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Biochemical and phytochemical decoding of the leaf bioactive compounds in Giloy (*Tinospora cordifolia)* through GC QTOF-Ms/Ms and GC MS

Umamaheshwar Y[1], P J Rathod[2*], Rukamsingh Tomar[3], Vala Ashishkumar[4], U K Kandoliya[5]
[1] Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India
[2] Associate Professor, Department of Biochemistry, ASPEE College of Agriculture Khapat Porbandar, Junagadh Agricultural University, Junagadh, Gujarat, India
[3] Principal Scientist, Indian Institute of Groundnut Research, Ivnagar Rd, Junagadh, Gujarat, India
[4] Assistant Professor, Department of Biotechnology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India
[5] Associate Professor, Department of Biochemistry, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

Abstract
Tinospora cordifolia commonly known as Giloy is a valuable medicinal plant used in Ayurvedic system of medicine by Ancient Hindu physicians the experiments conducted in Saurashtra region with decoding of phytochemicals presence in, three crude extracts (methanol, ethanol and hexane) prepared from the leaf tissue of *Tinospora cordifolia* also analysed through GC MS and GC Q-TOF MS MS. The results revealed the significant variation in solubility of compounds in different extracts as well as in number of compounds detected through high through put techniques. As experimental data suggested highest phytochemical in GC-MS followed by GC- QTOFs. It was found to be 127 compounds in GC MS profiling and remaining were ranges between 6 to 7 in GC-QTOF MS/MS analysis. The linearity of this peaks showed wider variabity in same compounds too. The higher amount of these compounds in methanolic extracts namely, Eugenol, 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, Hexadecenoic acid, Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters, 1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl). as well as hexane leaf extract of *Tinospora cordifolia* indicated the presence of 127 peaks and compounds. Out of them 94 are unique compounds. Methanolic extracts so wider variability in both the platform. Phytochemical screening tests revealed ethanolic extracts is best suitable for this plant. The biochemical composition showed that Moisture (63.16%), Total carbohydrates (20.7%), protein (3.98). While the total phenol was found 4.83% in leaf with antioxidants activity were shown to be 77.53% in *Tinospora cordifolia.*

Keywords: GC QTOF-Ms/Ms and GC MS, *Tinospora cordifolia*

Introduction

Tinospora cordifolia commonly known as Giloy is a valuable medicinal plant used in Ayurvedic system of medicine. Ancient Hindu physicians prescribed it for various diseases. Presently the tincture prepared from *Tinospora cordifolia* has received official recognition in the Indian Pharmacopoeia (Neeraja and Margaret, 2013, Gautam *et al*., 2020) [8, 7].

Description: The plant is an extensively spreading climbing shrub and attains height having several elongated spiraling branches. Leaves are simple and alternate in structure, have long petioles approximately 15 cm long, round and pulvinate. Lamina is ellipsoidal, 10–15 cm long or 10–15 cm broad and membranous with noticeable reticulum beneath. Flowers are unisexual and appear when plant is leafless and greenish yellow. Male flowers are clustered and female flowers are usually solitary. Fruits are in aggregate of one-three, egg-shaped and mostly orange-scarlet in colour. Plant stem is succulent, soft wooded, cylindrical, brown in colour and bitter in taste (Mishra *et al.*, 2014) [15].

Habitat: *Tinospora cordifolia* is a common plant of deciduous and dry forests, growing over herbs, shrubs and small trees. It grows in every kind of soil at varying climatic conditions. It usually prefers moderate sunlight and watering should be regular and moderately heavy. The stems of *Tinospora cordifolia* need support to climb on, preferably a tree like neem, mango etc. The plant is distributed throughout the tropical region of India up to 1,200 m above sea level. It is distributed from Uttarakhand to Assam, in north extending through West Bengal, Bihar, Karnataka and Kerala.

Phytochemistry: Tinospora cordifolia has many phytoconstituents belonging to various chemical classes (Nazir and Chauhan, 2018) [17]. such as Alkaloids (Berberine, Tembeterine, Choline, Tinosporin, Isocolumbin, Jatrorrhizine), Terenoids (Tinosporide, Furanolactone diterpene, Furanolactone clerodane diterpene, Furanoid diterpene, Tinosporaside), Diterpenoids lactone (Clerodane derivatives, Tinosporon, Tinosporides, Columbin), Sesquiterpenoids (Tinocordifolin), Glycosides (18-norclerodane glucoside, Furanoid diterpene glucoside, Cordiofoliosidem A, Cordiofolioside B, Palmatosides C), Steroids (Giloinsterol, ß-Sitosterol, Hydroxy ecdysone), Phenolics and Aliphatic compounds (Octosanol, Heptacosanol, Nonacosan-15-one). Lignin and polysaccharide have also been and characterized from different parts of *Tinospora cordifolia* (Mishra *et al*., 2013,2014) [14, 15]. The many researchers have detected biological activity in these plants. (Mutalik, and Maitreyee, (2011) [16]. Reviewed the *Tinospora cordifolia* (Gulvel) is traditionally used in multipurpose ayurvedic medicine for

centuries (Neeraja and Margaret,2013) [18]. The real treasures of bioactive compounds are known inspite of certain facts multi-diseases-curing compounds should knew from this plant. Researcher has tried to elucidates this with multiple approaches including GCMS, GCQTOF Ms /Ms and biochemical tests in this research with the objectives behind experiments was decoding the specific compounds extracted in specific solvents serves the variation in compounds detection or not? So, the aim was to clarifies the entire profiling of *Tinospora cordifolia* for phytochemical, biochemical and metabolites and their derivatives specifically in leaf tissue.

Materials and Methods

Preparation of the extract: The extraction for qualitative test, 5 gm of green leaf tissue of *Tinospora cordifolia* were crushed in mortar and pestle with the four different solvents of ethanol, methanol, hexane and aqueous extracts separately.

Qualitative test for phytochemicals

1. **Test for Carbohydrates (Molisch's test):** Molisch's test was performed as described by Foulger (1931). The 1 ml of leaf extract was taken and shaken vigorously with water and then filtered. To the aqueous filtrate was added few drops of Molisch's reagent (Molisch' reagent: Dissolve 10g of α-napthol in 100 ml of 95% alcohol). Followed by vigorous shaking again, 1 ml of concentrated H_2SO_4 was carefully added to form a layer below the aqueous solution. A brown ring at the interface indicated the positive.

2. **Test for Proteins (Biuret test):** Biuret test was followed by Gornall *et al*. (1949) [9]. 1ml of the leaf extract residue was taken test tubes and 1 mL of 40% sodium hydroxide solution is added. After adding a drop of 1% solution of copper sulphate, violet or pink colour is formed if protein is present.

3. **Test for Free amino acids (Ninhydrin test):** Ninhydrin test method was followed as per Yemm and Cocking (1955) [22].1 ml of leaf extract residue in test tubes to this add 0.25% w/v ninhydrin reagent is added and boiled for few minutes. The observation was recorded for formation of blue-violet colour. If test positive means the presence of amino acids.

4. **Test for Phenols (Ferric chloride test):** Ferric chloride test for phenol was performed as per Pasto and Johnson (1979). 1 ml of above extract was taken in test tube and add 20 drops of 5% of $Fecl_3$solution. After waiting for a few seconds. The test tubes shown formation of an intense colour ranging from purple to reddish brown. The observation shown the presence of phenols in given extracts

5. **Test for Saponins (Froth test):** Froth test for saponins was followed according to Kokate *et al*. (1994) [12]. 0.5 gm of crude dry powder of extract residue was taken in test tube and add 2 ml of distilled water and shaken vigorously and allowed to stand for 10 min. The observation recorded that stable froth appears; it depicts the presence of saponins.

6. **Test for Flavonoids (Alkaline reagent test):** Alkaline reagent test for flavonoids method was performed as described by Harbourne (1973) [3]. 1 ml of extract was taken in test tube and add few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid (3% Hydrochloric acid) and the observation recorded as presence of flavonoids.

7. **Test for Steroids (Liebermann-Burchard's test):** Liebermann-Burchard's test was used for steroids test as described by Cook (1961) [5]. 2 ml of extract taken in test tube and add 2 ml of chloroform and 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid (H_2SO_4) were added. The observation recorded that solution turns to red, then blue and finally bluish green in colour results the presence of steroids.

8. **Test for Cardiac glycosides (Keller-Kilian's test):** Keller-Kilian's test was used for Cardiac glycosides as described by Sim (1968) [21]. 1 ml of extract was taken in test tube and add 1 ml of $FeCl_3$ reagent (1 volume of 5% $FeCl_3$ and 99 volume of glacial acetic acid) and few drops of concentrated H_2SO_4 is added by sides of test tube. The observations recorded for the formation of greenish blue colour within a few minutes which indicates the presence of deoxy sugar of cardiac glycosides.

Biochemical constituents

1. **Moisture content:** 2 gm fresh leaf sample of *Tinospora cordifolia* were taken and transferred to petri dish and kept in the hot air oven at 105ºC for 24 hours and weighted until constant weight was obtained. After that moisture content were estimated as per formula and expressed as percent moisture (A.O.A.C, 1965) [2].

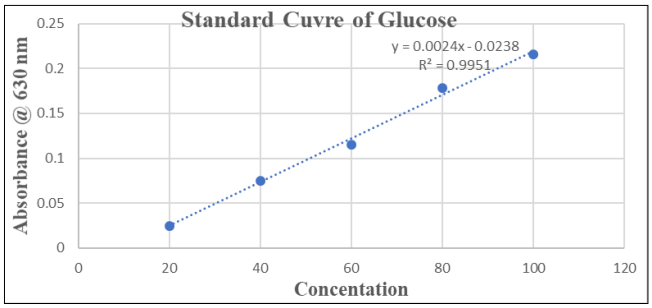
$$\text{Moisture (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight}} \times 100$$

Total Carbohydrates

Total carbohydrate estimation was determined by Anthrone reagent method (Hedge and Hofreiter, 1962) [11].

Extraction of sample: Leaf sample (0.5 gm) homogenized in hot 80% ethanol to remove sugars. Centrifuge and retain the residue. Dry the residue well over a water bath. To the dried residue add 5.0 ml of water and 6.5 ml of 52% perchloric acid. Store extract at 0º C for 20 minutes, centrifuge and save the supernatant. Extraction repeated using fresh perchloric acid and pooled the supernatant and made up to 100ml.

Determination of Total carbohydrates through spectrophotometric methods: Suitable aliquot (0.2 ml) was taken and made up the volume to 1 ml with distilled water. To that, 4 ml of anthrone reagent was added and heated for eight minutes in boiling water in water bath. The tubes were cooled rapidly and the absorbance was measured at 630nm. The carbohydrate was calculated by using glucose as standard. The amount of carbohydrate present in the sample was calculated in % using appropriate formula. The starch contents were calculated by using a standard curve.

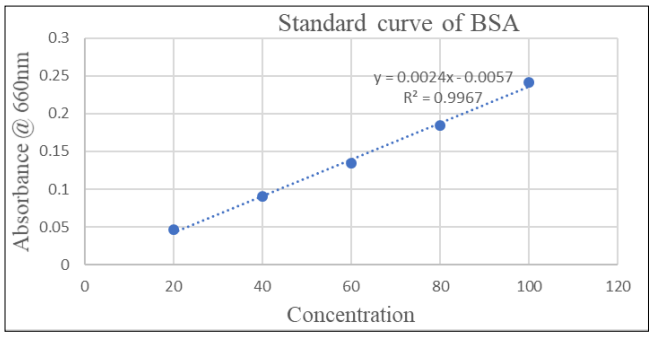


Graph 1: Standard graph of glucose

Total Soluble Protein: The total soluble protein was determined by Folin-Lowry method (Lowry *et al*., 1951) [13].

Extraction of sample: Leaf sample of *Tinospora cordifolia* (0.5 gm) homogenized in a mortar and pestle with 10 ml 0.1 N NaOH and kept in water bath for 6 hours at 65° C. After tubes were cooled, Centrifuge and collect the supernatant. Extraction repeated using fresh 0.1 N NaoH and pooled the supernatant and made up to 100ml.

Determination of Total soluble protein: Suitable aliquot (0.2 ml) was taken and made up to volume 1 ml with distilled water. To that, 5.0 ml of reagent C (Prepared by mixing 50 ml of reagent A with 1 ml of reagent B; A: 2 % Sodium carbonate in 0.1 N Sodium hydroxide. B: 0.5 % Copper sulphate in 1 % Sodium Potassium tartrate) was added and mixed properly. Tubes were kept in room temperature for 10 minutes and added 0.5 ml of reagent D (D: Folin ciocalteau reagent diluted with distilled water in 1:1 ratio), thoroughly mixed and kept for 30 minutes at room temperature. The absorbance was measured at 660 nm. The protein content was calculated by using Bovine Serum Albumin (BSA) as standard. The amount of protein present in the sample was calculated in % using appropriate formula.

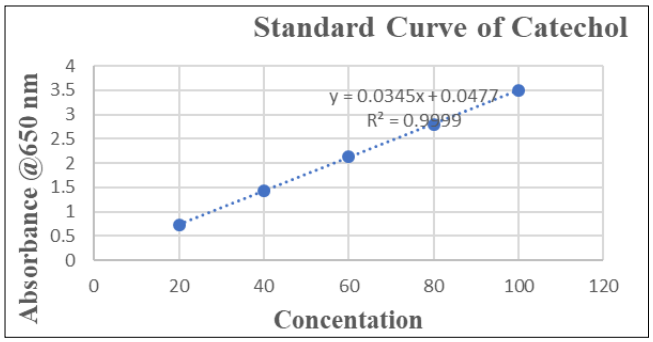


Graph 2: Standard graph of BSA

Total Phenols: The phenol estimation was determined by Folin-Ciocalteau reagent method (Bray and Thorpe, 1954) [4].

Extraction of sample: Leaf sample (0.5 gm) homogenized in a mortar and pestle with 5 ml 80% ethanol. Centrifuge the homogenate at 10,000rpm and save the supernatant and re-extract with 80% ethanol, centrifuge and pool the supernatant. Evaporate supernatant to dryness and dissolve residue in 5 ml distilled water.

Determination of Total phenol: Suitable aliquot (0.2 ml) was taken from ethanol extract taken made up the volume to 3 ml with distilled water. To that add 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and kept for 3 min. After that, 2 ml of 20% Sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for exactly one minute and cooled in ice water. The absorbance was measured at 650nm. The amount of phenol present in the sample was calculated in % using appropriate formula. A standard graph was prepared using catechol ranging between 10- 50µg concentrations.



Graph 3: Standard graph of catechol

Total Oil Content

Sample preparation: Dried leaf of sample of *Tinospora cordifolia* (5 gm) was finely powdered in mortar and pestle. Packed in the Whatman No 1 filter paper packet and label with pencil.

Determination of Total Oil Content by Soxhlet method: Sample packet was placed in the butt tubes of Soxhlet Extraction apparatus and poured 250 ml of Hexane to extraction chamber. Gently heating at 65ºC for 8 hours at 150 drops per minute. After extraction, the extraction flask is allowed to cooled and dismantled. Evaporate the Hexane remains in water bath until no odour of Hexane. Moisture outside the flask is removed and weighed it. After that total

oil content were estimated as per formula and expressed as percent of total oil (A.O.A.C, 1965). [2]

$$\text{Total oil (\%)} = \frac{\text{Weight of oil flask after extration -Weight of empty oil flask}}{\text{Weight of the dried material (Sample)}} \times 100$$

Antioxidant activity by DPPH method: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging activity using the stable radical DPPH (Blois, 1958) [3].

Extraction of sample: Leaf sample (0.5 gm) homogenized in a mortar and pestle 10 ml of methanol. Centrifuge the homogenate at 4000 rpm for 15 minutes, repeat the extraction and collect the supernatant and made up to 10 ml then dilute it to 100ml (1: 10).

Determination of Antioxidant activity by DPPH method: Suitable aliquot of (0.5 ml) is treated with 3 ml of DPPH dye (25 mg DPPH dye in 10 ml methanol, dilute to 1 ml in 100ml methanol). Incubate in dark for 30 minutes and absorbance measured at 517 nm in a UV-Spectrometer. Ascorbic acid used as control. The percent of Radical scavenging activity of the sample was calculated in % using appropriate formula.

$$\text{RSA (\%)} = \frac{\text{Absorbance of control-Absorbanceof test sample}}{\text{Absorbance of control}} \times 100$$

Decoding of bioactive compounds through Mass Spectrometry: GC–MS analysis of plants was carried out by standard producer (Gohlke and McLafferty, 1993) [8].

Extraction of sample: For extraction for GC-QTOF, 5 gm of green leaf tissue of *Tinospora cordifolia* were crushed in mortar and pestle with liquid nitrogen to fine powder. Then added 20 ml of four different solvents of methanol, ethanol and hexane were homogenized completely. Extracts were centrifuged at 5000 rpm, re-extracted and pooled out supernatant. Collected supernatant was sonicated for 15 minutes and supernatant was filtered in 2 mm syringe filter.

GC-QTOF analysis: Experiment was conducted using an Agilent 7200 GC-QTOF system equipped with a 7890A GC system connected with a detector quadrupole time of flight (Q-TOF) mass spectrometer was used to acquire mass spectral data. HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25-µm film thickness) was used with 50:1 split mode and ratio. 1 µl of extract sample was injected into the inlet of column. The injector temperature was maintained at 280 °C and detector at 290 °C. The oven temperature was set to 70 -135 °C with a 2 °C/min, hold for 10 min, hold for 10 min, from 135–220 °C with 4 °C/min, hold for 10 min, from 220 to 270 °C with a 3.5 °C/min and then hold for 20 min. Helium was used as the carrier gas at a flow rate of 1.9 ml/min. All volatile components were characterized by a comparison of their retention indices (RIs) with those previously published in the literature. The RIs were calibrated using a homologous series of n-alkanes (C7–C30) in a HP-5MS column under the same operating conditions. Further identification was performed by comparing the recorded mass spectra with the standard spectra in the NIST 14 Mass Spectral Library.

GC-MS analysis conditions: A Shimadzu Gas Chromatograph, GC-2010 system comprising of an AOC-20i auto-sampler and interfaced to a Mass Spectrometer (QP 2010 Plus) equipped with a polar fused capillary column, DB-Wax (100% Polyethylene Glycol, 30 mt Length × 0.25 mm ID × 0.25 µm df). For GC-MS detection, an Electron Ionization system was operated in Electron Impact (EI) mode with ionization energy of 70 eV. Helium gas (99.999% purity) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed with a split ratio of 50:1. The injector temperature was maintained at 250°C, the Ion-Source temperature at 230°C. The column oven temperature was programmed from 60°C with an increase of 12 °C/min to 150°C (isothermal for 1 min), then was increased at a rate of 5°C/min to 240°C (isothermal for 5 min). Mass spectra were taken for fragments ranging from 50 m/z to 1000 m/z. Identification of the Fatty Acid Methyl Ester was conducted by comparing the mass spectrum with NIST library. The compound showing more that 90% Similarity Index (SI) was identified and recorded. The FA composition was reported as a relative percentage of the total peak area.

Results and Discussions

The experiments data for phytochemical test are presented in table.1 and Biochemical composition in *Tinospora cordifolia* is presented in table.2.

Table 1: Test for phytochemicals in leaves of Giloy

Test for	Methanolic	Ethanolic	Hexane	Aqueous
Protein	-	+	-	+
Amino acids	-	+	-	+
Phenols	-	-	+	+
Saponins	-	-	-	+
Flavonoids	+	+	-	-
Steroids	+	+	-	-
Cardiac glycosides	+	+	-	+

The result of phytochemical screening tests revealed that diterpenes and carbohydrates are positive in all extracts of *T. cordifolia*, but flavonoids and saponins only present in methanol and ethanol extracts. TFC of *T. cordifolia* was higher in ethanolic leaves extracts than methanolic leaves extracts. Experimental data for phytochemical test showed mostly negative in hexane extract except phenols whereas, ethanolic extracts showed all positive except phenols and saponins and the aqueous extract also showed the presence proteins, amino acids phenol saponins and cardiac glycosides. Similar findings were observed by Garg and Garg (2018) [6]. while study with Giloy (*Tinospora cordifolia*) and also suggested studied the phytochemical compounds in leaves and They found leaf and stem extracts of *T. cordifolia* expressed the presence of several phytochemicals *viz*., flavonoids, amino acids, diterpenes, protein, saponins and carbohydrates.

These results also confirmed previously by Grover *et al*. (2013) [10]. studied phytochemical screening in giloy and reported that petroleum ether extract showed the presence of alkaloids, glycosides, carbohydrates, tannins, sterols, proteins and amino acids. The aqueous extract also showed the presence of alkaloids, glycosides, carbohydrates, tannins, proteins, and amino acids. And also, Pradhan *et al*. (2013) [20]. conducted phytochemical analysis of *Tinospora cordifolia* stem of varied thickness. Their results indicated

the presence of phenols, flavonoids, alkaloids, saponins, cardiac glycosides, steroids, carbohydrate and proteins by using different extraction solvents *viz*. methanol, petroleum ether, water, chloroform and ethyl acetate. Saponins were present only in aqueous and methanolic extracts of *Tinospora cordifolia.*

Table 2: Biochemical composition in *Tinospora cordifolia*

Moisture (%)	Total carbohydrates (%)	Total protein (%)	Total oil (%)	Total phenol (%)	Antioxidant activity (%)
63.16	20.07	3.98	3.63	4.83	77.53

The results of biochemical composition of leaf tissues showed the presence of Moisture (63.16), Total carbohydrates (20.7%), protein (3.98). While the total phenol was launch 4.83% in leaf. The antioxidants activities through DPPH methods were shown to be 77.53%

GCQ-TOF Ms /Ms analysis of *Tinospora cordifolia* leaf extracts

The results of GCQ-TOF Ms Ms analysis of Tinospora cordifolia stem extracts from methanol extracts are presented with Area %, MZ ratio (Mass) and RT in Table 3. The results of the methanolic Tinospora cordifolia leaf extracts indicated the presence of 20 peaks and compounds. The compounds detected from extracts were most of primary as well as secondary metabolites. The analysis of Tinospora cordifolia leaf extract were founds maximum area of in subsequent compounds and RT were detected *viz*., Eugenol (13.89), 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (27.117), Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters (17.843), Hexadecenoic acid, methyl ester (27.245), 1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z, E)]- (14.847) and Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (27.6), major compounds which are found to be aromatic and flavonoid compounds were shown higher peaks in chromatogram according to RT fig. 2 Similar findings also suggested by Gautam *et al*. (2020) [7]. analysed in stem powder of Tinospora cordifolia and the compounds detected *viz*., Hexadecenoic acid with higher peak value 29.71, Tetradecenal of 13.56, Hexadecanoic acid of 11.93 and Linolaidic acid of 8.79 retention time. The results are inversely matched with Albinjose *et al*. (2015) [1]. analysed in the leaf powder of Tinospora cordifolia and the compounds that found to be Hydroxymethyl colchicines (26.63), 17-(1,5. Dimethylhexyl)-10,13-dimethyl.3-styrylhexadecahydrocyclopenta(a)p henanthren-2-one (5.62), Propanic acid,2-(3-acetoxy-4,4,14-trimethylandrost.8.en.17. yl) (8.68) and Cyclopropanedodecanoic acid,2-octyl-, methyl ester (7.22).

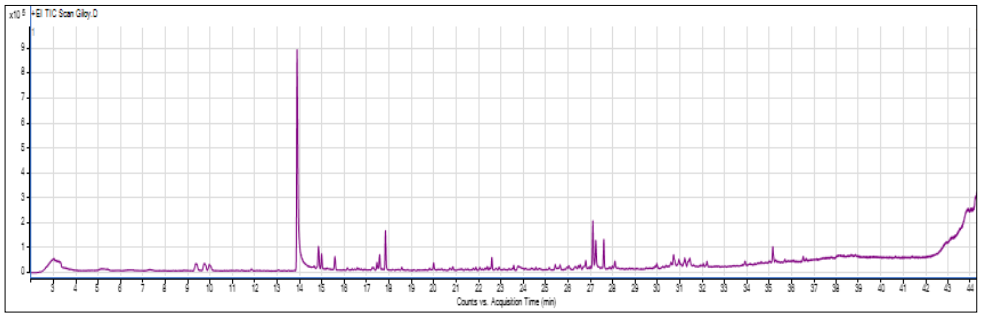


Fig 1: Chromatogram of methanolic extract of *Tinospora cordifolia*

Table 3. Total number of compounds and unique compounds in ethanolic extracts in different medicinal plants

The results of GCQ-TOF MS analysis of *Tinospora cordifolia* leaf extracts from ethanol extracts are presented with Area, MZ ratio (Mass) and RT in Table.5 And figure2. The results of the ethanolic *Tinospora cordifolia* leaf extracts indicated the presence of 7 peaks and compounds. The compounds detected from extracts were most of primary as well as secondary metabolites. The analysis of *Tinospora cordifolia* leaf extract was founding maximum area of in subsequent compounds and RT were detected *viz*., Methyltartronic (2.141), Dimethyl ether (2.722), Eugenol (13.886) and Hexadecanoic acid, ethyl ester (28.643), major compounds were belonging to alkyl esters, sesquiterpenoids and fatty acids, were shown higher peaks in the graph Fig. 2. Similar results were also suggested by Singh *et al* (2019) [1]. analysed in the ethanolic leaves extract of *Tinospora cordifolia* and found the compounds Eugenol (180.22), Hydroxyeugenol (168) and Hexa decanolic acid (256) with MZ ratio of compounds.

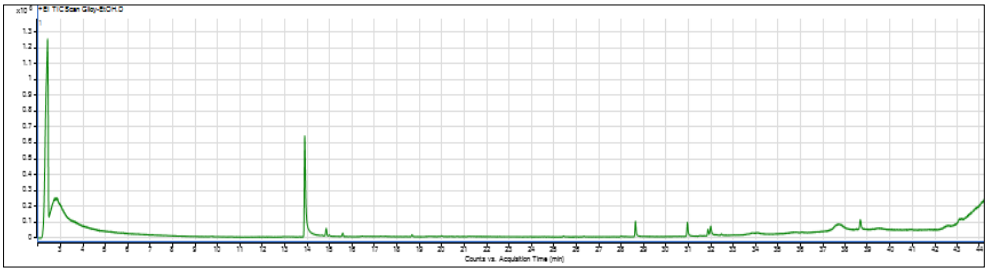


Fig 2: Chromatogram of ethanolic extract of *Tinospora cordifolia*

The results of GCMS (Shimadzu GCMS-QP2010Plus and Shimadzu GCMS-QP2010SE, Japan) analysis of *Tinospora cordifolia* hexane leaf extract is presented with compound name, area (%) and RT in Table.4. The results of the hexane leaf extract of *Tinospora cordifolia* indicated the presence of 127 peaks and compounds. Out of them 94 are uniques compounds and some were detected many times. The compounds detected from extracts were most of primary as well as secondary metabolites. Similar observation measured by Papitha *et al*. (2016) [19]. with GC MS platform. The analysis of *Tinospora cordifolia* hexane leaf extract was founds maximum area of in subsequent compounds and RT were detected *viz*., 6-Octadecenoic acid, (Z)- (19.503), 2,4-Decadienal, (E, E)- (9.884), 13-Octadecenal, (Z)- (26.82), 2,4-Decadienal, (E, E)- (9.451) and n-Hexadecanoic acid (17.69), major compounds were belonging to fatty acids, were shown higher peaks in the graph Fig. 3. The sample results of hexane extract were inversely matched with the results of Sharma *et al.* (2010) [1]. They analysed *Tinospora cordifolia* hexane leaf extract and found the compounds *viz*., methyl-9,12-octadecadienoate (23.17), methyl 9- octadecenoate (19.74), methyl hexadecanoate (16.28) and Methyl octadecanoate (5.52%).

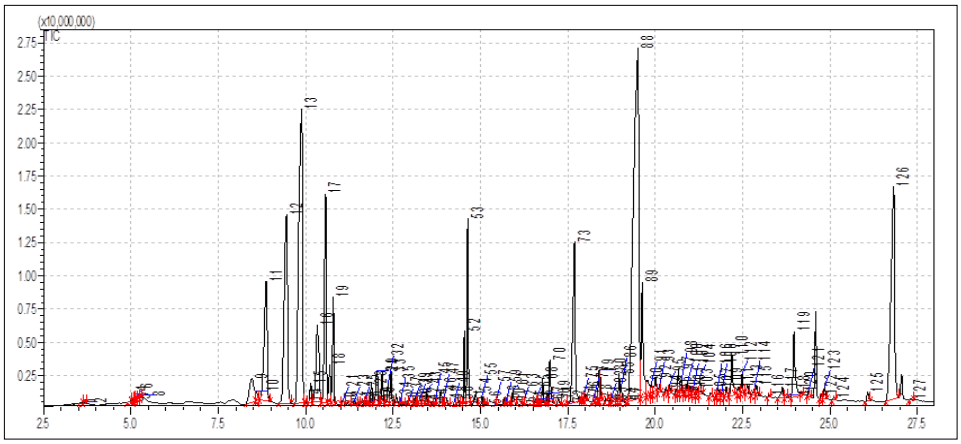


Fig 3: GC MS Chromatogram of hexane extract of *Tinospora cordifolia*

Table 3: Chemical compounds from methanolic leaf extract of *Tinospora cordifolia* through *GCMS*

Sl. NO	Name	Area	Mass	RT
1	Eugenol	3241393	164.1	13.89
2	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	380438	276.2	27.117
3	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	351448	306.2	17.843
4	Hexadecanoic acid, methyl ester	304747	270.3	27.245
5	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S- (Z, E)]-	262334	204.2	14.847
6	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	257484	292.2	27.6
7	Pentane, 2,2,3,4-tetramethyl-	185304	128.2	9.755
8	2,4-Difluorobenzoic acid, 2-nitro-5-fluorophenyl ester	153241	297	17.579
9	Hexane, 3,3-dimethyl-	153138	114.1	14.987
10	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1. alpha.,4. alpha.,4a. alpha., 10a.alpha.)-	130369	204.2	15.581
11	Undecane, 3,8-dimethyl-	114475	184.2	22.602
12	6-Octen-1-ol, 3,7-dimethyl-, formate	65792	184.1	30.97
13	Octane, 2,7-dimethyl	64555	142.2	28.103
14	Nonane, 3,7-dimethyl	63973	156.2	19.989
15	Hexane, 3,3-dimethyl	55954	114.1	17.461
16	Bicyclo [2.2.1] heptane, 1,3,3-trimethyl	50945	138.1	25.43
17	Hexane, 3,3-dimethyl	43790	114.1	23.578
18	Phthalic acid, cyclobutyl hexyl ester	43279	304.2	26.04
19	Hexane, 3,3-dimethyl	42823	114.1	25.643
20	Sulfurous acid, 2-ethylhexyl hexyl ester	40624	278.2	32.217

Table 4: Chemical compounds from hexane leaf extract of *Tinospora cordifolia*

Sl. No	Compound Name	RT	Area %
1	6-Octadecenoic acid, (Z)-	19.503	22.87
2	2,4-Decadienal, (E, E)-	9.884	13.15
3	13-Octadecenal, (Z)-	26.82	9.33
4	2,4-Decadienal, (E, E)-	9.451	7.63
5	n-Hexadecanoic acid	17.69	5.03
6	2-Undecenal	10.569	4.96
7	2-Decenal, (Z)-	8.868	4.53
8	8-Heptadecene	14.632	2.9
9	2-Decen-1-ol, (E)-	10.327	2.81
10	Octadecanoic acid	19.627	2.77
11	4-Heptenal	10.79	1.99

12	9-Octadecenoic acid	23.968	1.83
13	Cyclohexanone	8.459	1.39
14	Cyclododecene	14.537	1.04
15	9-Octadecenoic acid	22.193	0.92
16	2-Piperidinone, N-[4-bromo-n-butyl]-	12.04	0.81
17	cis-9-Hexadecenal	16.984	0.81
18	3,4Dehydro-dl-proline	10.686	0.75
19	Hexadecane	12.438	0.59
20	Santolina epoxide	12.19	0.56
21	9-Octadecenamide	19.773	0.5
22	Oleic acid TMS	20.005	0.46
23	Oleic Acid	18.387	0.44
24	Nonadecane	18.964	0.42
25	Santolina epoxide	12.356	0.41
26	Santolina epoxide	11.88	0.39
27	Hexadecanoic acid	18.412	0.39
28	E-2-Hexenyl E-2-octenoate	10.141	0.37
29	13-Tetradecenal	16.775	0.37
30	(1R,2R,3S,5R)-(-)-2,3-Pinanediol	13.864	0.35
31	1,3,12-Nonadecatriene	20.661	0.34
32	Hexadecanoic acid	22.478	0.33
33	Pentadecane	14.868	0.32
34	Heneicosane	20.744	0.3
35	E-3-Pentadecen-2-ol	23.634	0.3
36	9-Octadecenamide	21.278	0.29
37	7-Tetradecenal	15.926	0.28
38	5,5-Dimethyl-cyclohex-3-en-1-ol	12.586	0.25
39	Oleoyl chloride	24.846	0.25
40	2(3H)-Furanone, dihydro-5-tetradecyl-	19.023	0.24
41	Octadecane	19.878	0.22
42	2-Octyn-1-ol	8.577	0.21
43	Oleoyl chloride	21.791	0.19
44	9-Octadecenal	22.036	0.19
45	10-Methoxy-nb-. alpha. -methylcorynantheol	22.894	0.19
46	Heneicosane	26.076	0.19
47	9-Octadecene	13.451	0.17
48	9,17-Octadecadienal	16.91	0.17
49	Cyclopentadecanone	22.634	0.17
50	Oleyl Alcohol	22.839	0.16
51	9-Octadecenal	20.914	0.15
52	Hexatriacontane	22.383	0.15
53	Oxacycloheptadec-8-en-2-one	20.406	0.14
54	Cyclohexene	14.037	0.13
55	Tetradecanal	15.043	0.13
56	Cyclohexane	15.669	0.13
57	9-Hydroxy-2,2-dimethyl-dec-5-en-3-one	13.124	0.12
58	Hexadecanal	17.221	0.12
59	1-Cyclohexanol, 1-[5-hydroxy-4-methyl-2-hexenyl]	11.386	0.11
60	Pentadec-7-ene	13.264	0.11
61	1-Dodecen-3-ol	14.361	0.11
62	9-Octadecenal	18.001	0.11
63	2H-Pyran-2-one, tetrahydro-6-tridecyl-	21.202	0.11
64	Hexadecane	11.065	0.1
65	Hexadecanal	20.11	0.1
66	Oleic Acid	21.08	0.1
67	2(3H)-Furanone	24.387	0.1
68	Oleic Acid	12.116	0.09
69	8-Hexadecenal	20.307	0.09
70	Octadecanal	20.993	0.09
71	Z, Z-3,13-Octadecadien-1-ol	21.89	0.08
72	22-Tricosenoic acid	24.752	0.08
73	1-Decanol	11.8	0.07
74	5-Decen-1-ol	13.357	0.07
75	9,12-Octadecadienoic acid	18.31	0.07
76	9,12-Octadecadienoyl chloride	15.842	0.06
77	(Z)6, (Z)9-Pentadecadien-1-ol	16.691	0.06
78	n-Pentadecanoic acid	17.448	0.06
79	Z-5,17-Octadecadien-1-ol acetate	20.592	0.06
80	Tetrapentacontane	21.586	0.06
81	Nonanal	5.272	0.05

82	Dodecanal	11.196	0.05
83	Cyclohexane	13.17	0.05
84	11-Eicosenoic acid	13.601	0.05
85	trans-3-Nonen-2-one	13.937	0.05
86	5-Octadecene	15.726	0.05
87	Tetradecanal	16.16	0.05
88	Pentadecanoic acid	16.568	0.05
89	cis-1,2-Cyclododecanediol	18.77	0.05
90	Cyclopentadecanone	23.707	0.05
91	Hexadecanoic acid	24.167	0.05
92	Squalene	25.108	0.05
93	Nonanal	5.162	0.04
94	Nonanal	5.217	0.04
95	trans-3-Nonen-2-one	12.69	0.04
96	Hexadecane	13.696	0.04
97	9,12-Octadecadien-1-ol	14.448	0.04
98	4-Iodo-2-oxaadamantan-6-one	15.144	0.04
99	9,12-Octadecadienoic acid	21.743	0.04
100	Hexanoic acid	23.249	0.04
101	Triethylene glycol monododecyl ether	9.992	0.03
102	Cyclohexanone, 2-(1-methyl-2-nitroethyl)-	11.657	0.03
103	4-Nonenal	11.703	0.03
104	2,4-Dodecadienal	12.787	0.03
105	Undecane	14.227	0.03
106	3-Nonen-2-one	16.215	0.03
107	Octadecanal	18.226	0.03
108	Eicosanoic acid	18.583	0.03
109	1-Docosanol	18.724	0.03
110	Octadecanal	19.188	0.03
111	17-Pentatriacontene	27.309	0.03
112	2-Octenal	3.533	0.02
113	2-Octenal	3.662	0.02
114	Nonanal	5.04	0.02
115	Cyclopentanepropanol -	10.997	0.02
116	7-Hexadecene	13.052	0.02
117	2-Pentadecanone	16.416	0.02
118	9-Hexacosene	16.637	0.02
119	9,17-Octadecadienal	17.93	0.02
120	Hexadecadienoic acid	18.903	0.02
121	2-Pentadecanone	20.817	0.02
122	TRIDECANOL	4.933	0.01
123	Nonanal	5.1	0.01
124	4-Tridecene	11.518	0.01
125	Oxirane	12.915	0.01
126	9-Eicosyne	15.419	0.01
127	1-Docosanol	17.857	0.01

Table 5: Chemical compounds from ethanolic leaf extract of *Tinospora cordifolia*

Sl. No	Compound name	Area	Mass	RT
1	Methyltartronic acid	9685829	134	2.414
2	Dimethyl ether	4782171	46	2.722
3	Eugenol	2324319	164.1	13.886
4	Hexadecanoic acid, ethyl ester	284334	284.3	28.643
5	Phthalic acid, di(2-propylpentyl) ester	269259	390.3	38.68
6	Neophytadiene	262058	278.3	30.952
7	1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S-(Z, E)]-	129649	204.2	14.85

Table 4: Chemical compounds from Methanolic leaf extract of *Tinospora cordifolia* by GCMSQTOF

Name	Formula	Mass	RT	Height	Area	Score (Lib)	CAS ID
Neophytadiene	C_20H_{38}	278.3	30.952	8567	262058	86.92	504-96-1
Methyltartronic acid	$C_4H_6O_5$	134	2.414	692934	9685829	84.85	595-98-2
Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	390.3	38.68	28169	269259	84.35	1000377-93-5
Eugenol	$C_{10}H_{12}O_2$	164.1	13.886	146710	2324319	82.13	97-53-0
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S- (Z, E)]-	$C_{15}H_{24}$	204.2	14.85	4487	129649	77.6	75023-40-4
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.3	28.643	9577	284334	75.75	628-97-7
Dimethyl ether	C_2H_6O	46	2.722	105318	4782171	67.5	115-10-6

Table 5: Chemical compounds from Ethanolic leaf extract of *Tinospora cordifolia*

Name	Formula	Mass	RT	Height	Area	Score (Lib)	CAS ID
1,5-Cyclodecadiene, 1,5-dimethyl-8-(1-methylethenyl)-, [S- (Z, E)]-	$C_{15}H_{24}$	204.2	14.847	9160	262334	89.52	75023-40-4
Hexane, 3,3-dimethyl-	C_8H_{18}	114.1	14.987	14197	153138	87.9	563-16-6
Undecane, 3,8-dimethyl-	$C_{13}H_{28}$	184.2	22.602	10138	114475	86.37	17301-30-3
Eugenol	$C_{10}H_{12}O_2$	164.1	13.89	202465	3241393	84.76	97-53-0
2,4-Difluorobenzoic acid, 2-nitro-5-fluorophenyl ester	$C_{13}H_6F_3NO_4$	297	17.579	37235	153241	84.74	1000357-60-1
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	292.2	27.6	25369	257484	84.74	6386-38-5
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	$C_{19}H_{30}O_3$	306.2	17.843	71016	351448	82.15	166273-38-7
Hexane, 3,3-dimethyl-	C_8H_{18}	114.1	17.461	6243	55954	79.94	563-16-6
Pentane, 2,2,3,4-tetramethyl-	C_9H_{20}	128.2	9.755	8093	185304	78.41	1186-53-4
Hexane, 3,3-dimethyl-	C_8H_{18}	114.1	23.578	4089	43790	77.85	563-16-6
Nonane, 3,7-dimethyl-	$C_{11}H_{24}$	156.2	19.989	6864	63973	77.84	17302-32-8
Bicyclo [2.2.1] heptane, 1,3,3-trimethyl-	$C_{10}H_{18}$	138.1	25.43	2501	50945	77.44	6248-88-0
Octane, 2,7-dimethyl-	$C_{10}H_{22}$	142.2	28.103	5651	64555	76.72	1072-16-8
1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1. alpha.,4. alpha.,4a. alpha., 10a.alpha.)-	$C_{13}H_{20}N_2$	204.2	15.581	4386	130369	76.4	1000221-85-9
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.3	27.245	10451	304747	74.69	112-39-0
Hexane, 3,3-dimethyl-	C_8H_{18}	114.1	25.643	5218	42823	74.53	563-16-6
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276.2	27.117	18625	380438	73.08	82304-66-3
Phthalic acid, cyclobutyl hexyl ester	$C_{18}H_{24}O_4$	304.2	26.04	11296	43279	71.65	1000314-90-1
6-Octen-1-ol, 3,7-dimethyl-, formate	$C_{11}H_{20}O_2$	184.1	30.97	2286	65792	70.28	105-85-1
Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	278.2	32.217	4172	40624	69.2	1000309-20-2

Conclusion

This study analysed three crude extracts (methanol, ethanol, hexane) from the leaf tissue of *Tinospora cordifolia* plants by using GC MS and GC Q-TOF MS MS plate form. The methanolic extract was found to have higher amounts of certain compounds such as Eugenol and Pentanoic acid. The study analysed the phytochemical composition of Giloy leaves and found the presence of alkaloids, glycosides, carbohydrates, and proteins. The results showed the presence of flavonoids, amino acids, diterpenes, proteins, and saponins in leaf and stem extracts of *T. cordifolia*. The study also reported the presence of compounds such as Hexadecanoic acid, Tetradecenal, and Linoleic acid in stem powder of *Tinospora cordifolia*. The analysis of leaf extracts showed the presence of compounds like Eugenol, Hexadecanoic acid, and 1,5-Cyclodecadiene. Overall, the study provides valuable insights into the bioactive compounds present in Giloy leaves and their potential health benefits.

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