Journal of Spices and Aromatic Crops
Vol. 32 (1): 90-100 (2023)
10.25081/josac.2023.v32.i1.8592

Indian Society for Spices

Exploring the therapeutic potential of volatile bioactive compounds from different parts of *Tinospora cordifolia*: a gas chromatography-mass spectrometry (GC-MS) study

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Received 06 April 2023; Revised 20 May 2023; Accepted 22 May 2023

Abstract

Chemicals utilised in the pharmaceutical business can be found in large quantities in medicinal plants. Crude plant herbal extracts are frequently utilised because they combine a variety of secondary metabolites or phytochemical elements with significant therapeutic potential. The main objective of the this research was to identify the medicinally significant phytoconstituents from different economic parts of *Tinospora cordifolia* employing GC-MS. The stem, leaf, and root, respectively, had a total of 93, 74, and 53 peaks, which translates to a sizable number of phytocompounds with therapeutic value. Our results show that oleic acid, columbin, 10E,12Z-octadecadienoic acid, 9- octadecenoic acid (Z), 2, 3 - dihydroxypropyl ester, n-hexadecanoic acid, and 9,12,15-octadeca trieno were found in various parts of *T. cordifolia*. The results provide credence to the use of *Tinospora cordifolia* in conventional medical practice for a range of ailments.

Keywords: GC-MS; *Tinospora cordifolia*, phytochemical, medicinal

Introduction

The chemical compounds in herbal plants have a range of physiological impacts. They contain a vast variety of chemicals with potential medicinal qualities (Clarke, 1997). A growing number of clinical disorders are being treated with herbal plants (Kumar *et al*., 2013). As all societies have employed herbs at some point in the past, herbal drugs is the oldest type of medical care that humans are aware of. It made an important contribution to the development of contemporary civilization (Rajaratnam *et al*., 2014). Several commonly used treatments in today's world have herbal roots. From ancient times, higher vegetation have been a primary source of therapeutic derivatives that are essential to maintaining human health (Rajpara *et al*., 2009). Researchers in the areas of biotechnology and pharmacotherapy are interested in medicinal plants since most firms that manufacture drugs rely on them for creation of pharmaceutical ingredients, especially low molecular weight organic compounds known as secondary compounds (Zhang *et al*., 2020). Several of these metabolites have been employed in medications and are identified to have intriguing biological properties (Rajpara *et al*., 2009).

A huge deciduous climbing shrub from the Menispermaceae family, *Tinospora cordifolia* may be found throughout the tropical Indian subcontinent. There are roughly 450 species and 70 genera in it. The *Tinospora* genus has 32 species, among which *Tinospora sinensis, Tinospora malabarica, Tinospora crispa, Tinospora glabra* and *Tinospora uliginosa,* are a few of the more significant ones. One of the most significant and useful plants that is frequently found in India is *Tinospora cordifolia*, also referred as Giloy in Hindi, Guduchi in Sanskrit, Seenthil kodi in Tamil (Akhilraj *et al*., 2023a; Rajpara *et al*., 2009). Gulvel in Punjabi, Gulancha in Bengali, Gilo in Oriya, Gulbel in China and Amritu in Marathi (Singh *et al*., 2003; Rajpara *et al*., 2009). The plant can be found throughout India, ranging from the Sikkim mountains in the northeast to Kerala in the south. It can also be located in the Himalayas and at an altitude of around 1000 feet. It is also found in various countries in Asia like China, the Philippines, Sri Lanka, Malaysia, Thailand, as well as in Africa.

It is frequently grown as a flowering plant and is easily multiplied via stem hard wood or semi hard wood cuttings. It may also be cultivated by planting matured seeds during the rainy season, however seedling development is observed to be slower than that of plants produced from cuttings (Srivastava, 2011; Papitha *et al*., 2016). A perennial climbing shrub with alternating heart-shaped leaves and a succulent stalk, giloy has therapeutic potential (Rajpara *et al*., 2009).

It is well known that *Tinospora* is a well-liked traditional medicinal plant, and many scientists have discovered that it makes an intriguing source for numerous investigations. Anti-inflammatory, hepatoprotective, anti-neoplastic activity and immunomodulatory stand out among the many described therapeutic qualities (Pradhan *et al*., 2013; Pachaiappan *et al*., 2018). This plant is known as Rasayana and contributes to a significant Ayurveda medication (Chulet and Pradhan, 2009). This plant is said to have a wide range of ethnobotanical and medicinal properties. As traditional medicine is used by more than 80% of the community in third-world nations, it plays a significant part in the health care system (Turner, 2007). In order to scientifically validate indigenous herbal plants or find bioactive compounds that can be used as therapeutic drugs, it is necessary to identify the active principles in therapeutic plants because, as stated by the World Health Organization, herbal medicines are quite susceptible to extinction (Joshi and Kaur, 2016).

During the past ten years, GC-MS has consolidated its position as a major technical platform for research into the phytochemical profiles of bioactive molecules in different plant species. For the purpose of identifying different phytoconstituents in the test sample, gas-liquid chromatography and mass spectroscopy are combined in a process known as GC-MS. In current years GC-MS investigation has been generally used for the determination of volatile essential oil, non-polar components, lipids, fatty acids, terpenoids, alkaloids and steroids. *Tinospora cordifolia* was analyzed for the phytochemical constituents utilizing GC-MS, solvent extracts were prepared using methanol (Akhilraj *et al*., 2023b). This study can be used as a reference source of important data on the quantity and variety of phytoconstituents found in *Tinospora cordifolia* which can aid in the creation of innovative medications. To confirm the presence of several medicinal substances, *Tinospora cordifolia* was subjected to screening for phytochemicals utilizing GC-MS analysis in the current study.

Materials and methods

T. cordifolia extract preparation

T. cordifolia was acquired from the TNAU Campus' Botanical Garden in Tamil Nadu. The stem, leaves and root samples were thoroughly cleaned with distilled water. The plant parts were dried in partial shade at room temperature for one week and coarsely powdered. Then powdered samples were extracted with HPLC grade methanol using microwave assisted extraction (MAE) method.

Samples were crushed and sieved, in a 24 mesh filter for MAE. The sieved material (20 g) was taken in a 500 mL narrowed flask to which mixture of water and 200 millilitres of methanol (80% (v/v)) was added and mixed thoroughly, allowing the drug to dissolve in the solvent. The irradiation frequency and extracting temperature were both adjusted at 480 W for 4 min. After the completion of extraction process, the volumetric flask was taken-out of the oven and the content was redissolved in methanol. The yield of the extract (% w/w) was then estimated after concentrating the extract in a water bath (Satija *et al*., 2015). All fractions were dried in a rotary evaporator and stored in a refrigerator for further analysis.

GC – M S analysis

The samples that had been derivatized were analysed using a Shimadzu single quadrupole GC-MS-QP2020 NX Gas Chromatograph-Mass Spectrometer (GC-MS). One µl of the sample was injected at 250 °C injection temperature. The temperature range was programmed to begin at 40 °C for 2 min, thereafter increased by 8 °C per min for 10 min to 320 °C. A splitting ratio of 1:30 was used to inject the samples. The configuration of the spectrometer was as follows: The mass spectrum at 40 m/z to 600 m/z, ionization chamber temperature 220 °C, the interaction at 300 °C, and the fluid cut at 6.0 min.

Analysis of data

To profile the metabolites, we employed the Shimadzu Post run Tester v2020. Component detection and mass spectrum analysis were done using National Institute of Standards and Technology (NIST) v2020. The Classy Fire autocomplete feature was used to categorize the identified metabolites (Djoumbou Feunang *et al*., 2016).

Results and discussion

GC - MS study of the methanolic extracts from three economic parts of *Tinospora cordifolia viz.,* stem, leaf and root revealed the presence of 93,74 and 53 compounds, respectively.

Traditional healers used the entire plant to treat a variety of diseases. In case of stem (Table 1) out of these 93 compounds, the maximum peak area was shown by oleic acid (20.36%) followed by columbin (7.28%) and 10E,12Z-octadecadienoic acid (6.17%).

Table 1. Constituents of stem extract of *Tinospora cordifolia.*

Peak no.	Retention time (RT)	Area (%)	Compound	Mol. formula	Mol. weight
3	9.164	2.14	4H – pyran – 4 - one, 2 ,3 – dihydro -3, 5 – dihydroxy – 6 – methyl -	$C_6H_8O_4$	144
5	11.653	1.30	1,2,3-propanetriol, 1-acetate	$C_5H_{10}O_4$	134
25	28.333	5.49	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
35	31.528	6.17	10E,12Z-octadecadienoic acid	$C_{18}H_{32}O_2$	280
36	31.674	20.36	Oleic acid	$C_{18}H_{34}O_2$	282
37	32.067	2.58	Octadecanoic acid	$C_{18}H_{36}O_2$	284
47	39.489	2.42	Hexadecanoic acid, 2 – hydroxy – 1 - (hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330
51	41.174	7.28	Columbin	$C_{20}H_{22}O_6$	358
59	42.268	1.44	1H – 2 - indenone, 2, 4, 5, 6, 7, 7a – hexahydro-3-(1-methylethyl)-7a-methyl	$C_{13}H_{20}O$	192
61	42.541	3.27	(R, 1E, 5E, 9E) - 1, 5, 9 – trimethyl -12 - (prop – 1 –en – 2 - yl) cyclotetradeca-1, 5, 9 – triene	$C_{20}H_{32}$	272
62	42.683	4.66	9,12-octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354
63	42.782	5.57	9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356
75	44.773	2.55	Squalene	$C_{30}H_{50}$	410
85	46.103	1.31	1-heptacosanol	$C_{27}H_{56}O$	396
87	46.810	3.64	Gamma sitosterol	$C_{29}H_{50}O$	414

The peak area, retention time, molecular formula & molecular weight of the constituents present in leaves are presented in Table 2. The maximum peak area was shown by 9, 12, 15 - octadecatrienoic acid, (Z, Z, Z) (13.12%) followed by octadecenoic acid (12.75 %) and gamma sitosterol (9.14%).

Table 2. Constituents of leaf extract of *Tinospora cordifolia.*

Peak no.	Retention time	Area (%)	Compound	Mol. formula	Mol. weight
14	25.872	1.69	Neophytadiene	$C_{20}H_{38}$	278
19	28.300	4.22	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
22	31.117	3.83	Phytol	$C_{20}H_{40}O$	296
23	31.486	3.78	9,12-octadecadienoicacid (Z,Z)-	$C_{18}H_{32}O_2$	280
24	31.613	13.12	9,12,15-octadecatrienoic acid, (Z, Z, Z)	$C_{18}H_{30}O_2$	278
25	31.662	12.75	Octadecanoic acid	$C_{18}H_{34}O_2$	282
26	32.043	2.19	Octanoic acid-TMS	$C_{18}H_{36}O_2$	284
40	39.488	1.35	2-Propyl-5-hydroxy-pentanoic acid-2TMS	$C_{14}H_{32}O_3Si_2$	304
45	41.147	1.72	Methyl linoleate	$C_{19}H_{34}O_2$	294
48	42.538	1.05	Octenedioic acid-2TMS	$C_{14}H_{28}O_4Si_2$	316
49	42.625	3.57	Methyl arachidonate	$C_{21}H_{34}O_2$	318
50	42.800	5.11	9,19 – cyclolanostan – 3 - ol, 24 –methylene - , (3.beta.)-	$C_{31}H_{52}O$	440
51	42.963	4.32	(R, 1E, 5E, 9E) - 1, 5, 9 – trimethyl – 12 - (prop – 1 – en – 2 - yl) cyclotetradeca -1, 5, 9 – triene	$C_{20}H_{32}$	272
53	43.245	2.22	Oleic acid-TMS	$C_{21}H_{42}O_2Si$	354
54	43.384	4.71	Methyl cis-13,16-docosadienate	$C_{23}H_{42}O_2$	350
59	44.775	2.21	2- (Furan – 3 - yl) - 7, 8 – dihydroxy -6a, 7, 10b – trimethyl - 2, 4a, 5, 6, 8, 9, 10, 10a – octahydro -1H-	$C_{20}H_{28}O_5$	348
61	45.200	4.96	Obtusifoliol	$C_{30}H_{50}O$	426
63	45.879	1.77	3,4-dihydroxymandelic acid-4TMS	$C_{20}H_{42}O_4Si_4$	458
70	46.827	9.14	Gamma sitosterol	$C_{29}H_{50}O$	414
72	47.729	1.57	24-noroleana-3,12-diene	$C_{29}H_{46}$	394

Major constituents identified in the root samples are presented in Table 3. Major constituents with medicinal use include columbin (59.15%) followed by (R, 1E, 5E, 9E) - 1,5,9 –trimethyl – 12 - (prop – 1 – en – 2 - yl) cyclotetradeca -1, 5, 9 triene (11.34%).

Table 3. Constituents of root extract of *Tinospora cordifolia.*

Peak no.	Retention time	Area (%)	Name	Mol. formula	Mol. weight
12	27.278	2.44	Dimethyl palmitamine	$C_{18}H_{39}N$	269
15	28.292	1.17	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256
19	31.472	1.09	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280
20	31.591	1.75	Oleic acid	$C_{18}H_{34}O_2$	282
30	41.325	59.15	Columbin	$C_{20}H_{22}O_6$	358
36	42.287	1.76	1H – 2 - indenone, 2, 4, 5, 6, 7, 7a – hexahydro – 3 - (1 - methylethyl) - 7a - methyl	$C_{13}H_{20}O$	192
37	42.595	11.34	(R, 1E, 5E, 9E) - 1, 5, 9 – trimethyl – 12 - (prop - 1 – en – 2 -yl) cyclotetradeca - 1, 5, 9 – triene.	$C_{20}H_{32}$	272
39	42.764	1.59	9-octadecenoic acid (Z)-,2, 3 - dihydroxypropyl ester	$C_{21}H_{40}O_4$	356
45	44.159	3.70	1, 7 – dimethyl – 4 - (propan – 2 - ylidene) tricyclo[4.4.0.02,7] decan-3-one	$C_{15}H_{22}O$	218
48	44.759	1.03	Squalene	$C_{30}H_{50}$	410

Present study investigated the virtues of the *Tinospora cordifolia* plant's stem, leaf and root as a herb with pharmaceutical significance. Oleic acid is believed to modulate a number of physiological functions and some researchers even point to a protective impact against cancer, autoimmune disorders, and inflammatory illnesses in addition to its capacity to speed up curing of lesions. Although the contribution of oleic acid in immunological reactions is debatable, consuming diets high in olive oil may improve the immune reaction linked to a greater efficacy in elimination of microbes like fungus and bacteria via disrupting with numerous immune system mechanisms like neutrophils, lymphocytes and macrophages (Sales-Campos *et al.*, 2013). Columbin, an orthorhombic crystal structure furanoid diterpenoid, has been effectively isolated from the *T. cordifolia* ethyl acetate segment. It may enhance learning and retention, postpone the neurodegenerative process and prevent some forms of cancer because of its ability to preferentially inhibit the cholinergic enzyme. Consequently, the substance may be used as a possible lead for the creation of new medications to treat Alzheimer's disease (Onoja *et al.*, 2021; Singh, 2021). Columbin is utilized to target the yellow fever virus envelope protein, and it was discovered that its binding energy was -6.43 kcal/mol. According to this, columbin may be able to prevent viral entrance into the host cell (Wojcicki *el al.,* 1991).

The human body benefits from the omega-9 unsaturated fatty acids that 9-octadecenoic acid (Z) is a part of. Unsaturated fatty acids lower cholesterol by activating cholesterol acetyltransferase, as is widely recognized (Gnanavel and Saral, 2011). Antipyretic, anticonvulsant, antibacterial, and analgesic effects are present in 2, 3-dihydroxypropyl ester (Ravi and Krishnan, 2017). The discovered cytotoxic effect of N-hexadecanoic acid is hypothesised to be caused by the relationship it has with DNA topoisomerase I based on the findings of docking results, and it can be further investigated for its cytotoxic and anticancer properties with additional cancer treatment target proteins (Aparna *et al*., 2012). The traditional medicinal practice of India, Ayurveda, uses n-hexadecanoic acid to treat the signs and symptoms of rheumatoid arthritis (Prabha and Bushra, 2019). 2, 3 - dihydroxypropyl ester of 9, 12 - octadecadienoic acid (Z, Z) reduces inflammation and lowers cholesterol. It is also hepatoprotective, nematicide, insectifuge, antihistaminic, anti-acne, anti-eczema inhibitor of 5 - alpha reductase, arthritic and anti-inflammatory (Sivaranjani *et al*., 2021).

According to Sivaranjani *et al*. (2021), gamma sitosterol found in *Ulva reticulata* has antidiabetic, anti- angeogenic, anticancer, antimicrobial, anti- inflammatory, anti-diarrheal, and antiviral properties. (R,1E,5E,9E)-1,5 9– trimethyl -12 - (prop – 1 – en – 2 - yl) cyclotetradeca 1, 5, 9 - triene, which is also present in *Commiphora wightii*, has historically been used to treat rheumatoid arthritis, obesity, and lipid abnormalities (Francis *el al.,* 2004). Different researchers reported that (E) – 4 - (3 – hydroxyprop – 1 – en – 1 - yl) – 2 - methoxyphenol (coniferyl alcohol, CAS: 458 - 35 - 5, ZINC: 12359045) showed the greatest resistance to rSAHH (S-adenosyl-homocysteine hydrolase) (IC50= 34 nM) among the substances that ChemMapper as well as SciFinder Scholar analysed and evaluated (Hao *et al*., 2017). Squalene is largely utilized for supportive therapy in a range of malignancies since its inhibiting effects have been demonstrated and impact on the propagation of cancer and a significant anti - tumor action, immune system, skin conditions, detoxification, and cholesterol (Gunes, 2013). Inhibitors of 17-beta-hydroxysteroid dehydrogenase, aryl hydrocarbon hydroxylase, testosterone hydroxylase, aromatic amino acid decarboxylase activity, arachidonic acid and uric acid synthesis may all be found in hexadecanoic acid, 2 – hydroxyl – 1 - (hydroxymethyl) ethyl ester (Mohammad *et al*., 2019).

Harmful effect on male reproduction in Wistar rats due to cadmium chloride was lessened by *plukenetia conophora. Plukenetia conophora* which contains 4H – pyran – 4 - one 2, 3 – dihydro - 3, 5 – dihydroxy – 6 - methyl, which may be the cause of the efficacious effects (Olaniyan *et al*., 2018). The nature of 1, 2, 3 - propanetriol, 1 - acetate was antidipogenic. Oleic acid was found to be useful in the management of cutaneous papillomas (Bhat, 2017). It has already been noted that the long-chain primary fatty alcohol 1-heptacosanol exhibits nematocidal, anticancer, antioxidant, and antibacterial properties (Everlyne *et al*., 2015). According to Rajagopal *et al*. (2014) the substance 9, 12, 15 - octadecatrienoic acid (Z, Z, Z) is an omega 3 fatty acid that has anti-inflammatory, antidiabetic, and therapeutic properties for eczema, hypocholesterolemia and has other properties such as nematicide, pesticide, anticancer, anti-inflammatory, antiviral, antibacterial, as well as antihistamic and antioxidant properties. Numerous bioactivities, such as anti-anxiety, immune-modulating, metabolism- modulating, and apoptosis inducing, antioxidant, autophagy anti-nociceptive, anti- inflammatory, cytotoxic, and antimicrobial, are displayed by phytol and its derivatives (Islam *et al*., 2018).

The properties of octadecanoic acid include antiviral, anti-inflammatory, 5-reductase inhibitor, hypocholesterolemic, and propecia (Duru and Maduka, 2021). According to reports, neophytadiene is an antibacterial chemical that can be used to treat rheumatoid arthritis, headaches, and various skin conditions (Suresh *et al*., 2010). 24-noroleana-3,12-diene- retains anti-oxidant activities (Huang *et al*., 2022). Hexadecanoic acid holds anti-inflammatory, vasodilator, release of insulin–stimulation, anti-diabetic, anti-bacterial and anti-fungal properties (Channabasava *et al*., 2014).

Conclusion

Chemicals utilized in the pharmaceutical business can be found in large quantities in medicinal plants. In the case of herbal medicines, crude plant extracts are frequently utilized because they combine a variety of secondary metabolites or phytochemical elements with significant therapeutic potential. The primary objective of the study was to employ GCMS to identify the medicinally significant phytoconstituents that are present in *T. cordifolia* methanol extracts. The stem, leaf, and root respectively, had a total of 93, 74, and 53 peaks, which translates to a sizable number of phytocompounds with therapeutic value. This investigation has led us to the conclusion that *T. cordifolia* can have significant applications in traditional medicine. The GCMS Technique has shown that there were several significant phytoactive compounds in the various economically valuable parts of *Tinospora cordifolia*, including oleic acid, columbin, 10E,12Z-octadecadienoic acid, 9 - octadecenoic acid (Z)-, 2, 3 - dihydroxypropyl ester, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid. The examination of the many phytochemical constituents of *Tinospora cordifolia* support the usage of this plant to cure a variety of ailments. These results provide credence to the use of *Tinospora cordifolia* in conventional medical practice for a range of ailments.

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