Comparative GCMS Analysis of Hexane Extracts of Male and Female Stems of *Tinospora cordifolia* Miers ex Hook. F. and Thoms

Anisha Bano[1], Harcharan Singh Dhaliwal[2], Vivek Sharma[1]*

[1]Department of Botany, Akal College of Basic Sciences, Eternal University, Baru Sahib-173101 (H.P.) India.

[2]Department of Biotechnology, Akal College of Agriculture, Eternal University, Baru Sahib-173101 (H.P.) India.

Abstract:

Tinospora cordifolia Miers ex Hook. F. and Thoms is a versatile medicinal plant which has the ability to cure many diseases. Being a dioecious plant, the present study was the first attempt to compare volatile compounds in hexane extract of male and female stems of *T. cordifolia*. Total 50 compounds were identified in male and 47 in female stem. Further, on the basis of RI value 8 compounds (Octanoic acid, methyl ester; 2-Decadienal, (E)-; 2,4-Decadienal; Dodecanoic acid, methyl ester; Tetradecanoic acid methyl ester; Hexadecanoic acid, methyl ester; 9,12-Octadecanoic acid (Z,Z)-, methyl ester and Octadecanoic acid, methyl ester) were confirmed in female and 6 compounds (Octanoic acid, methyl ester; Dodecanoic acid, methyl ester; Tetradecanoic acid, methyl ester; Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester and Octadecanoic acid, methyl ester) were confirmed in male. Hexadecanoic acid, methyl ester found to be major component in stem and its percentage was higher in female plant (36.20%) as compared to male stem (30.74%). It has anti-inflammatory, hypocholesterolemic and anti-arthritic activities. The percentage of 9, 12-octadecadienoic acid, methyl ester and octadecanoic acid, methyl ester was found to be higher in male (12.89 and 10.23% respectively) as compared to female (5.24 and 9.65% respectively).

Keywords: Hexadecanoic acid, Methyl ester, Retention Index, *T. cordifolia*.

*Corresponding author: Dr. Vivek Sharma, vivek03sharma@rediffmail.com

Introduction

Tinospora cordifolia Miers ex Hook. F. and Thoms (Menispermaceae) is an important diploid, dioecious climbing shrub which has numerous pharmaceutical and nutraceutical applications (Bhan 2016). The plant possesses adaptogenic, immunomodulatory (Sharma et al. 2012), anti-diabetic (Patel et al. 2012), hepatoprotective (Kavitha et al. 2011), neuroprotective (Kosaraju et al. 2014), radioprotective, cytoprotective (Patel et al. 2013), anti-cancer (Leyon and Kuttan 2004) and anti-oxidant properties (Premanath and Lakshmidevi 2010). The plant has the ability of cleaning and detoxifying whole body via liver and it can also rejuvenate and strengthen the body. The medicinal importance of this plant is attributed to the presence of alkaloids, diterpenoid lactones, glycosides, sesquiterpenoid, steroids, polysaccharides and aliphatic compounds. Stem is the most important part of plant which has pharmacologically and nutraceutically one of the most important applications. Despite of all this, its roots, leaves and fruits are also pharmacologically important. Its roots and leaves have anti-oxidant and anti-diabetic properties (Pandey et al. 2012; Singh et al. 2013). The anti-diabetic and immunomodulatory potential of stem is well documented in traditional and modern scientific literature (Upadhyay et al. 2010; Sangeetha et al. 2013). In Ayurvedic Pharmacopoeia, stem of *T. Cordifolia* has been approved as medicine due to its high alkaloid content. Stem starch and extract alone or in combination with honey is used as tonic, in skin diseases and fever (Reddy et al. 2009; Bhatt et al. 1987). In a study, hexane, ethyl acetate and methanol extract of *T. cordifolia* stem were found have anti-diabetic activity in streptozotocin induced diabetic rats by reducing blood glucose level (Rajalakshmi et al. 2009). Seven active compounds have also been isolated and characterized from *T. cordifolia* stem which known to have immunomodulatory activity (Sharma et al. 2012).

Though great attention has been paid to isolate secondary metabolites from various parts of *T. cordifolia* but no or little attention has been paid on qualitative and quantitative distribution of secondary metabolites in male and female plants, as the plant is dioecious in nature. As we know different phytoconstituents of an herbal extract, act as multi-component therapeutic system which can affect the health of an organism through complex and multi targeted interactions. Variations in the levels of these phytoconstituents can affect the quality of herbal extracts which could lead to inconsistent medicinal properties. Plant metabolomic approaches

like HPLC, HPTLC, LCMS, GCMS and NMR spectroscopy can be effective and excellent tools for the quality control of medicinal plants and their products (Bhatia et al. 2013).

Initial screening of medicinal plants by these analytical techniques provides basic information about compounds present in plant and helps in selecting biologically active plants. In recent years, GCMS has commonly been employed for identification of various bioactive compounds present in medicinal plants (Juszczak et al. 2019; Satapute et al. 2019 & Fan et al. 2018). GCMS is one of the most sensitive, fast and highly accurate analytical technique which require a small volume of plant extract and can detect various compounds like alcohols, alkaloids, amino acids, long chain hydrocarbons, nitro compounds, organic acids, steroids and esters (Razack et al. 2015). Very few reports (Bajpai et al. 2016; 2017) are available in literature which can give an idea of phytochemical variations in male and female plant. In best of knowledge, there was not even a single report of comparing the phytochemical composition of hexane extract of male and female stem of *T. cordifolia* through GCMS. Hence, in the present study, GCMS technique was used for the detection and identification of biologically active compounds in male and female stem of *T. cordifolia*.

Materials and Methods

Collection of Plants and Identification

Stems of male and female plants of *T. cordifolia* were collected from Bilaspur district (31.33^0N 76.75^0E, 673m/2,208ft) of Himachal Pradesh, North India in the month of July. The plants were identified by Botany Department, Eternal University Baru Sahib, Himachal Pradesh and accessions (62590 & 62591) were submitted at Herbarium of Punjabi University Patiala, Punjab, India.

Chemicals

All the solvents (Hexane, Methanol and Sulphuric acid) were of analytical grade, purchased from Merck, Life Science Pvt. Ltd. Mumbai, India while NaCl and K_2SO_4 were purchased from HiMedia.

Preparation of n-Hexane Extract

Stems of male and female plants were shade dried for 10-15 days and were powdered mechanically using electrical blander. About 100 g of powdered plants material were soaked in 300mL of hexane and kept for 24 hours at room temperature. After 24 hours, the extracts were filtered through Whatman filter paper No. 1. The process was repeated thrice and the collected extracts were dried under vacuum at 32^0C using rotavapor (Rotavapor R-210, Buchi, Switzerland) to give 0.404 g of n-hexane extract.

Gas Chromatography Mass Spectrometry (GCMS)

For GCMS analysis hexane extract of both the plants were derivatized individually so that maximum number of components can be converted into volatile form. For derivatization 10mg of hexane extract was taken in a two neck round bottom flasks and was dissolved in 4ml of 1%H_2SO_4 in dried methanol. The two necked round bottom flask was heated at 50^0C on water bath under nitrogen atmosphere for 12 hours. After 12-hour heating, to the solution added 4mL of aqueous NaCl (4%) and the derivatives were extracted twice with 10mL of hexane in separating funnel. After the collection of hexane layer, to the remaining solution added K_2SO_4 and again partition was done with hexane. The collected hexane layers were dried and subjected to GCMS analysis. The gas chromatography mass spectrometry (GCMS) analysis of the hexane extract was performed using a Shimadzu QP 2010 equipped with AOC-5000 auto-injector using a ZB-5MS (J&W Scientific, Folsom, CA, USA) capillary column (30 m × 0.25 mm i.e., 0.25 μm thickness). The GC oven temperature was 40°C for 4 minutes and then to 220°C at 4°C/minute and held for 15 minutes. Injector temperature, 250°C; interface temperature, 250°C; acquisition mass range, 40–800 amu; ionization energy, 70 eV. Helium was used as carrier gas. A standard solution of n-alkanes (C_9-C_{24}) was used to obtain the retention index (RI) with those reported in literature. The metabolites were identified by comparing their mass spectra (MS) with NIST library, WILEY library data base and Adams libraries (Stein 1990; Adams 2004).

Results and discussion

The TIC (Total Ion Count) of gas chromatograms of hexane extract of both male (Figure 1.) and female (Figure 2.) stem have shown separation of 50 peaks belonging to different compounds. In case of male stem, all the 50 compounds were identified by matching their mass and mass fragments with NIST and WILEY library data base (Table 1.) while, in case of female stem, total 47 compounds were identified (Table 2.).

These compounds belong to different classes like: hydrocarbons (Hexadecanoic acid, methyl ester), aromatic hydrocarbons (Benzene, (1-Butylheptyl)-), medium chain aldehydes (Hexanal), alcohols (Artemisia alcohol) and carbonyl compounds (Methyl-4-oxooctanoate). For the further confirmation, RI values were matched with Adams library and eight compounds were confirmed in female stem while six compounds were confirmed in male stem (Table 3.). Comparative analysis of these compounds in male and female stem (Figure 3.) have shown the presence of octanoic acid, methyl ester; dodecanoic acid, methyl ester; tetradecanoic acid, methyl ester; hexadecanoic acid, methyl ester; 9,12-octadecadienoic acid (Z,Z)-, methyl ester and octadecanoic acid, methyl ester in both male and female stem, while, 2-decadienal, (E)-; 2,4-decadienal were only present in female stem in low percentage (0.45 and 0.47% respectively). (E,E)-2,4-decadienal and (E)-2-decenal are polyunsaturated fatty aldehydes which were also present in methanolic wood extract of *Ailanthus altissima* and shown significant nematocidal activity against *Meloidogyne javanica* (Caboni et al. 2012).

Hexadecanoic acid, methyl ester was found to be the major compound in both male and female stem but its percentage was higher in female stem (36.20%) as compared to male stem (30.74%) (Figure 3.). Hexadecanoic acid, methyl ester (Synonym: Palmitic acid, methyl ester; Methyl palmitate) is a saturated fatty acid methyl ester (C16:0). Fatty acids play an important role in various life processes. One of their important roles is structural, as constituents of phospholipids which are important components of cell membrane (Hanaka et al. 2020; Venancio et al. 2022). Zhukow studied the role of palmatic acid in structure and functions of plant cell membranes. He suggested a relationship, when polarity of lipid fraction rose, the content of palmatic acid of fraction increases and among the phospholipid, the greatest content of palmitic acid is usually associated with phosphatidyl inositol (Zhukow, 2015). Palmitic acid has also the ability to reduce the incidence of soil-borne diseases in root exudates and promote the growth of some crop plant. Its application decreases the population of *Fusarium oxysporum* f.s.p. *niveum*, reduces the disease severity of *Fusarium* wilt and

promote the growth of watermelon. It also changes the rhizosphere microbial composition of watermelon (Ma et al. 2021). Palmitic acid, methyl ester (PME) is a potent vasodilator and can also acts as novel neuroprotective agent against cardiac arrest. Immediate administration of 0.02 mg/kg PME after cardiopulmonary resuscitation enhances cerebral blood flow *in vivo*. Additionally, it also alleviates neuronal cell death and promotes functional outcomes in asphyxia cardiac arrest (Hui-Chao Lee et al. 2018). It also inhibits cardiac arrest induced neural-inflammation and mitochondrial dysfunction (Yin-Chin Wu et al. 2021). It also exhibits anti-inflammatory, hypocholesterolemic, hepatoprotective, nemiticidal, antihistaminic, anti-eczemic, anti-acne, α reductase inhibitor, anti-androgenic, anti-arthritic and anti-coronary properties (Krishnamoorthy and Subramaniam 2014).

The percentage of 9,12-octadecadienoic acid, methyl ester (12.89%); octadecanoic acid, methyl ester (10.23%), tetradecanoic acid, methyl ester (3.07%) and dodecanoic acid, methyl ester (1.15%) was found to higher in male stem (Figure 3.). While the percentage of octanoic acid, methyl ester (0.95%) was found to be higher in female stem. Mass fragments of some of these compounds are given in Figure 4. Tetradecanoic acid, methyl ester has antibacterial and antifungal activity while octadecanoic acid, methyl ester known to have antimicrobial activity (Belakhdar et al. 2015). 9,12-octadecadienoic acid, methyl ester is known to be an excellent anticancer compound (Yu et al. 2005; Abubakar and Majinda 2016).

In best of our knowledge, the present analysis was the first attempt to comparatively study the metabolites present in hexane extracts of male and female stem of *T. cordifolia* through GCMS. Though, Albinjose et al. 2015, has also done the GCMS analysis of various extracts of *T. cordifolia* irrespective of gender of plant and identified total 40 compounds from different extracts (11-petroleum ether, 15-chloroform and 14-methanol). In our study, we were able to identified 50 compounds in male and 47 compounds in female stem from single hexane extract. In present study, derivatization of hexane extract was done by acid catalyzed methylation of analytes which result in formation of methyl esters. Derivatization makes the analytes volatile so that they can be eluted at reasonable temperatures without thermal decomposition which is important for GC analysis (Knapp, 1979). Methylation, makes the methyl esters of fatty acids, so in this study most of the identified compounds were fatty acid methyl esters (FAMs) which were present in higher percentage. Derivatization could be one of the reasons for separation of large number of compounds in hexane extract. In another study, 15 compounds were identified from methanolic stem extract showing the

presence of inositol, brucine, 1-deoxy-, trans-sinapyl alcohol and (E)-4-(3-hydroxy-prop-1-en-1-yl)-2-methoxyphenol etc. in higher proportion (Modi et al. 2021). Sinha et al. 2017, has detected 64 compounds in methanolic stem extract but still the percentage of hexadecanoic acid, methyl ester (1.10%) and n-hexadecanoic acid (23.12%) were low as compared to present study.

Such qualitative and quantitative distribution of compounds is a serious issue in quality control of herbal formulations, as each compound has its own biological importance and difference in the quantity of compounds with in the species can greatly affect the efficacy of their biological activities. Only 7% known taxon of different orders and family show dioecy (Renner et al. 1995) and very little attention has been paid to study the effect of dioecy on qualitative and quantitative distribution of plant metabolites. The present study is such an attempt to study the distribution of secondary metabolites in male and female stem of *T. cordifolia*.

Conclusion

So it become important to carefully select the plant material with desired activity for the herbal product formulations so that quality of formulations can be maintained for the high degree of efficacy. The analytical techniques like GCMS could be an effective tool for the quantification of compounds in plant material along with HPLC and LCMS. In the present study, comparative GCMS analysis of male and female stem is an attempt to summarize that which compounds are present in higher quantity in which gender so that the quality of herbal formulations can be controlled.

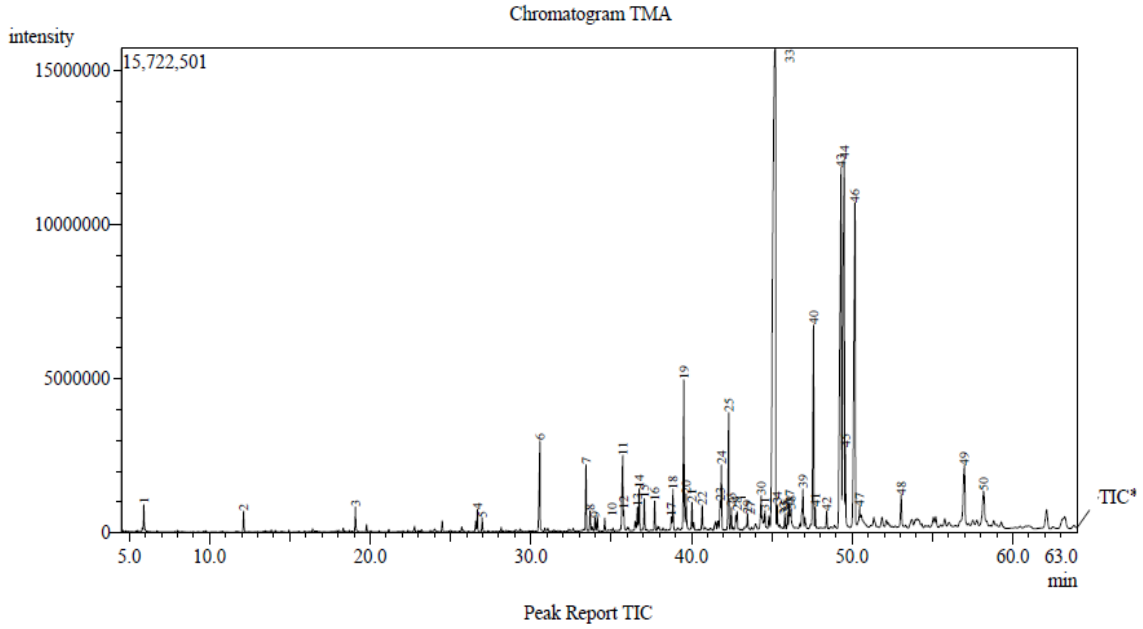


Figure 1. Gas chromatogram of hexane extract of male stem of *T. cordifolia.*

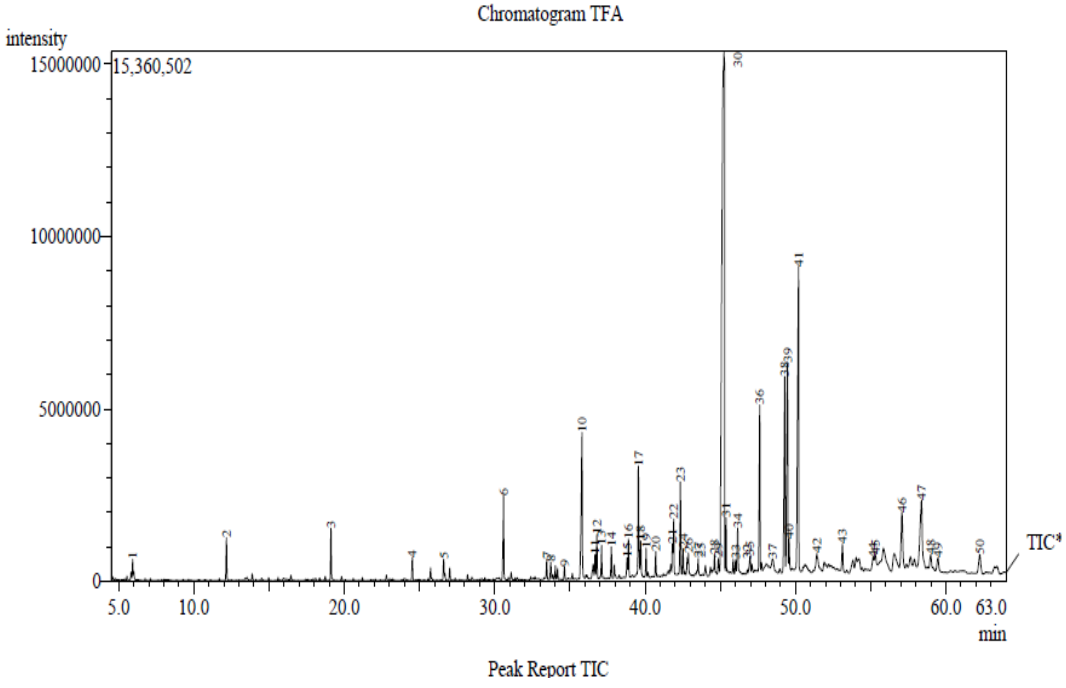


Figure 2. Gas chromatogram of hexane extract of female stem of *T. cordifolia.*

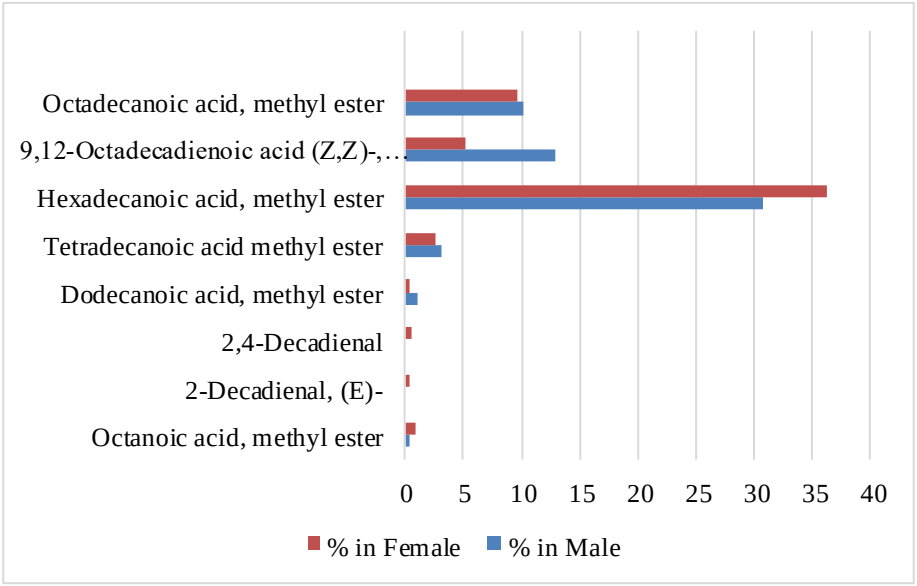
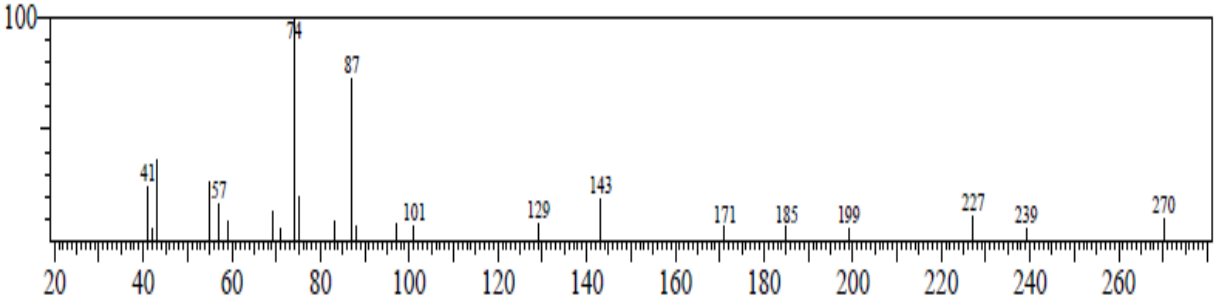
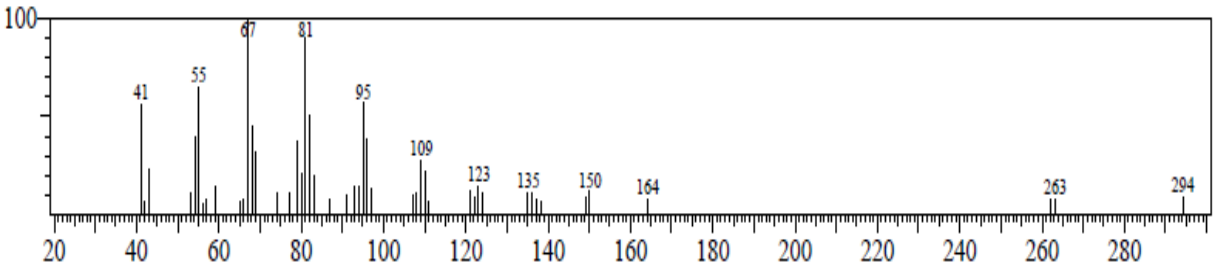


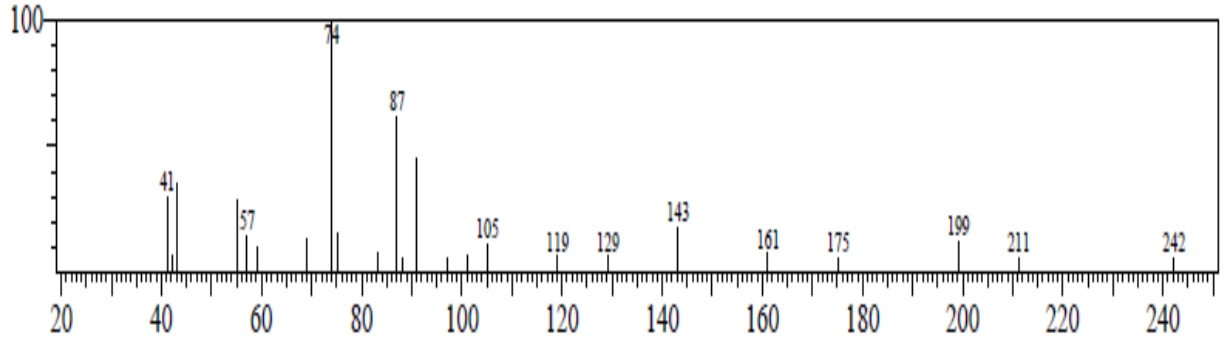
Figure 3. Comparative analysis of compounds confirmed in the hexane extract of male and female stems of *T. cordifolia* on the basis RI value.



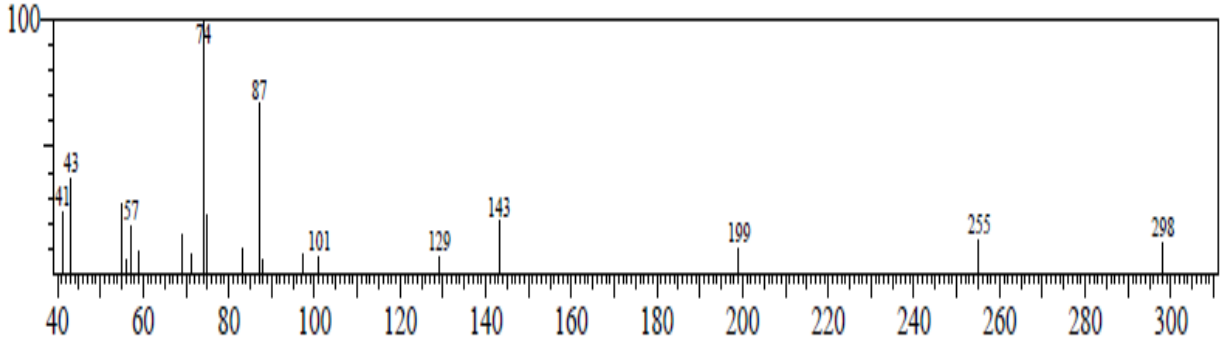
Hexadecanoic acid, methyl ester



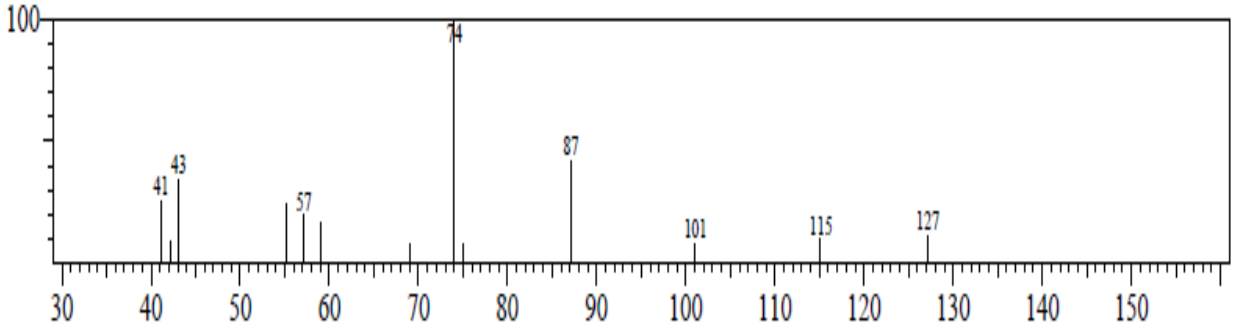
9,12-Octadecadienoic acid, methyl ester



Tetradecanoic acid, methyl ester



Octadecanoic acid, methyl ester



Octanoic acid, methyl ester

Figure 4. Mass fragments of some of compounds identified from *T. cordifolia.*

Table 1. Compounds Identified in Hexane Extract of Male Stem of *T. cordifolia* by comparing mass spectra with NIST and Willey data base.

Peak Number	Retention Time (RT)	%	Compound Name
1	5.905	0.38	Hexanal
2	12.119	0.35	2-Heptenal, (Z)-
3	19.079	0.39	Octanoic acid, methyl ester
4	26.666	0.33	Undecanoic acid, methyl ester
5	26.970	0.23	Methyl 4-oxooctanoate
6	30.561	1.96	Nonanoic acid, 9-oxo-, methyl ester
7	33.432	1.15	Dodecanoic acid, methyl ester
8	33.691	0.36	Benzene, (1-butylhexyl)-
9	33.992	0.20	Benzene, (1-propylheptyl)-
10	34.135	0.26	Nonanedioic acid, dimethyl ester
11	35.722	1.73	7-Hexadecenoic acid, methyl ester, (Z)-
12	35.782	0.28	Benzene, (1-methylnonyl)-
13	36.631	0.43	Benzene, (1-pentylhexyl)-
14	36.746	0.75	Benzene, (1-butylheptyl)-
15	37.062	0.54	Benzene, (1-propyloctyl)-
16	37.711	0.52	Benzene, (1-ethylnonyl)-
17	38.742	0.26	Methyl 12-oxo-9-dodecenoate
18	38.849	0.71	Benzene, (1-methyldecyl)-
19	39.519	3.07	Tetradecanoic acid, methyl ester
20	39.666	0.65	Benzene, (1-butyloctyl)-
21	40.026	0.43	Benzene, (1-propylnonyl)-
22	40.663	0.42	Benzene, (1-ethyldecyl)-
23	41.777	0.53	Benzene, (1-methylundecyl)-
24	41.859	1.14	5-Octadecenoic acid, methyl ester
25	42.314	2.33	Pentadecanoic acid, methyl ester
26	42.478	0.40	Benzene, (1-butylnonyl)-
27	42.757	0.21	2-Pentadecanone, 6,10,14-trimethyl-

28	42.834	0.29	Benzene, (1-propyldecyl)-
29	43.480	0.21	Benzene, (1-ethylundecyl)-
30	44.332	0.53	7-Hexadecenoic acid, methyl ester, (Z)-
31	44.570	0.22	Benzene, (1-methyldodecyl)-
32	44.823	0.24	14-.Beta.-H-pregna
33	45.201	30.74	Hexadecanoic acid, methyl ester
34	45.334	0.28	9,12-Octadecadienoyl chloride, (Z,Z)-
35	45.810	0.27	1,2-Benzenedicarboxylic acid, dibutyl ester
36	45.970	0.27	(+,-)-6-Hepten-4-olide
37	46.094	0.50	Bicyclo[3.1.0]hexane-2-undecanoic acid, methyl ester
38	46.192	0.31	9-Octadecenoic acid (Z)-
39	46.933	0.97	cis-10-Heptadecenoic acid, methyl ester
40	47.597	4.45	Heptadecanoic acid, methyl ester
41	47.708	0.32	2-Undecyl-tetrahydropyran
42	48.414	0.30	Artemisia alcohol
43	49.309	12.89	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
44	49.501	11.55	9-Octadecenoic acid (Z)-, methyl ester
45	49.578	1.21	10-Octadecenoic acid, methyl ester
46	50.170	10.23	Octadecanoic acid, methyl ester
47	50.464	0.22	Palmitaldehyde, diallyl acetal
48	53.051	0.77	Nonadecanoic acid, methyl ester
49	56.984	2.08	Methyl 18-methylnonadecanoate
50	58.178	1.15	Cyclohexanone

% = Percentage of compound

Table 2. Compounds identified in hexane extract of female stem of *T. cordifolia* by comparing mass spectra with NIST and Willey data base.

Peak Number	Retention Time (RT)	%	Compound Name
1	5.928	0.36	Hexanal
2	12.167	0.85	2-Heptenal, (Z)-
3	19.116	0.95	Octanoic acid, methyl ester
4	24.523	0.45	2-Decenal, (E)-
5	26.612	0.47	2,4 Decadienal
6	30.591	1.87	Nonanoic acid, 9-oxo-, methyl ester
7	33.457	0.36	Dodecanoic acid, methyl ester
8	33.723	0.37	Benzene, (1-butylhexyl)-
9	34.639	0.26	Benzene, (1-ethyloctyl)-
10	35.809	4.47	7-Hexadecenoic acid, methyl ester, (Z)-
11	36.671	0.41	Benzene, (1-pentylhexyl)-
12	36.788	0.83	Benzene, (1-butylheptyl)-
13	37.107	0.64	Benzene, (1-propyloctyl)-
14	37.754	0.62	Benzene, (1-ethylnonyl)-
15	38.799	0.41	Methyl 12-oxo-9-dodecenoate
16	38.893	0.78	Benzene, (1-methyldecyl)-
17	39.554	2.63	Tetradecanoic acid, methyl ester
18	39.705	0.75	Benzene, (1-butyloctyl)-
19	40.065	0.55	Benzene, (1-propylnonyl)-
20	40.705	0.53	Benzene, (1-ethyldecyl)-
21	41.820	0.57	Benzene, (1-methylundecyl)-
22	41.900	1.05	5-Octadecenoic acid, methyl ester
23	42.346	2.08	Undecanoic acid, 10-methyl-, methyl ester
24	42.521	0.51	Benzene, (1-butylnonyl)-
25	42.804	0.35	2-Pentadecanone, 6,10,14-trimethyl-
26	42.879	0.40	Benzene, (1-propyldecyl)-
27	43.528	0.26	Benzene, (1-ethylundecyl)-

28	44.612	0.31	Benzene, (1-methyldodecyl)-
29	44.866	0.39	14-.BETA.-H-PREGNA
30	45.242	36.20	Hexadecanoic acid, methyl ester
31	45.384	0.81	7,10-Hexadecadienoic acid, methyl ester
32	45.856	0.27	1,2-Benzenedicarboxylic acid, dibutyl ester
33	46.015	0.27	(+,-)-6-Hepten-4-olide
34	46.152	1.05	Bicyclo[3.1.0]hexane-2-undecanoic acid, methyl ester
35	46.973	0.53	methyl dihydromalvalate
36	47.621	3.70	Heptadecanoic acid, methyl ester
37	48.465	0.42	ND
38	49.279	5.24	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
39	49.470	6.03	9-Octadecenoic acid (Z)-, methyl ester
40	49.574	0.67	10-Octadecenoic acid, methyl ester
41	50.199	9.65	Octadecanoic acid, methyl ester
42	51.417	0.62	Hexanoic acid, heptadecyl ester
43	53.116	0.76	Nonadecanoic acid, methyl ester
44	55.142	0.46	ND
45	55.303	0.38	Cyclopropanebutanoic acid,
46	57.071	2.49	Methyl 18-methylnonadecanoate
47	58.381	4.16	Cyclohexanone
48	58.982	0.48	Tricyclo[20.8.0.0E7,16]Triacontan
49	59.475	0.49	ND
50	62.250	0.83	Methyl 18-methylicosanoate

% = Percentage of compound, ND = Not detected

Table 3. Compounds confirmed on the basis of RI value in hexane extract of male and female stem of *T. cordifolia.*

Compound Name	**Formula and Molecular Weight**	**RI_R**	**Female**		**Male**	
			RI_C	**%**	**RI_C**	**%**
Octanoic acid, methyl ester	$C_9H_{18}O_2$ (158)	1123	1127	0.95	1126	0.39
2-Decadienal, (E)-	$C_{10}H_{18}O$ (154)	1260	1266	0.45	ND	ND
2,4-Decadienal	$C_{10}H_{16}O$ (152)	1315	1322	0.47	ND	ND
Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$ (214)	1524	1524	0.36	1523	1.15
Tetradecanoic acid methyl ester	$C_{15}H_{30}O_2$ (242)	1722	1725	2.63	1724	3.07
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ (270)	1921	1934	36.20	1932	30.74
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$ (294)	2095	2093	5.24	2094	12.89
Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$ (298)	2124	2127	9.65	2126	10.23

RI_R = Value of retention index for compounds given in Adams library, RI_C = Calculated value of retention index for given compounds, % = Percentage of compound, ND = Not detected

References

Abubakar, M.N. & Majinda, R.R.T. (2016). GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus anbolensis* (DC). *Medicines*, 3(3), 2-9.

Adams, R.P. (2004). Identification of Essential Oil Component by Gas chromatography/ Quadrupole Mass spectroscopy. Allured Publishing Corporation, Illinois, U.S.A.

Adams, R.P. (2007). Identification of essential oil components by gas chromatography mass spectroscopy. Allured publ. Crop. Carol Steam. IL.

Albinjose, J., Jasmine, E., Selvankumar, T. & Srinivaskumar, K.P. (2015). Bioactive compounds of *Tinospora cordifolia* gas chromatography-mass spectrometry (GC-MS*). IJMRD,* 2(1), 88-97.

Bajpai, V., Kumar, S., Singh, A., Singh, J., Negi, M.P.S., Bag, S.K., Konwar, R. & Kumar, B. (2017). Chemometric based identification and validation of specific chemical markers for geographical, seasonal and gender variations in *Tinospora cordifolia* stem using HPLC-EST-QTOF-MS analysis. *Phytochem Anal.,* DOI 10.1002/pca.2673.

Bajpai, V., Singh, A. Chandra, P. Negi, M.P.S. Kumar, N. & Kumar, B. (2016). Analysis of phytochemical variations in dioecious *Tinospora cordifolia* stems using HPLC/QTOF MS/MS and UPLC/QqQ_{LIT}-MS/MS. *Phytochem Anal.,* 27, 92-99.

Belakhdar, G., Benjouad, A. & Abdenneb, E.H. (2015). Determination of some bioactive chemical constituents from *Thesium humile* Vahl. *J. Mater. Environ. Sci.,* 6(10), 2778-2783.

Bhan, M. (2016). Phytochemical and pharmacological evaluation. *Int. J. Adv. Sci. Res.,* 1(9), 8-11.

Bhatia A., Bharti, S. K., Tewari, S. K., Sidhu, O. P. & Roy, R. (2013). Metabolic profiling for studying chemotype variations in *Withania somnifera* (L.) Dunal fruits GC-MS and NMR spectroscopy. *Phytochemistry,* 93,105-115.

Bhatt, R.P. & Sabin S.D. (1987). Contribution to the ethnobotanics of Khedbra region of North Gujrat. *J. Econ. Taxon. Bot.,* 9, 138-145.

Caboni, P., Ntalli, N.G., Aissani, N., Cavoski, I. & Angioni, A. (2012. Nematicidal activity of (E,E)-2,4-Decadienal and (E)-2-Decenal from *Ailanthus altissima* against *Meloidogyne javanica*. *J. Agric. Food Chem*., 60, 1156-1151.

Fan, S., Chang, J., Zong, Y., Hu, G. & Jia, J. (2018). GC-MS analysis of the composition of the essential oil from *Dendranthema indicum* Var. *Aromaticum* using three extraction methods and two columns. *Molecules*, 23, 576.

Hanaka, A. & Reszczynska, E. (2020). Lipid composition in plant membranes. *Cell Biochem. Biophys*., 78(4), 401-414.

Hui-Chao Lee, R., Couto e Silva, A., Possoit, H.L.E., Lerner, F.M., Chen, P.Y., Azizbayeva, R., Citadin, C.T., Yu-Cin Wu, C., Neumann, J.T. & Lin, H.W. (2019). Palmitic acid methyl ester is a novel neuroprotective agent against cardiac arrest. *Prostaglandin Leukot. Essent. Fatty Acids*, 147, 6-14.

Juszczak, A.M., Zovoko-Koncic, M. & Tomczyk, M. (2019). Recent trends in the application of chromatographic techniques in the analysis of Luteolin and its derivatives. *Biomolecules*, 9, 731.

Kavitha, B.T., Shruthi, S.D., Rai, S.P., & Ramachandra, Y.L. (2011). Phytchemical analysis and hepatoprotective properties of *Tinospora cordifolia* against carbon tetrachloride-induced hepatic damage in rats. *J. Basic Clin. Pharm.*, 2(3), 139-142.

Knapp, D.R. (1979). Handbook of analytical derivatization reaction. Wiley & Sons, New York, 10.

Kosaraju, J., Chinni, S., Roy, C.P., Kannan, E. & Antony, A.S. (2014). Neuroprotective effect of *Tinospora cordifolia* ethanol extract on 6-hydroxy dopamine induced Parkinsonism. *Indian J. Pharmacol.*, 46(2), 16-180.

Krishnamoorthy, K. & Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC-MS. *Int. Sch. Res. Notices*, 1-13. http://dx.doi.org/10.115/2014/567409

Leyon, P.V. & Kuttan, G. (2004). Inhibitory effect of polysaccharide from *Tinospora cordifolia* on experimental metastasis. *J. Ethanopharmacol.*, 90, 233-237.

Ma, K., Kou, J., Rahman, M.K.U., Du, W. & Liang, X. (2021). Palmitic acid mediated change of rhizosphere and alleviation of Fusatium wilt disease in watermelon. *Saudi J. Biol. Sci.*, 28, 3616-3623.

Modi, B., Koirala, N., Arya, S.P., Shrestha, J., Koirala, S., Upadhyaya, J., Basnyat, R.C., Nassan, A., Alqarni, M. & Batitha, G.E.S. (2021). *Cell. Mol. Biol.*, 67(1), 50-57.

Pandey, M., Chikara, S.K., Vyas, M. K., Sharma, R., Thakur, G.S. & Bisen, P.S. (2012). *Tinospora cordifolia*: A climbing shrub in health care management. *Int. J. Pharm. Bio. Sci*., 3(4), 612-628.

Patel, A., Bigoniya, P., Singh, C.S. & Patel, N.S. (2013). Radioprotective and cytoprotective activity of *Tinospora cordifolia* stem enriched extract containing cordifolioside-A. *Indian J. Pharmacol*., 45(3), 237-243.

Patel, M. B. & Mishra, S.M. (2012). Magnoflorine from *Tinospora cordifolia* stem inhibits α-glucosidase and is antiglycemic in rats. *J. Funct. Foods*, 4,79-86.

Premanath, R. & Lakshmidevi, N. (2010). Studies on antioxidant activity of *Tinospora cordifolia* (Miers.) leaves using in vitro models. *J. Am. Sci.*, 6(10), 736-743.

Rajalakshmi, M., Eliza, J., Priya, C.E., Nirmal, A. & Daisy, P. (2009). Anti-diabetic properties of *Tinospora cordifolia* stem extracts on streptozotocin-induced diabetic rats. *Afr. J. Pharm. Pharmacol.*, 3(5), 171-180.

Razack, S., Kumar, K.H., Nallamuthu I., Naika, M. & Khanum, F. (2015). Antoxidant, biomolecule oxidation protective activities of *Nardostachys jatamansi* DC and its phytochemical analysis by RP-HPLC and GC-MS. *Antioxidan*t, 4, 185-203.

Reddy, C.S., Reddy, K.N., Murthy, E.N. & Raju, V.S. (2009), Traditional medicinal plants in Seshachalam hills. *J. Med. Plant. Res.*, 3, 408-412.

Renner, S.S. & Ricklefs, R.E. (1995). Dioecy and its correlates in the flowering plants. *Am. J. Bot.*, 82, 596-606.

Sangeetha, M.K., Mohana Priya, C.D. & Vasanthi, H.R. (2013). Anti-diabetic property of Tinospora cordifolia and its active compound is mediated through the expression of Glut-4 in L6 myotubes. *Phytomedicine*, 20, 246-248.

Satapute, P., Murali, K.P., Kurjogi, M. & Jogaiah, S. (2019). Physiological adaptation and spectral annotation of Arsenic and Cadmium heavy metal-resistant and susceptible strain *Pseudomonas taiwanensis*. *Environ. Pollut.*, 251, 555-563.

Sharma, U., Bala, M., Kumar, N., Singh, B., Munshi, R. K. & Bhalerao, S. (2012). Immunomodulatory activity compounds from *Tinospora cordifolia*. *J. Ethanopharmacol*., 141, 918-936.

Singh, C.S., Singh, A.K., Khandelwal, S. & Vishwkarma. (2013). Anti-diabetic activity of ethanolic extract of *Tinospora cordifolia* leaves. *IJDDHR*, 3(1), 601-604.

Sinha, A. (2017). Phyto-chemical studies of methanol extracts of *Tinospora cordifolia* stem by GC-MS. *WJPR*, 6(4), 1319-1326.

Stein, S.E. (1990). Mass spectral database and software, version 3.02. National Institute of Standards and Technology (NIST), Gaitherdurg, MD.

Upadhyay, A.K., Kumar, K., Kumar, A. & Mishra, H.S. (2010), *Tinospora cordifolia* (Wild.) Hook f. and Thoms (Guduchi)-validation of Ayurvedic pharmacology through experimental and clinical studies. *Int. J. Ayurveda Res.*, 1, 112-121.

Venancio, A. & Guimaraes, A. (2022). The potential of fatty acids and their derivatives as antifungal agents: a review. *Toxin*, 14(3), 188.

Yin-Chin Wu, C., Couto e Silva, A., Citadin., C.T., Clemons, G.A., Acosta, C.H., Knox, M.S., Rodgers, K.M., Hui-Chao Lee, R. & Lin, H.W. (2021). Palmitic acid methyl ester inhibits cardiac arrest-induced neuroinflammation and mitochondrial dysfunction. *Prostaglandins Leukot Essent Fatty Acids*, 165, 102227.

Yu, F.R., Lian, X.Z., Guo, H.Y., McGuire, P.M., Li, R.D., Wang, R. & Yu, F.H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901cells. *J. Pharm. Sci.*, 8, 528-53.

Zhukow, A.V. (2015). Palmitic acid and their role in the structure and functions of plant cell membranes. *Russ. J. Plant Physiol.*, 62(5), 706-713.

Acknowledgements

The authors are also grateful to His Holiness Baba Iqbal Singh Ji, Former President, The Kalgidhar Trust and Founder Chancellor of Eternal University (H.P.), Dr. Davinder Singh, Hon'ble Vice Chancellor, Eternal University, Baru Sahib (Himachal Pradesh) India for providing necessary laboratory facilities.

Statement of ethics

Ethical approval was not required, as we have not conducted any research work on animal models as well as human trials, so there is no need for getting any permission from ethical committee.

Conflict of interest statement

We declare that we have no conflict of interest for this research publication.

Funding sources: No funding received.

Author contributions

Anisha Bano[1]: Performed all the experiments in the laboratory by her own, this particular assignment was given to her as research work for compilation of data and report. That's why she was assigned as first author for this publication.

Harcharan Singh Dhaliwal[2]: Guided the first author to perform some research work and compilation of data on morphology and phytochemistry.

Vivek Sharma[1*]: Acted as major advisor for this research assignment, guided the first author to do research work on phytochemical analysis as well as biological activities. Also actively involved for compilation and preparation of data as well as communication of this research article to the journal of interest.

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Anisha Bano], [Harcharan S. Dhaliwal] and [Vivek Sharma]. The first draft of the manuscript was written by [Vivek Sharma] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.