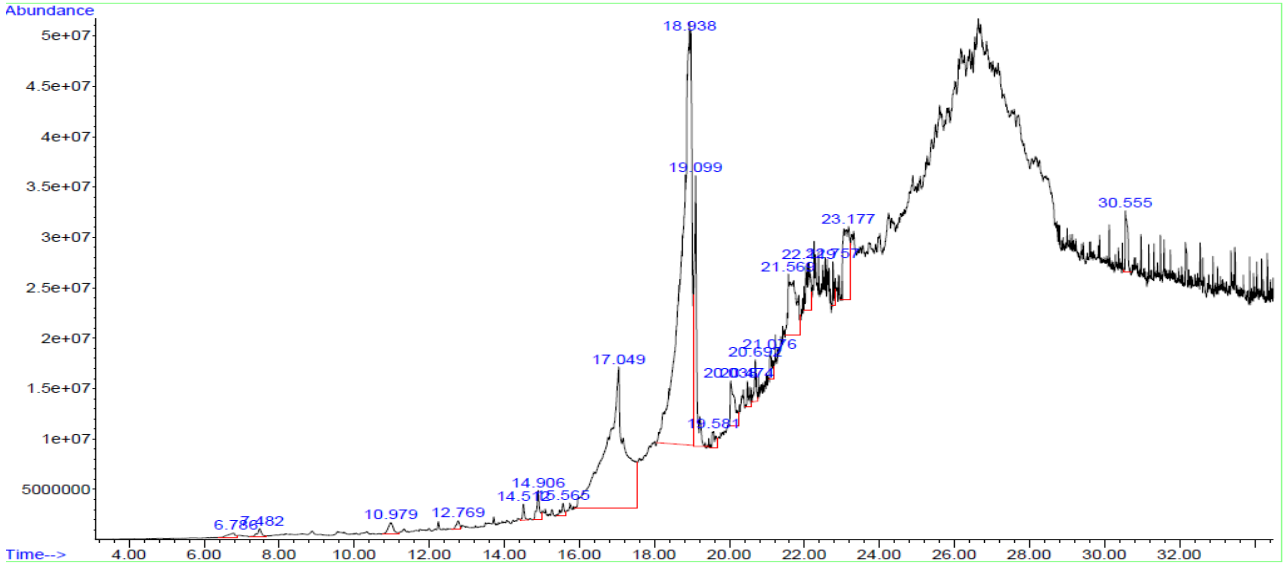

Fig. 3. Chromatogram obtained from the GC-MS with the methanol extract of *Tylophora indica* roots

The predominant chemical constituents in methanolic and hexane stem extract are as 2-[N-Benzyloxycarbonyl-(S)-phenylalanylamino]-4-methylpentan-1-ol, 1(Tetrahydropyranyloxy)hepta-2,6-diyn-4-ene, N-(2-Hydroxyethyl)-2-oximinoethanamide, 2,4-dimethoxybicyclo[3.2.1]octane, 1-Phenylhex-5-en-2-one, Cyclohexanol, 1-methyl-4-(1-methylethenyl)-acetate, 1-Tridecene, 1-Formyl-2,2,6-trimethyl-3-cis-(3-methylbut-2-enyl)-5-cyclo, 1,3,3-trimethyl cis, exotricyclo [3.3.0.0(2,4)]octan-6-one, tert-Butyl 8-Methyl-10-azabicyclo[4.3.1]deca-3,7-diene-10-carboxylate, Naphthalene,1,2,3,5,6,7,8,8Aoctahydro-1,8A-dimethyl-7-(1-Methylethenyl), 1H-Cycloprop[e]azulene,decahydro-1,1,7-trimethyl-4-methylene, Trichloroacetic acid,tridecyl ester, 6-Amino-3,4,7-triphenylpyrido[2',3':4,5]thieno[2,3-c]pyridazine, 3-Butyl-4-phenylfuran-2,5-dione, 4-hydroxytetradec-2-ynal, 1,11-Dodecadiene, Methyl 1,3-dihydro-2H-isobenzofuran-4-carboxylate, 3-(Allylamino)-5-hydroxy-1H-indole-3-carbonitrile, (2S,5R)-2-Isopropyl-5methylhept-6-en-1-ol, 1-Hexanol, 2-ethyl-, 2-Butanone, 3, 3-dimethyl-1- (methylsulfonyl) -, O-[(methylami)], 7-methyl-tetracyclo[4.1.0.0(2,4).(03,5) heptanes, Nonane, Undecane, 2, 6-dimethyl-, Heptane, 3-ethyl-2-methyl-, Decane, (1R,1"R,6S,9s,9"S)-dispiro (bicyclo[4.2.1]non-3-ene-9, 2'-o, Trifluoroacetyl-α-terpineol, 4-isopropenyl-1-methyl-1-cyclohexene, 3-Octen-2-ol, (E)-, 4-Azido-2-methylbenzoic acid, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Pentatriacontane and γ-Tocopheryl methyl ether. Squalene, the main compound in the methanolic extract of stem, is a triterpene with multiple biological properties, including antioxidant and anticancer activity (Spanova & Daum, 2011; Kim & Karadeniz, 2012).

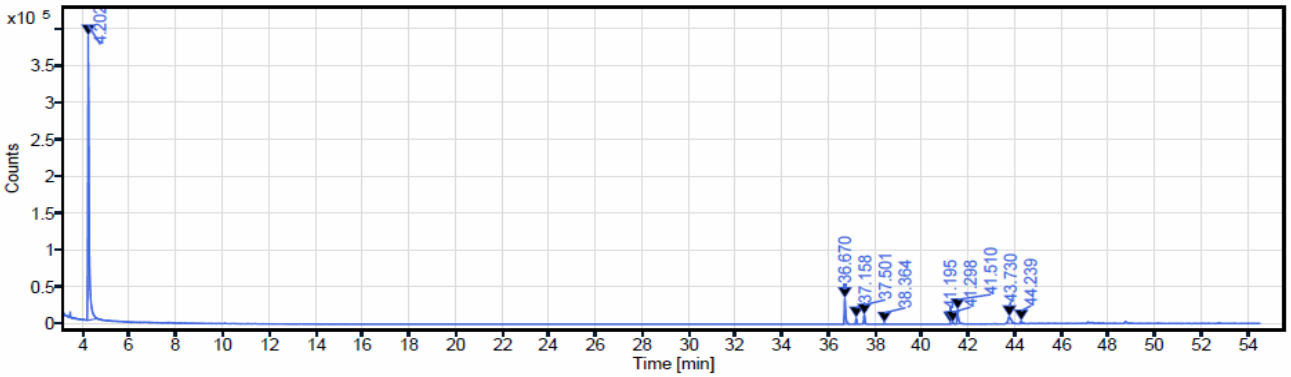

Fig. 4. Chromatogram obtained from the GC-MS with the hexane extract of *Tylophora indica* leaves

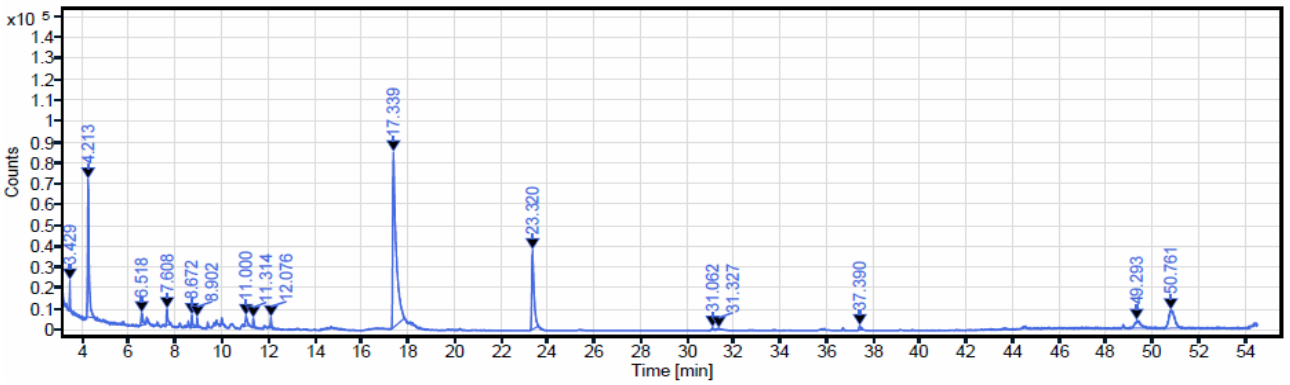

Fig. 5. Chromatogram obtained from the GC-MS with the hexane extract of *Tylophora indica* leaves

The leading chemical compounds were obtained in methanolic and hexane root extracts are as nonanoic acid, 2-undecenal, cis-9-hexadecenal, 9-hexadecen-1-ol,(z)-, Z,E-2,13-octadecadien -1-ol, cis-11-hexadecenal, oleyl alcohol, n-hexadecanoic acid, oleic acid, cis-vaccenic acid, benzenamine,4-methoxy-N-(triphenyl phosphoranylidene)-, B(9a)-homo-19-norpregna-9(11),9a-dien-16-ol, 20-one, Purine-2,6-dione,1,3-di methyl-8-(morpholin-4-yl)-7-(2-oxo-2-phenyl ethyl)-3,7-dihydro-, eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy] methyl]ethyl ester, Benzaldehyde, 2-hydroxy-5-nitro-,2-iodophenylhydrazone, 1,6-dihydroxy-3-methylanthraquinone, o,o'-bis(trimethylsilyl)-, Iron, cyclopentadienyl-ethyl-1,2-di isopropyl phosphinoethane, propyl-piperazine, methyl cyclopentane, trans-13-octadecenoic acid, cis-13-octadecenoic acid, 9-octadecenoic acid (z)-, 2, 3- dihydroxy propyl ester, 2,3-dihydroxypropyl elaidate, N'-(2-bromobenzylidene) -2-(1-bromo-2-naphthyloxy) butyrohydrazide, acetic acid, 8-acetoxy-6-benzenesulfonyl-2-thia-6-aza-adamantan-4-yl ester, quinoline, 6, 8- dichloro-4-bromoacetyl-2-(3-acetoxyphenyl)-, 9-Octadecenoic acid (z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester and 1,2-dihydro(4H) anthra(1,2-d)(1,3) oxazine-7,12-dione.

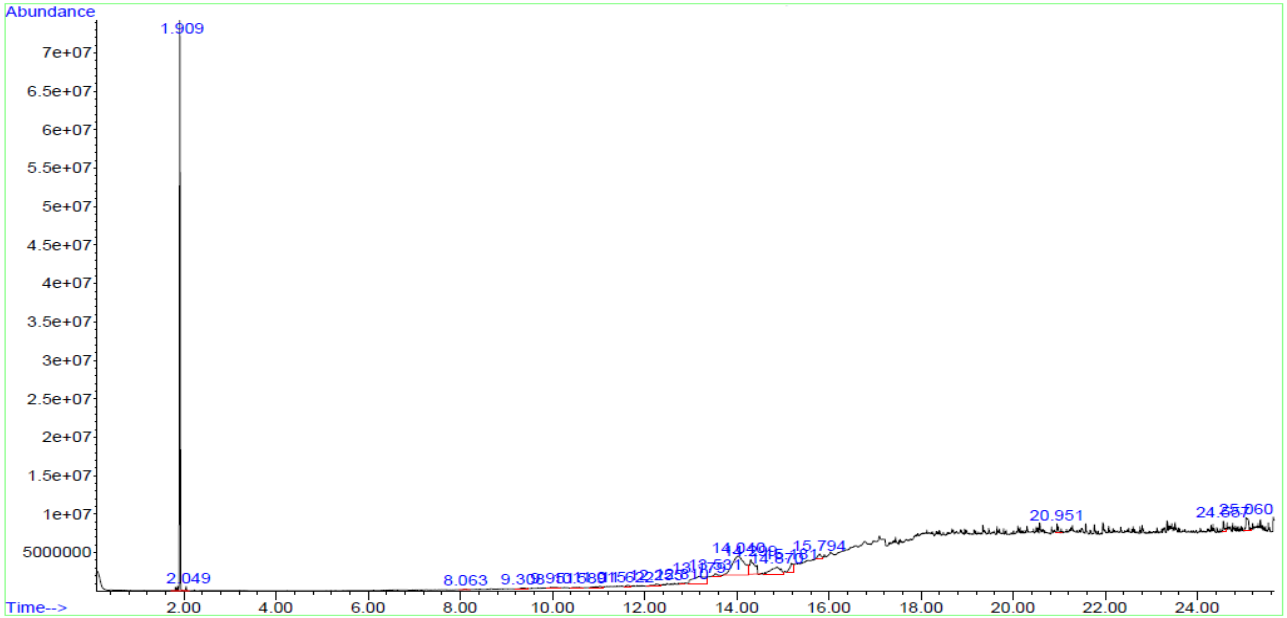

Fig. 6. Chromatogram obtained from the GC-MS with the Hexane extract of *Tylophora indica* roots

Oleic acid, which is the main active compound in the roots, is a fatty acid found in olive oil, sunflower oil, and other vegetable oils. There have been numerous studies showing that it can reduce heart disease risk, improve brain function and helpful in the inhibition of the production of uric acid (Carrillo *et al*., 2012; Sales-Campos *et al*., 2013; Sidana & Joshi, 2013; Karacor & Cam, 2015). The methanolic root extract contains a compound called 9-Hexadecen-1-ol that inhibits the production of

tumor necrosis factors. The nonanoic acid also serves as an acidulant, giving foods a tart, sour, or acidic flavor or enhancing their perceived sweetness. An arachidonic acid inhibitor, vaccenic acid is found in hexane and methanol extractions of the root and has been reported to exhibit anti-carcinogenic properties *in vivo* as well as causing apoptosis in many cancers cell lines *in vitro* (Lim *et al*., 2014; Willie *et al.,* 2021).

CONCLUSION

Tylophora indica has a long history of traditional use in Ayurvedic and Unani medicine for various therapeutic purposes. It possesses a variety of pharmacological properties, including antiasthmatic, anti-inflammatory, anticancer, and antidiarrheal effects. This plant contains bioactive compounds, such as alkaloids, flavonoids, and tannins. This study analyzed the leaves, stems, and roots of *T. indica* for their secondary metabolites, and methanol extracts identified the highest number of compounds, followed by hexane extracts. GC-MS analysis revealed 39 phytochemical compounds in the leaves, stems, and roots of *T. indica* that may contribute to its medicinal properties. Levomenthol, squalene, cyclohexanol, methyl cyclopentane, nonanoic acid and oleic acid were identified as important compounds in the study. There are numerous biological activities associated with these compounds, which may make them useful as treatments for various disorders. Some of the bioactive compounds found in *T. indica* may be used as potential drug formulations, and more research is needed to possibly develop novel drugs using these compounds. It can therefore be concluded that *T. indica* contains an array of bioactive compounds that could be useful for drug development, and more research is needed to discover its full potential.

ACKNOWLEDGEMENTS

The authors are highly grateful to the Head, Department of Botany, Dayalbagh Educational Institute for their encouragement and continuous support. A grant from UGC-SAP provided support for this work (UGC letters No. F. 5-8/2O18/DSR-l (SAP-ll).

REFERENCES

Azcue JM. 1996. Comparison of different cleaning procedures of root material for analysis of trace elements. *International Journal of Environmental Analytical Chemistry*. vol 62(2): 137–145. doi: https://doi.org/10.1080/03067319608027060.

Bembde NS, Meshram PV, Patil MK, Shaikh J. 2020. The Preliminary Phytochemical Analysis of Ethanolic extract of *Tylophora indica*. *Research Journal of Pharmacognosy and Phytochemistry*. vol 12(1): 1–6. doi: http://dx.doi.org/10.5958/0975-4385.2020.00001.1.

Carrillo C, Cavia MDM, Alonso-Torre S. 2012. Role of oleic acid in immune system; mechanism of action; a review. *Nutrición Hospitalaria. vol* 27(4): 978–990. doi: http://dx.doi.org/10.3305%2Fnh.2012.27.4.5783.

Craig RD, Bateman RH, Green BN, Millington DS. 1979. Mass spectrometry instrumentation for chemists and biologists. *Philosophical Transactions of the Royal Society A, Mathematical, Physical, and Engineering Sciences*. vol 293(1400): 135–155. doi: https://doi.org/10.1098/rsta.1979.0085.

Enegide C, Okhale SE. 2023. Ethnomedicinal, phytochemical, and pharmacological review of asclepiadaceae. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine.* vol 2(1): 3–18. doi: https://doi.org/10.4103/jpdtsm jpdtsm_100_22.

Gantait S, Kundu S. 2017. Neoteric trends in tissue culture-mediated biotechnology of Indian ipecac [*Tylophora indica* (Burm. f.) Merrill]. *3 Biotech.* vol 7: 1–15. doi: https://doi.org/10.1007/s13205-017-0865-8.

Government of India. 1972. Wildlife (Protection) Act 1972. New Delhi: National Informatics Centre (NIC), Ministry of Electronics & Information Technology, Government of India. https://www.india.gov.in/.

Gupta M, Mukhtar HM, Ahmad S. 2010. Phyto-pharmacological and plant tissue culture overview of *Tylophora indica* (burm f.) Merill. *Journal of Pharmaceutical Science and Research*. vol 2(7): 401–411.

Gupta V, Guleri R, Gupta M, Kaur N, Kaur K, Kumar P, Anand M, Kaur G, Pati PK. 2020. Anti-neuroinflammatory potential of *Tylophora indica* (Burm. f) Merrill and development of an efficient *in vitro* propagation system for its clinical use. *Plos One*. vol 15(3): 1–20. doi: https://doi.org/10.1371/journal.pone.0230142.

Gururani R, Patel S, Yaduvanshi N, Dwivedi J, Paliwal S, Sharma S. 2020. *Tylophora indica* (Burm. f.) Merr: An insight into phytochemistry and pharmacology. *Journal of Ethnopharmacology*. vol 262: 1–18. doi: https://doi.org/10.1016/j.jep.2020.113122.

Harit D, Sharma RA. 2017. Isolation, identification and characterization of flavonoids from *Nyctanthes arbortristis* and *Tylophora indica. World Journal of Pharmaceutical Research*. vol 6(12): 1243-1254. doi: https://doi.org/10.20959/wjpr201712-9759.

Hussein RA, El-Anssary AA. 2019. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal Medicine*. vol 1(3): 11–30. doi: http://dx.doi.org/10.5772/intechopen.76139.

Karacor K, Cam M. 2015. Effects of oleic acid. *Medical Science and Discovery*. vol 2(1): 125–132. doi: https://doi.org/10.17546/msd.25609.

Kaur H, Anand M, Goyal D 2014. HPTLC based analysis of tylophorine from cultures and *in vitro* regenerated plants of *Tylophora indica*- an endangered medicinal plant. *International Journal for Pharmaceutical Research Scholars*. vol 3(2): 91–95.

Kaur H, Singh K. 2012. A brief phytopharmacological overview of *Tylophora indica*-an endangered medicinal plant. *International Journal of Pharmaceutical Sciences and Research*. vol 3(11): 4073–4076.

Kim SK, Karadeniz F. 2012. Biological importance and applications of squalene and squalane. *Advances in Food and Nutrition Research*. vol 65: 223–233. doi: https://doi.org/10.1016/B978-0-12-416003-3.00014-7.

Kour N, Gupta M. 2015. Utility of *Tylophora indica* as antiasthmatic plant: A review. *Journal of Plant Development Sciences*. vol 7(12): 869–873.

Lim JN, Oh JJ, Wang T, Lee JS, Kim SH, Kim YJ, Lee HG. 2014. Trans-11 18: 1 vaccenic acid (TVA) has a direct anti-carcinogenic effect on MCF-7 human mammary adenocarcinoma cells. *Nutrients*. vol 6(2): 627–636. doi: https://doi.org/10.3390/nu6020627.

Maheshwari M, Vijayarengan P. 2020. Phytochemical screening and GC-MS analysis of *Tylophora indica*. International *Journal of Botany Studies*. vol 5(3): 255–257.

Mamani R, Alhaji NM. 2019. GC-MS analysis of phytocomponents in methanolic extract of *Coleus aromaticus*. *Journal of Pharmacognosy and Phytochemistry.* vol 8(4): 106–109.

Nazar S, Hussain MA, Khan A, Muhammad G, Bukhari SNA. 2020. Alkaloid-rich plant *Tylophora indica*; current trends in isolation strategies, chemical profiling and medicinal applications. *Arabian Journal of Chemistry*. vol 13(8): 6348–6365. doi: https://doi.org/10.1016/j.arabjc.2020.05.037.

Prasad M, Srivastava JN, Dantu PK, Ranjan R. 2019. Medicinal plants of DEI herbal garden, Dayalbagh: A survey. *Journal of Pharmacognosy and Phytochemistry*. vol 8(5): 6–22.

Ranemma M, Nagendram E, Niranjan S, Reddy AN, Mohan CH. 2017. Phytochemical analysis, antibacterial and antioxidant activity of *Tylophora indica*. *International Journal of Current Microbiology and Applied Sciences.* vol 6(2): 487–493. doi: http://dx.doi.org/10.20546/ijcmas.2017.602.055.

Rani AS, Patnaik S, Sulakshanaand G, Saidulu B. 2012. Review of *Tylophora indica*-an antiasthmatic plant. *FS Journal of Research in Basic and Applied Science*. vol 1(3): 20–21.

Raut S, Raut S, Ghadai A. 2012. Phytoceutical evaluation and antimicrobial properties of *Eclipta alba* and *Tylophora indica*. *International Journal of Microbiology Research*. vol 4(5): 227–230.

Sales-Campos H, de Souza PR, Peghini BC, da Silva JS, Cardoso CR. 2013. An overview of the modulatory effects of oleic acid in health and disease. *Mini-reviews in Medicinal Chemistry*. vol 13(2): 201–210. doi: https://doi.org/10.2174/138955713804805193.

Samaddar T, Nath S, Halder M, Sil B, Roychowdhury D, Sen S, Jha S. 2012. Karyotype analysis of three important traditional Indian medicinal plants, *Bacopa monnieri*, *Tylophora indica* and *Withania somnifera*. *The Nucleus*. vol 55: 17–20. doi: https://doi.org/10.1007/s13237-012-0048-2.

Sharma BK. 2022. Reproductive biology of *Gymnema sylvestre* and *Tylophora indica* (Asclepiadaceae) in Central India. *International Journal for Research Trends and Innovation*. vol 7(7): 1–7. doi: https://doi.org/10.6084/m9.doione.IJRTI2207159.

Sidana J, Joshi LK. 2013. Recycle HPLC: A powerful tool for the purification of natural products. *Chromatography Research International.* vol 2013: 1–8. doi: http://dx.doi.org/10.1155/2013/509812.

Spanova M, Daum G. 2011. Squalene–biochemistry, molecular biology, process biotechnology, and applications. *European Journal of Lipid Science and Technology*. vol 113(11): 1299–1320. doi: https://doi.org/10.1002/ejlt.201100203.

Thakur AV, Ambwani S, Ambwani TK, Ahmad AH, Rawat DS. 2018. Evaluation of phytochemicals in the leaf extract of *Clitoria ternatea* Willd. through GC-MS analysis. *Tropical Plant Research*. vol 5(2): 200–206. doi: https://doi.org/10.22271/tpr.2018.v5.i2.025.

Vani P, Mohan C, Reddy AN. 2021. Photochemical analysis and antibacterial activity *Tylophora indica* (Burm. F.) Merill. *Journal of Pharmacognosy and Phytochemistry*. vol 10(3): 91–93. doi: https://doi.org/10.22271/phyto.2021.v10.i3b.14063.

Vanitha A, Vijayakumar S, Ranjitha V, Kalimuthu K. 2019. Phytochemical screening and antimicrobial activity of wild and tissue cultured plant extracts of *Tylophora indica*. *Asian Journal of Pharmacy and Pharmacology*. vol 5(1): 21–32. doi: https://doi.org/10.31024/ajpp.2019.5.1.3.

Vivean PR, Kumar V, Manigandan LS, Sasikala M, Parthibhan P. 2014. *Tylophora indica*—A Mini Review. *International Journal of Frontiers in Science and Technology*. vol 2(4): 58–66.

Wade AG, Crawford GM, Young D, Corson S, Brown C. 2019. Comparison of diclofenac gel, ibuprofen gel, and ibuprofen gel with levomenthol for the topical treatment of pain associated with musculoskeletal injuries. *Journal of International Medical Research.* vol 47(9): 4454–4468. doi: https://doi.org/10.1177/0300060519859146.

Willie P, Uyoh EA, Aikpokpodion PO. 2021. Gas chromatography-mass spectrometry (GC-MS) assay of bio-active compounds and phytochemical analyses in three species of Apocynaceae. *Pharmacognosy Journal*. vol 13(2): 383–392. doi: http://dx.doi.org/10.5530/pj.2021.13.49.