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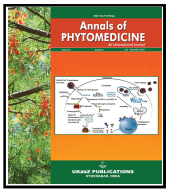

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Preliminary phytochemical screening, FTIR and GC-MS analyses of aqueous, ethanolic and methanolic extracts of stem of *Tinospora cordifolia* (Willd.) Miers for search of antidiabetic compounds

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Abstract

Phytochemicals present in different medicinal plants have shown immense therapeutic potential against number of diseases including diabetes. *Tinospora cordifolia* (Willd.) Miers, a well known medicinal plant, has a high medicinal importance. The study is aimed to fractionate, identify and compile the structure and medicinal properties of each phytochemical of *T. cordifolia* from the available literature, so that the compounds with antioxidant and antihyperglycemic potential can be studied further using *in vivo* system. The extracts of *T. cordifolia,* prepared in water, ethanol and methanol, were fractionated and the phytochemicals were identified by FTIR and GC-MS. The structure and properties of each component were compiled from the available literature, so that the compounds with antioxidant and antihyperglycemic effects could be studied further. Qualitative tests for the presence of carbohydrates, proteins, tannins, phenolics, glycosides, alkaloids and flavanoids were performed in extracts of *T. cordifolia*. The peaks obtained in FTIR spectra of various extracts of *T. cordifolia* were identified for presence of different functional groups. The phytocomponents resolved by GC-MS in extracts of *T. cordifolia* are known to contribute to its antioxidant, cancer-preventive, hypercholesterolemic, nematicidal, antifungal, antidiabetic, and hepatoprotective effects. Highest amounts of proteins, tannins, phenol and flavanoids were extracted in water while carbohydrates and glycosides were maximally present in methanolic extract and glycoside in ethanolic extract. Comparative phytochemical analysis of aqueous, ethanolic and methanolic extracts of *T. cordifolia* showed differential distribution of antioxidant and antidiabetic compounds. Further validation of these effects under *in vivo* system may give leads for the development of herbal alternative for diabetes.

1. Introduction

Plants play a very important role in maintaining the health of communities and individuals in general and have found medicinal usage in folk and other systems of medicine since time immemorial (Ahn, 2017; Pan *et al.,* 2014). Approximately 3-4 billion people living in developing countries depend on traditional herbal medicines which represent approximately 88% of the world's population who rely for their primary healthcare on traditional medicines (Jaiswal and Williams, 2016; Vaidya and Devasagayam, 2007). World Health Organization (WHO) has defined medicinal plant as any type of plant whose one or more parts are used for therapeutic and/ or prophylactic purpose (WHO, 2005). The development of traditional herbal medicines has been influenced by different cultural and geographical locations where they were developed first. The major concern in development of herbal medicine is the holistic approach to life, maintaining balance between mind, body and the environment with emphasis on the good health rather than prevention or cure of diseases (Karmi *et al.,* 2015; Ekor, 2014). Natural products, such as plant extracts, either as standardized therapeutic regimen or isolated pure compound provides ultimate opportunity for discovery of new drugs because of unmatched chemical diversity, affordable cost and ease of availability (Sofowora *et al.,* 2013). The phytochemical analysis of *T. cordifolia* plant is important because of its immense medicinal value against number of ailments, thus great interests of the pharmaceutical companies for the development of new formulations with therapeutic potential against various diseases.

There are many different systems of traditional medicine known, all of which were aimed on maintaining the health rather than curing a disease. However, due to spread of various diseases, the focus of medical practitioners and researchers had been shifted towards search of herbal remedies for cure of such diseases and all efforts of extensive research have been diverted in new direction. The welfare of mankind is possible only when well-developed efficient medical system of cure and control of various diseases is easily available to the common people and masses. Herbal medicines provide most

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economic, easily available and safe system of cure of various diseases that it is in practice since ancient times and has progressively been enriched by research and learned practitioners. Benefits of ancient systems of traditional medicine are well recognized in present era. Lots of drugs used in modern medicine have derived their origin from plant sources (NMPB, 2016; Mosihuzzamn, 2012; Balunas and Kinghom, 2005; Rates, 2001; Balandrin *et al.,* 1985). *T. cordifolia* stands out as an exceptional herb with a multitude of medicinal benefits (Saha and Ghosh, 2012), belongs to the family, *Menispermaceae*, which is easily grown in different varieties of soil ranging from acidic to alkaline and requires humidity (Albinjose *et al.,* 2015). Guduchi, the Sanskrit name of *T. cordifolia*, means one which protects the entire body; another term *amrita* is attributed to its ability to impart vitality, longevity and youthfulness (Dhama *et al*., 2017). The leaves afford a good fodder for cattle. *T. cordifolia* is recommended for fever, edema, burning sensation, diabetes, general immunostimulant, anemia, skin diseases and hepatoprotection, *etc.* (Kaur *et al.*, 2009). *T. cordifolia* is a plant which has been scientifically validated in various animal models for antioxidant, anti-inflammatory, immunomodulatory, hypoglycemic and other pharmacological activities (Panchabhai *et al.,* 2008; Adhvaryu *et al.,* 2007; Zhao *et al.,* 1991). *T. cordifolia* is used for treatment of different ailments as well as general tonic for prevention of illness by maintenance of balance between body, mind and environment rather than responding to symptoms of diseases. In our previous studies, the extracts of stem of *T. cordifolia* prepared in water, ethanol and methanol showed antihyperglycemic, antioxidant and hepatoprotective activities in rats with experimental diabetes (Dubey and Srivastava, 2017). Despite very extensive studied plant due to its medicinal value, detailed literature search has shown some gaps in the phytochemical analysis with special focus on antidiabetic and antioxidant compounds in *T. cordifolia*. The present study is aimed to extract the phytoconstituents in water, ethanol and methanol according to their polarity and their identification by fourier transform infrared spectroscopy (FTIR) and gas chromatography-mass spectroscopy (GC-MS), both techniques are very powerful analytical tools for fractionation and identification of complex mixtures. Attempts have also been made to identify the constituents extracted in water, ethanol and methanol by qualitative phytochemical tests and to collect and compile the information about the medicinal properties of isolated phyto-components for future validation.

2. Materials and Methods

2.1 Collection of *T. cordifolia* stem and preparation of extracts

The dried stem of *T. cordifolia* were purchased from the local herbal market (identified and authenticated by CSIR-National Institute of Science Communication and Information Resources, New Delhi, Botanist: Dr. Sunita Garg, Voucher Specimen Number: NISCAIR/RHMD/3415-16), dried in shade and powdered with the help of mixer grinder. The extract of *T. cordifolia* was prepared by the method of Khan and Srivastava (Khan and Srivastava, 2012). Separately 30 g stem powder of *T. cordifolia* was suspended in 100 ml of water, ethanol and methanol, kept on magnetic stirrer for 1 h and incubated at 5°C in a refrigerator for 24 h. Next day, the extracts were placed on magnetic stirrer for 1 h, filtered with Whatman filter paper (Grade 2) and the filtrates were dried and the powder obtained was used in the study.

2.2 Phytochemical screening

The phytocomponents present in the extracts of the stem of *T. cordifolia* prepared in water, ethanol and methanol, were qualitatively analyzed for the presence of carbohydrates, protein, tannins and phenol, glycosides, flavonoids and alkaloids in detail as per the standard protocols (Lee *et al.,* 2013; Dhandapani and Babna, 2008). Molisch's test was performed by the method described by Foulger (1931), while other tests of carbohydrates were performed by standard procedures. Qualitative test for proteins was performed by the Biuret method (https://en.wikipedia.org/wiki/Biuret_test) while ninhydrin test was performed by the method described by Robert (1965). Other phytochemicals were detected by the method described by Ezeonu and Ejikene (2016).

2.3 Fourier transform infrared spectroscopic (FTIR) analysis

Fourier transform infrared spectroscopic (FTIR) analysis of the extracts was carried out by the method of Tao *et al.* (2015), using Spectrum II, Perkin Elmer FTIR. Aqueous, ethanolic and methanolic extracts of *T. cordifolia* were oven dried to get powders of the different solvent extracts to be used for FTIR analyses. Approximately, 10 mg dried extracts powder was used to prepare translucent sample disc by KBr pellet method. The method exploits the property that alkali halide becomes plastic when subjected to pressure that form a sheet that is transparent in the IR region. The sample was mixed with 200 mg fine KBr powder, pulverized and put into disc forming die, dried at 110°C for 2-3 h. A force of approximately 8 tons was applied under vacuum, degassing was performed to remove air and moisture from KBr. Analysis of the sample-KBr disc was carried out by scanning the samples in the wave number range of 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1}.

2.4 Gas chromatography-Mass spectrometry

2.4.1 Preparation of sample for GC-MS

The powder obtained after extraction of 30 g dried stem of *T. cordifolia* in water, ethanol and methanol, was dissolved in 2 ml methanol, 100 µl sample was taken and dissolved in 1300 µl methanol (dilution 1:14) and was used for GC-MS analysis.

GC-MS analysis was carried out by the method described by Adeoye-Isijola *et al.* using the fused silica capillary column composed of dimethyl poly siloxane, and helium as carrier gas at a constant flow rate of 1 ml/min (Adeoye-Isijola *et al.,* 2018*)*. The eluted components were detected in the mass detector.

3. Results

The herbal medicines are gradually gaining popularity due to their effectiveness, low cost, ease of availability and above all minimum or no side effects when compared with synthetic chemical drugs. Present study is carried out to screen the phytochemicals present in stem of *T. cordifolia* using different solvents, *viz.*, water, ethanol and methanol, in the light of their antidiabetic and antioxidant properties.

3.1 Qualitative phytochemical analysis of the extracts of *T. cordifolia* stem prepared in water, ethanol and methanol

The results of qualitative phytochemical analyses of aqueous, ethanolic and methanolic extracts of stem of *T. cordifolia* are given in Table 1 and Figure 1. All the three extracts tested for different phytochemicals showed the presence of alkaloids, flavonoids, glycosides, carbohydrates, proteins, tannins and phenols. The results presented in Table 1 clearly showed that all the three solvents are equally efficient in extracting carbohydrates while reducing sugars were maximally extracted in methanol; ketoses in water and pentoses in water and methanol equally. Water seems to be the best solvent for proteins and peptides, followed by ethanol while methanolic extract has not shown the presence of proteins tested by biuret method; the amino acids have been extracted equally in all the three solvents. Glycosides were maximally extracted in methanol while flavonoids were extracted only in aqueous extract. Alkaloids were maximally extracted in ethanolic fraction followed by methanol and water (Table 1).

3.2 FTIR-analysis

FTIR is the most powerful technique to identify the functional groups present in different extracts of *T. cordifolia*. The absorption maxima in the IR spectrum help to identify the functional groups of the active components present in different extracts (Maobe and Nyarango, 2013). When the extracts were analyzed into the FTIR, the different functional groups of the components were separated and were shown as peaks in the FTIR spectra. FTIR analysis of all the three extracts of the stem of *T. cordifolia* is presented in Table 2 and Figure 2. The results of FTIR analysis confirmed the presence of alcohol, aldehyde, alkyne, alkene, amines and ester. The absorbance bands analysis in bioreduction process are observed in the region between 400-4000 cm^{-1}.

3.3 GC-MS analysis

For fractionation of aqueous, ethanolic and methanolic extracts of *T. cordifolia*, a powerful separation technique, gas chromatography combined with powerful identification technique of mass spectroscopy (GC-MS) is used. GC-MS analysis of all the three extracts of the stem of *T. cordifolia* is presented in Table 3 and in Figure 3. In the GC-MS analysis of various extracts of *T. cordifolia* stem, a total of 65 components have been detected. Individually, aqueous extract contained 15 compounds, ethanolic extract contained 18 compounds, and methanolic extract contained 32 compounds (Table 3). Beta amyrin, androstan-17-one, and 3-ethyl-3-hydroxy- (5. Alpha) are common compounds present in all three extracts of *T. cordifolia,* however maximum amount of beta amyrin is extracted in aqueous, followed by ethanolic and methanolic extracts approximated by the obtained peak areas (2.64, 2.13 and 2.11 in aqueous, ethanolic and methanolic extracts, respectively Tabel 4). Medicinal properties of each components isolated and identified by GS-MS have been compiled on the basis of available information (Table 5).

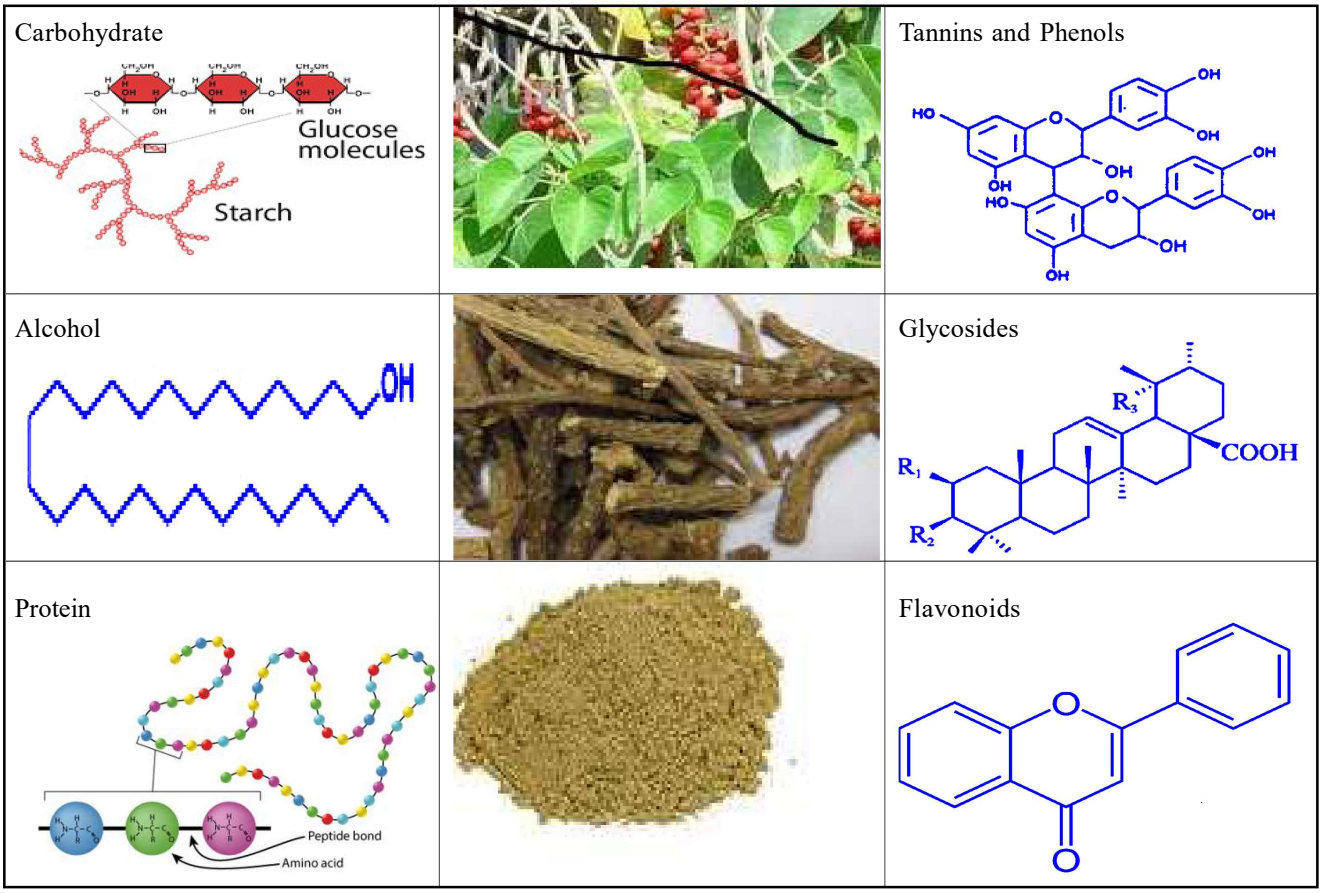


Figure 1: *Tinospora cordifolia*: Plant, dried stem and its powder.

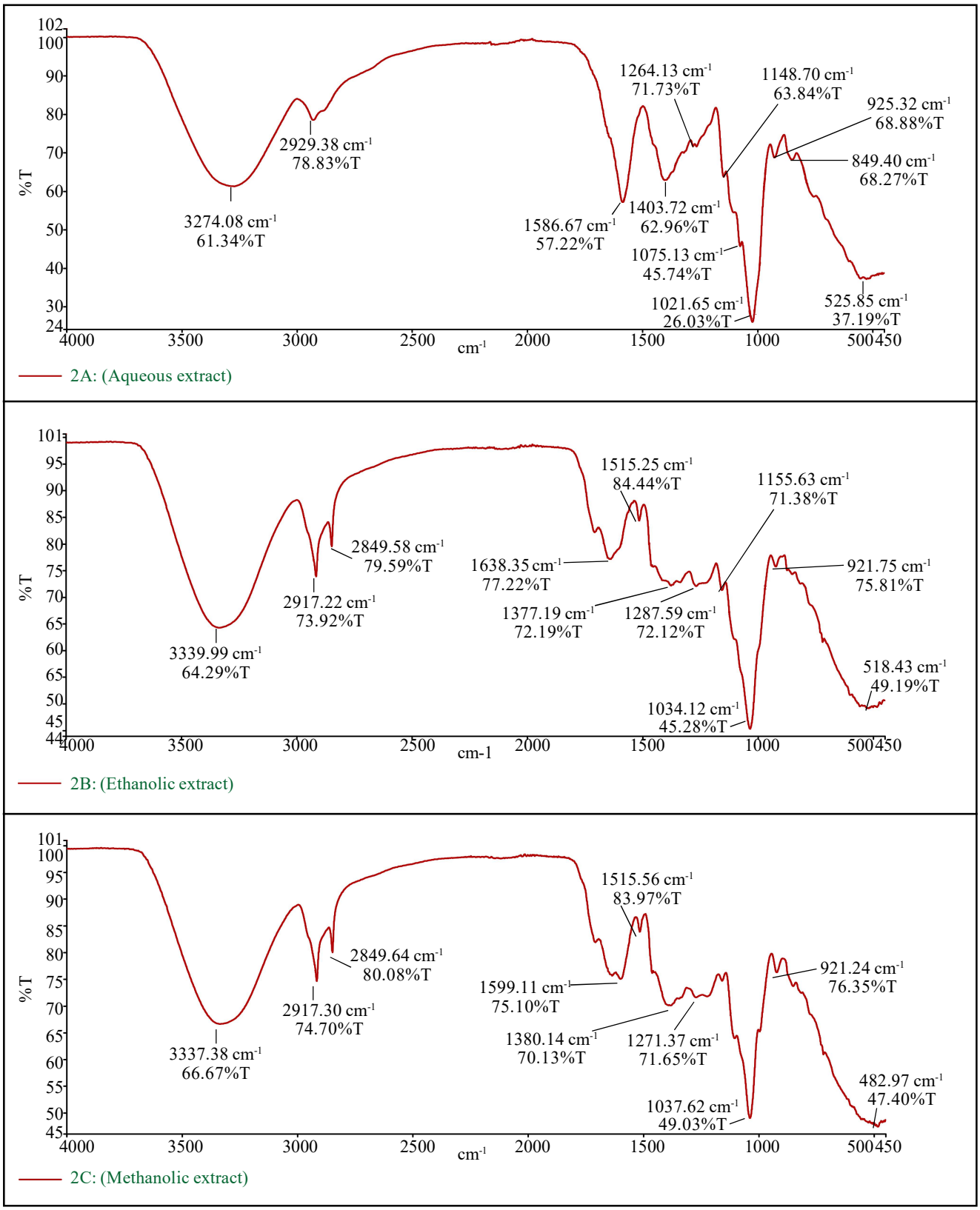


Figure 2: FTIR Chromatogram of aqueous, ethanolic and methanolic extract of *T. cordifolia*.

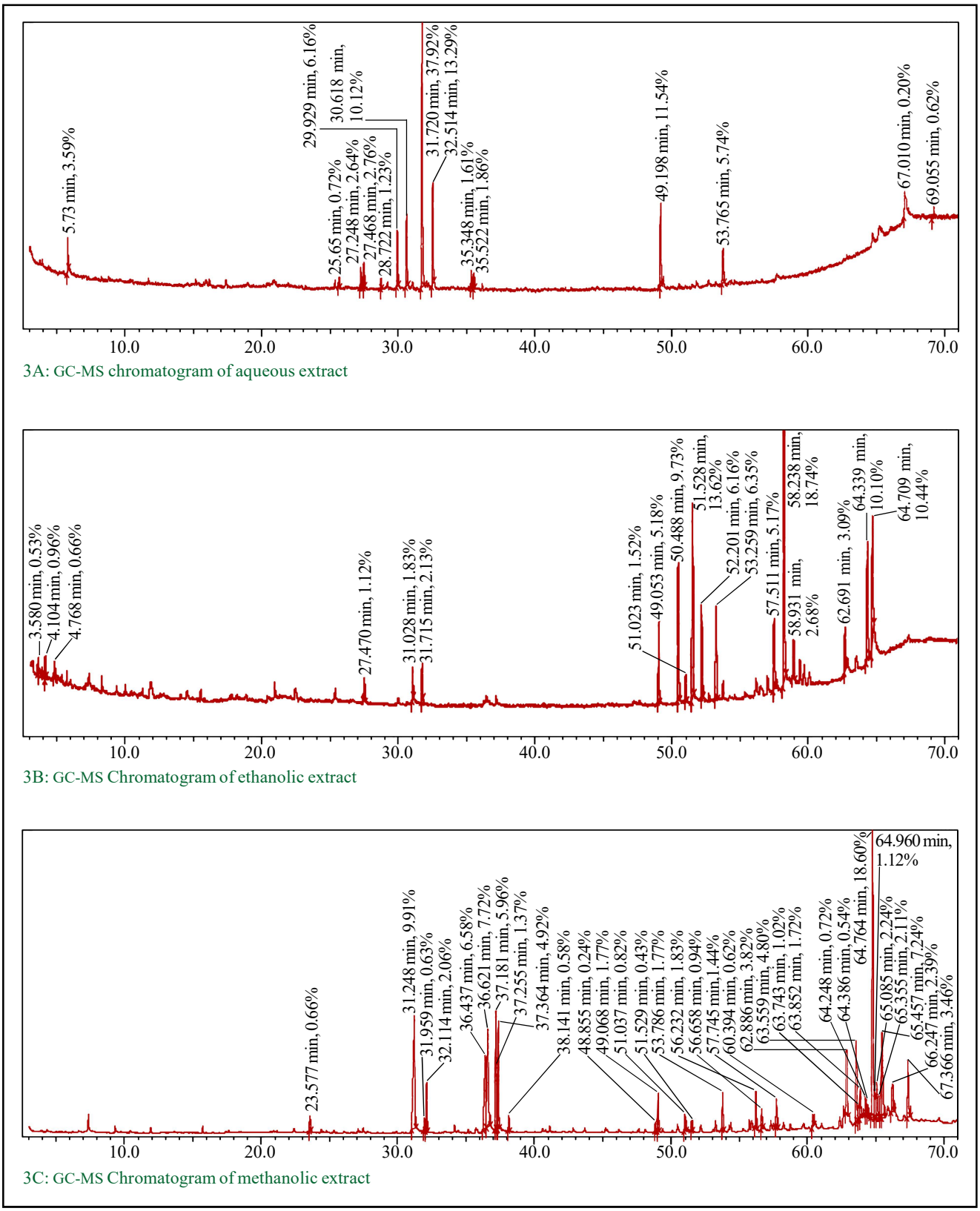


Figure 3: GC-MS Chromatogram of aqueous, ethanolic and methanolic extracts of *T. codifolia*.

Table 1: Qualitative phytochemical screening of aqueous, ethanolic and methanolic extracts of the stem of *T. cordifolia*

Constituents	Test	Results					
		Negative control	Std (0.1%)	Std (1%)	Aqueous extract	Ethanolic extract	Methanolic ext.
Carbohydrates	Molisch's test	–	++	+++	+++	+++	+++
	Fehling's test	–	++	+++	–	++	+++
	Benedict's test	–	+	+++	+++	++	+++
	Seliwanoff's test	–	+	+++	+++	+	++
	Bial's test	–	++	+++	+++	++	+++
Protein	Biuret's test	–	+	+++	+++	+	–
	Ninhydrin's test	–	+	+++	+++	+	+++
Tannin and phenol's	Ferric chloride test	–	+++	+++	++	+	++
	Lead acetate test	–	+	+++	+++	+	++
Glycosides	Keller-Killani test	–	++	+++	+++	+++	+++
	Borntrager's test	–	++	+++	++	+	+
	Legal's test	–	++	+++	–	+	++
Flavanoids	Shinoda's test	–	+	+++	++	–	–
Alkaloid's test	Wagner's test	–	++	+++	+++	+++	+++
	Hager's test	–	+	+++	+	+++	++
	Mayer's test	–	+	+++	+	+++	+++
	Dragendroff's test	–	++	+++	+++	+++	+++

- Signs:- absent; + slight presence; ++ moderate color; +++ intense color
- Standards used for carbohydrate: Glucose for Molish's, Fehling's and Benedict test; fructose for Selwinoff's test and xylose for Bial's test; for protein: bovine serum albumin for Biuret's and ninhydrin's tests; for tannins and phenol: catechol for ferric chloride and lead acetate test; for glycosides: sucrose for Keller- Killani, Borntrager's and Legal's tests; for flavanoids: for Shinoda's test standard used as quercetin; and for alkaloids: caffeine was used as standard Wagner's, Hager's, Mayer's and Dragendroff's tests.
- Each test was performed three times and the results were complied on the basis of intensity of color observed.

Table 2: Peaks observed in FTIR spectra of aqueous, ethanolic and methanolic extracts of *T. cordifolia* and corresponding probable functional group

	Aqueous extract		Ethanolic extract		Methanolic extract	
S.No.	Observed peak (wave number cm^{-1})	Functional groups	Observed peak (wave number cm^{-1})	Functional groups	Observed peak (wave number cm^{-1})	Functional groups
1	3274.08	Hydroxyl compound	3339.99	Hydroxyl compound	3337.38	Hydroxyl compound
2	2929.38	Alkene	2917.22	Methyl group	2917.30	Methyl group
3	1586.67	Aromatic	2849.58	Alkane	2849.64	Alkane
4	1403.72	Nitrosamine	1638.35	Conjugated Alkene	1599.11	Aromatic ring
5	1264.13	Alcohols, Esters	1515.25	Phenol ring	1515.56	Phenol ring
6	1146.70	Aliphatic ether	1377.19	Phenol	1380.14	Phenol
7	1075.13	Amines	1287.59	Ester	1271.37	Aromatic ester
8	1021.65	Alkyl amine	1034.12	Amines	1037.62	Amines
9	925.32	Ether	1155.63	Ester	921.24	Ether
10	849.40	Alkene	921.75	Ether	487.97	Halogen compound
11	525.85	Halogen compound	518.43	Halogen compound	____	____

- The FTIR of Perkin Elmer Spectrum II, was used for the present study.
- IR spectra of aqueous, ethanolic and methanolic extract of *T. cordifolia* were scanned as function of % Transmittance versus wave number (cm^{-1}).
- The functional groups giving the particular peak were identified from the reported values.
- The experiment was repeated twice.

Table 3: Phytochemicals resolved by GC-MS in aqueous, ethanolic and methanolic extracts of *T. cordifolia*

S.No.	Name of the compound	Aqueous extract		Ethanolic extract		Methanolic extract	
		Rt (min)	PA (%)	Rt (min)	PA (%)	Rt (min)	PA (%)
1.	2-Piperidinone	5.73	3.59	–	–	–	–
2.	Tridecanoic acid,12-methyl-, methyl ester	25.65	0.72	–	–	–	–
3.	Beta amyrin	27.248	2.64	–	–	–	–
4.	Calcitriol	27.468	2.76	–	–	–	–
5.	Ethyl tridecanoate	28.722	1.23	–	–	–	–
6.	Hexadecanoic acid, methyl ester	29.929	6.16	–	–	–	–
7.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5. Alpha	30.618	10.12	–	–	–	–
8.	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8dio-	31.720	37.92	–	–	–	–
9.	1,Z-5,E-7-Dodecatriene	32.514	13.29	–	–	–	–

S.No.	Name of the compound	Aqueous extract		Ethanolic extract		Methanolic extract	
		Rt (min)	PA (%)	Rt (min)	PA (%)	Rt (min)	PA (%)
10.	8-Azabicyclo[3,2,1]octan-3-ol, 8-methyl-endo-	35.348	1.61	–	–	–	–
11.	7-Hexadecenoic acid, methyl ester,(Z)	35.522	1.86	–	–	–	–
12.	Alfa-Copaene	49.198	11.54	–	–	–	–
13.	1'-Vinyl-1'-2'-3'-4'-tetrahydrospiro[1,3]dioxolar	53.765	5.74	–	–	–	–
14.	Phthalic acid	67.010	0.20	–	–	–	–
15.	Heptacosanol	69.055	0.62	–	–	–	–
16.	1-Butanamine, 3-methyl-N-(3-methylbutalide	–	–	3.580	0.53	–	–
17.	Butanedioicacid,monomethyl ester	–	–	4.104	0.96	–	–
18.	1-(+)-Lactic acid, tert-butyldimethylsilyl ester	–	–	4.768	0.66	–	–
19.	5-Isopropylidene-4, 6-dimethylnona-3,6,8 trien-	–	–	27.470	1.12	–	–
20.	Pentadecanoic acid	–	–	31.028	1.83	–	–
21.	Beta amyrin	–	–	31.715	2.13	–	–
22.	1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-	–	–	49.053	5.18	–	–
23.	2,2,6beta, 7-Tetramethylbicyclo[4,3,0]nona-1	–	–	50.488	9.73	–	–
24.	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-Dimethyl	–	–	51.023	1.52	–	–
25.	Androstan-17-one, 3-ethyl-3-hydroxy-,(5. Alpha)	–	–	51.528	13.62	–	–
26.	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-aza	–	–	52.201	6.16	–	–
27.	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl	–	–	53.259	6.35	–	–
28.	Cyclopentanecarboxamide, 2-[1-(2-butenyl)-3-	–	–	57.511	5.17	–	–
29.	Beta-Vatirenene	–	–	58.238	18.74	–	–
30.	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-	–	–	58.931	2.68	–	–
31.	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclo	–	–	62.691	3.09	–	–
32.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5. Alpha	–	–	64.339	10.10	–	–
33.	Beta-Sitosterol	–	–	64.709	10.44	–	–
34.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenyl	–	–	–	–	23.577	0.66
35.	1-(+)-Ascorbic acid 2,6-dihexadecanoate	–	–	–	–	31.248	9.91
36.	Benzenemethanol, 2,5-dimethoxy-, acetate	–	–	–	–	31.959	0.63
37.	Hexadecanoic acid, methyl ester	–	–	–	–	32.114	2.06
38.	Ethyl 9,12- hexadecadienoate	–	–	–	–	36.437	6.58
39.	9-Octadecenoic acid,1,2,3-propanetriyl ester, (EEE)	–	–	–	–	36.621	7.72
40.	n-Propyl 9,12-octadecadienoate	–	–	–	–	37.181	5.96
41.	Octadecanoic acid	–	–	–	–	37.255	1.37
42.	9-Octadecanoic acid, 1,2,3-propanetriyl ester, (EEE)	–	–	–	–	37.364	4.92
43.	Heptadecanoic acid, 15-methyl-, ethyl ester	–	–	–	–	38.141	0.58
44	Ethyl tridecanoate	–	–	–	–	48.855	0.24
45	1H-3a, 7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene	–	–	–	–	49.068	1.77
46	1,4- Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-Octahydro-11,11-dimethyl-	–	–	–	–	51.037	0.82
47	Cycloprop[e]indene-1a,2(1H)-dimethanol,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl-	–	–	–	–	51.529	0.43
48	1-Methoxy-5-methyl-5-phenyl-7-oxabicyclo[4.	–	–	–	–	53.786	1.77
49	Benzene, 1,2-bis(1-buten-3-yl)-	–	–	–	–	56.232	1.83
50	Benzene,(2-ethyl-4-methyl-1,3-pentadienyl)-,	–	–	–	–	56.658	0.94
51	Benzene,(2-ethyl-4-methyl-1,3-pentadienyl)-,	–	–	–	–	57.745	1.44
52	1-Heptacosanol	–	–	–	–	60.394	0.62
53	3 beta-Hydroxy-5-cholen-24-oic acid	–	–	–	–	62.886	3.82
54	Stigmasterol	–	–	–	–	63.559	4.80
55.	1-Tetradecene n-tetradec-1-ene	–	–	–	–	63.743	1.02
56.	(+)- Lariciresinol	–	–	–	–	63.852	1.72
57.	Obtusifoliol	–	–	–	–	64.248	0.72
58.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha)	–	–	–	–	64.386	0.54
59.	3.beta-Hydroxy-5-cholen-24-oic acid	–	–	–	–	64.764	18.60
60.	5-Isopropylidene-4, 6-dimethylnona-3,6,8-trien-	–	–	–	–	64.960	1.12
61.	14- Oxatricyclo[9.2.1.0(1,10)]tetradecane,2,6,6,10,11-pentamethyl	–	–	–	–	65.085	2.24
62.	beta-Amyrin	–	–	–	–	65.355	2.11
63.	9,19-Cyclo-9.beta-lanostane-3.beta,25-diol	–	–	–	–	65.457	7.24
64.	(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.0	–	–	–	–	66.247	2.39
65.	9,19-Cyclolanostan-3-ol,24-methylene-,(3.beta	–	–	–	–	67.366	3.46

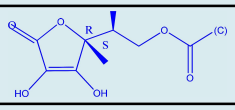
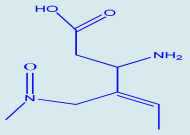
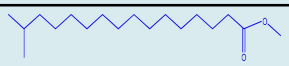
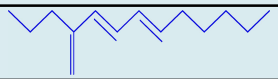
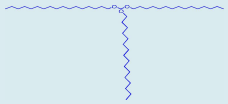
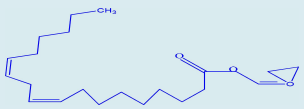
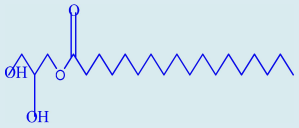
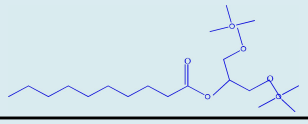
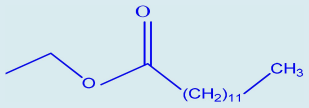
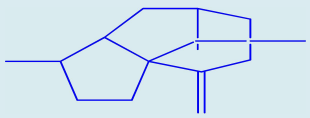
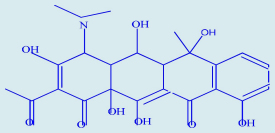
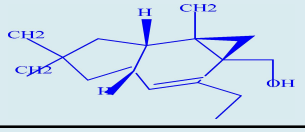
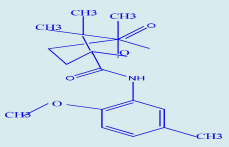
- Rt: retention time in min and PA: peak area in %.
- List of photochemical resolved on the basis of retention time and quantitated on the basis of peak area.

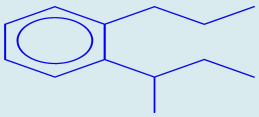
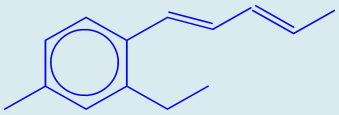
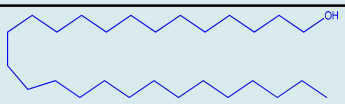
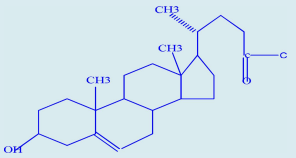
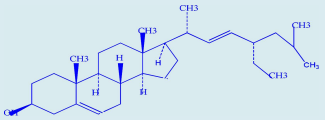
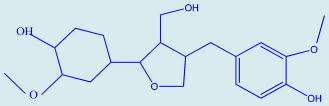
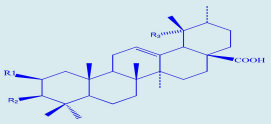
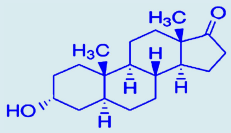
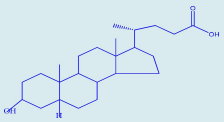
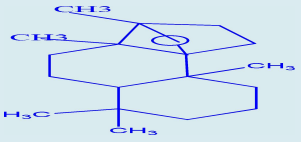
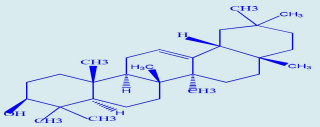
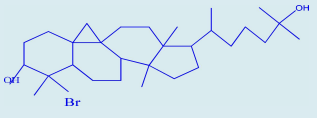
Table 4: Comparison of compound present in different extracts (aqueous, ethanolic and methanolic) of *T. cordifolia* stem

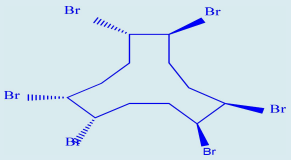
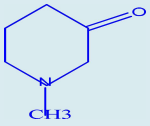
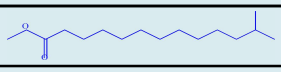
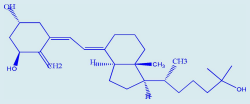
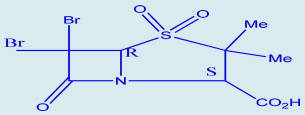
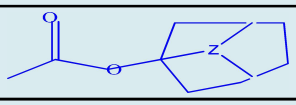
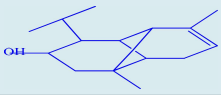
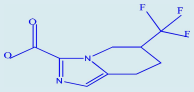
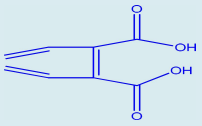
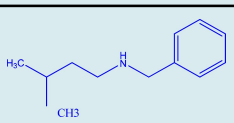
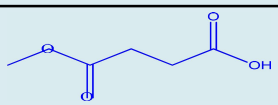
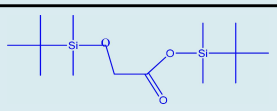
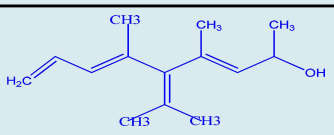
S.No.	Name of the compound	Type of solvent for extraction		
		Water	Ethanol	Methanol
1.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenyl	-	-	P
2.	1-(+)-Ascorbic acid 2,6-dihexadecanoate	-	-	P
3.	Benzenemethanol, 2,5-dimethoxy-, acetate	-	-	P
4.	Hexadecanoic acid, methyl ester	p	-	P
5.	Ethyl 9,12- hexadecadienoate	-	-	P
6.	9-Octadecenoic acid,1,2,3-propanetriyl ester, (EEE)	-	-	P
7.	n-Propyl 9,12-octadecadienoate	-	-	P
8.	Octadecanoic acid	-	-	P
9.	Heptadecanoic acid, 15-methyl-, ethyl ester	-	-	P
10.	Ethyl tridecanoate	p		P
11.	1H-3a, 7-Methanoazulene, octahydro-1,9,9-trimethyl-4methylene	-	P	P
12.	1,4- Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-Octahydro-11,11 dimethyl	-	p	P
13.	Cycloprop[e]indene-1a,2(1H)-dimethanol,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl	-	-	P
14.	1-Methoxy-5-methyl-5-phenyl-7-oxabicyclo[4.	-	-	P
15.	Benzene, 1,2-bis(1-buten-3-yl)-	-	-	P
16.	Benzene,(2-ethyl-4-methyl-1,3-pentadienyl)-,	-	-	P
17.	1-Heptacosanol	p	-	P
18.	3 beta-Hydroxy-5-cholen-24-oic acid	-	-	P
19.	1-Tetradecene n-tetradec-1-ene	-	-	P
20.	(+)- Lariciresinol	-	-	P
21.	Obtusifoliol	-	-	P
22.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha)	p	p	P
23	3.beta-Hydroxy-5-cholen-24-oic acid	-	-	P
24.	14-Oxatricyclo[9.2.1.0(1,10)]tetradecane,2,6,6,10,11-pentamethyl	-	-	-
25.	beta-Amyrin	P	p	P
26	9,19-Cyclo-9.beta-lanostane-3.beta,25-diol	-	-	P
27.	(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.0	-	-	P
28.	Piperidinone	p	-	P
29.	Tridecanoic acid 12-methyl ester	P	-	-
30.	Calcitriol	P	-	-
31.	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-dio	p	-	-
32.	1,Z-5,E-7-Dodecatriene	p	p	-
33.	8-Azabicyclo[3,2,1]octan-3-ol, 8-methyl-, endo-	P	-	-
34.	Alfa-Copaene	p	-	-
35.	1′-Vinyl-1′-2′-3′-4′-tetrahydrospiro[1,3]dioxolar	P	-	-
36.	Phthalic acid	p	-	-
37.	1-Butanamine, 3-methyl-N-(3-methylbutalide	-	P	-
38.	Butanedioicacid,monomethyl ester	-	P	-
39.	1-(+)-Lactic acid, tert-butyldimethylsilyl ester	-	p	-
40.	5-Isopropylidene-4, 6-dimethylnona-3,6,8-trien-	-	P	-
41.	Pentadecanoic acid	-	P	-
42.	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-aza	-	P	-
43.	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl	-	P	-
44.	Cyclopentanecarboxamide, 2-[1-(2-butenyl)-3-	-	P	-
45.	Beta-Vatirenene	-	P	-
46.	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-	-	P	-
47.	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclo	-	P	-
48.	Beta-Sitosterol	-	p	-

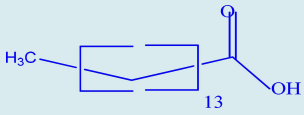
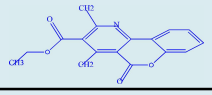
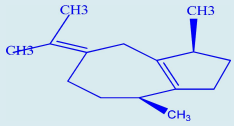
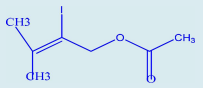
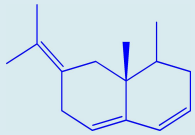
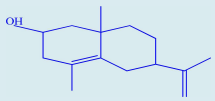
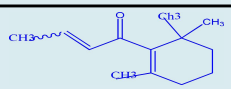
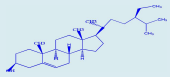
- Comparison of compounds resolved and identified by GC-MS in different extracts of *T. cordifolia*.
- P: presence of compound.

Table 5: Chemical formula, molecular weight and structure of all compounds resolved and identified in aqueous, ethanolic and methanolic extracts of *T. cordifolia*

S.No	Name of the compound	Structure	Chemical formula	Molecular weight	Medicinal properties
1.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenyl		$C_{10}H_{12}O_3$	180.2	Antimicrobial, antioxidant, anti-inflammatory
2.	Benzenemethanol, 2,5-dimethoxy-, acetate		$C_{11}H_{14}O_4$	168.2	Antibacterial activity
3.	Hexadecanoic acid, methyl ester		$C_{18}H_{36}O_2$	284.5	Antioxidant, pesticide, hypocholesterolimic
4.	Ethyl 9,12- hexadecadienoate		$C_{18}H_{32}O_2$	280.5	Ntioxidant, pesticide, hypocholesterolemic
5.	9-Octadecenoic acid,1,2,3-propanetriyl ester, (EEE)		$C_{57}H_{104}O_6$	885.4	Antispasmodic, immunomodulatory
6.	n-Propyl 9,12-octadecadienoate		$C_{21}H_{38}O_2$	322.5	Emulsifying agent, soaps, paint
7.	Octadecanoic acid		$C_{18}H_{36}O_2$	284.5	Antifungal, Antitumor, antibacterial
8.	Heptadecanoic acid, 15 methyl-, ethyl ester		$C_{20}H_{40}O_2$	312.5	Antioxidant, Hypocholesterolemic, Antiandrogenic, Hemolytic
9.	Ethyl tridecanoate		$C_{15}H_{30}O_2$	242.4	Antibacterial,antifungal
10.	1H-3a, 7Methanoazulene, octahydro-1,9,9-trimethyl-4methylene		$C_{15}H_{24}$	204.4	Anti-inflammatory, antiprotozoal,antibacterial and antimicrobial
11.	1,4-Methanocycloocta[d] pyridazine,1,4,4a,5,6,9,10,10a-Octahydro-11,11 dimethyl		$C_{20}H_{30}O$	286.5	Anti allergicantidepressive, antihypertensive, antiinflammatory
12.	Cycloprop[e]indene-1a,2(1H)-dimethanol,3a,4,5,6,6a,6b-hexahydro-5,5,6b-trimethyl		$C_{15}H_{24}O_2$	236.4	Anti-inflammatory
13.	1-Methoxy-5-methyl-5-phenyl-7-oxabicyclo[4.		$C_{18}H_{23}$	317.4	Antitubercular agent

S.No	Name of the compound	Structure	Chemical formula	Molecular weight	Medicinal properties
14.	Benzene, 1,2-bis-(1-buten-3yl)-		$C_{14}H_{18}$	186.3	Antineoplastic agent
15.	Benzene,(2-ethyl-4-methyl-1,3-pentadienyl)-,		$C_{14}H_{18}$	186.3	Antineoplastic agent, antiviral, washing hair
16.	1-Heptacosanol		$C_{27}H_{56}O$	396.7	Used against skin cancer, antioxidant, anticancer, antimalarial
17.	3 beta-Hydroxy-5-cholen-24-oic acid		$C_{24}H_{38}O_3$	374.6	Agreegation behavior, cholesterol dissolution, proliferation and apoptosis in colan
18.	Stigmasterol		$C_{29}H_{48}O$	412.7	Antioxidant, hypoglycemic, antidiabetic, anti-inflammatory
19.	(+)- Lariciresinol		$C_{20}H_{24}O_6$	360.4	Antifungal
20.	Obtusifoliol		$C_{30}H_{50}O$	426.7	Conjuctivitis, eye disease, blurred vision, asthma, ascaris
21.	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha)		$C_{21}H_{34}O_2$	318.5	Hypocholesterolemic, nimeticide, pesticide
22.	3.beta-Hydroxy-5-cholen-24-oic acid		$C_{24}H_{40}O_3$	376.6	Hepatoprotective, fat absorption,cholesterol excretion
23.	14-Oxatricyclo [9.2.1.0(1,10)] tetradecane,2,6,6,10,11-pentamethyl		$C_{18}H_{30}$	262.4	Wound healing, antiscarring
24.	beta-Amyrin		$C_{30}H_{50}O$	426.7	Antidiabetic, anti-inflammatory, anticancer
25.	9,19-Cyclo-9.beta-lanostane-3.beta,25-diol		$C_{30}H_{52}O_2$	444.7	Hypoglycemic, antidiabetic

S.No	Name of the compound	Structure	Chemical formula	Molecular weight	Medicinal properties
26.	(1S,6R,9S)-5,5,9,10-Tetramethyltricyclo[7.3.0		$C_{12}H_{18}Br_6$	641.7	Antiinflammatory, antibiotic,aiding memory
27.	Piperidinone		C_5H_9NO	99.1	Antifungal
28.	Tridecanoic acid 12-methyl ester		$C_{15}H_{30}O_2$	242.4	No activity reported
29.	Calcitriol		$C_{27}H_{44}O_3$	416.7	Hyperparathyroidism, absorption of calcium from the stomach
30.	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-dio		$C_{13}H_{13}N_5S_5O_2$	383.4	Healing property
31.	1,Z-5,E-7-Dodecatriene		$C_{12}H_{20}$	164.3	Antitumour, antibacterial, skin disease
32.	8-Azabicyclo[3,2,1]octan-3-ol, 8-methyl-, endo-		$C_7H_{13}NO$	127.2	Antimuscarinic property
33.	Alfa-Copaene		$C_{15}H_{24}$	204.4	Antioxidant, antiproliferative, antimicrobial
34.	1'-Vinyl-1'-2'-3'-4'-tetrahydrospiro[1,3]dioxolar		C_4H_6O	70.1	Antidiabetic, Increased immunity, hypoglycemic
35.	Phthalic acid		$C_8H_6O_4$	166.1	Synthesis of dyes, perfumes and other organic compound
36.	1-Butanamine, 3-methyl-N-(3-methylbutalide		$C_7H_{14}O_2$	130.2	Antioxidant, prevent heart disease,antidote for cyanide poisoning
37.	Butanedioicacid,monomethyl ester		$C_5H_8O_4$	132.1	A molecular entity capable of donating a hydron acceptor
38.	1-(+)-Lactic acid, tert-butyldimethylsilyl ester		$C_{15}H_{34}O_3Si_2$	318.6	Prevent skin disease
39.	5-Isopropylidene-4, 6-dimethylnona-3,6,8-trien-		$C_{14}H_{22}$	206.3	Hypertension, cough, headache

S.No	Name of the compound	Structure	Chemical formula	Molecular weight	Medicinal properties
40.	Pentadecanoic acid		$C_{15}H_{30}O_2$	242.4	Antioxidant, antifungal, microbial activity
41.	3-Amino-6-methyl-6,7-dihydro-9H-5-oxa-9-aza		$C_6H_8N_2$	108.1	Antiinflammatory, antitumor, serotonin activity
42.	Azulene,1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl		$C_{15}H_{24}$	204.4	Apoptosis, cellcycle, antifungal, antimicrobial
43.	Cyclopentanecarboxamide, 2-[1-(2-butenyl)-3-		$C_{14}H_{18}O_4$	250.3	Obesity, diabetes, fatty acid synthesis
44.	Beta-Vatirenene		$C_{15}H_{22}$	202.3	Anti-inflammatory, antiprotozoal, antibacterial and antimicrobial.
45.	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-		$C_{15}H_{24}O$	220.4	Anti inflammatory, antiprotozoal, antibacterial and antimicrobial
46.	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclo		$C_{18}H_{26}O$	258.4	Antioxidant, free radical scavenging activity
47.	Beta-Sitosterol		$C_{29}H_{50}O$	414.7	Enhancing sexual activity

- The structure, molecular weight and medicinal properties of resolved compounds are compiled from the available literature.

4. Discussion

T. cordifolia is a plant with very high medicinal value against a large number of ailments (Sharma *et al.,* 2014; Mishra *et al.,* 2013; Saha and Ghosh, 2012). Researchers across the globe have developed great interest in this plant because of many of its reported medicinal activities like antineoplastic, anti-HIV, anticancer, hepatoprotective, immunomodulatory, antileprotic, antistress, antiarthritic, antiallergic, antidiabetic, antispasmodic, anti-inflammatory, *etc*. A large number of bioactive phytocomponents, *viz*., alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from all parts of the plant including root, stem or the whole plant (Kavitha *et al.,* 2011; Ahmad *et al.,* 2010). For extraction of phytochemicals from *T. cordifolia* in the present study, water, ethanol and methanol were selected which are commonly used solvents for extraction. Many secondary metabolites including phenolics, alkaloids, glycosides, flavanoids and carbohydrates are soluble in methanol while ethanol is good solvent for extraction of alkaloids, saponins, tannins, phenolics, glycosides, proteins and amino acids. Methanol and ethanol are neither too toxic nor too volatile (B.P. 64.7° C and 78.4° C, respectively). Mostly methanol and ethanol are used for extraction of various polar compounds while certain non-polar compounds are also soluble in these solvents (polarity indices of methanol and ethanol are 6.6 and 5.2, respectively) (IIoki-Assango *et al.,* 2015).

Different groups of compounds have been detected in all the three solvents used for extraction including alkaloids, flavonoids, glycosides, carbohydrates, proteins, tannins and phenols. These secondary metabolites are very useful compounds being extensively used in medicine as anticancer, in heart diseases, analgesics, antimalarials, antibiotic, antibacterial, *etc.*, neutraceuticals, antidotes for reducing poisoning's by toxic compounds, in research as metabolic inhibitors for tracing various pathways, *etc*. Highest amount of alkaloids were detected only in ethanolic extract while proteins, carbohydrates, tannins and phenols are present in higher amounts in aqueous extract when compared with ethanolic or methanolic extracts of *T. cordifolia* while flavonoids were present only in aqueous extract. The presence of wide range of phytochemicals of medicinal importance in different species of *Tinospora* has been compiled earlier also (Chi *et al.,* 2016). The starch obtained from the stem, is highly nutritive and digestive and is used in many diseases. Starch is present throughout the parenchyma of the stem (Sharma *et al*., 2013). It is a tonic, useful in the treatment of dysentery, chronic diarrhea and other diseases of

gastrointestinal tract and protects the liver from damage by various drugs and chemicals. This is especially useful when the liver has been exposed to various toxins. Results indicate the presence of many phyto-components in the extracts prepared in all three solvents. These compounds may contribute the activities like antioxidant, cancer-preventive, hypocholesterolemic, nematicidal, antifungal, antidiabetic, hepatoprotective, *etc*. Hence, the stem of *T. cordifolia* is worthy for further investigation for being used in natural drug developments.

Beta amyrin present in all the three extracts is reported for its antidiabetic properties (Singh *et al*., 2009). The other compounds reported for their antidiabetic properties are 1-vinyl-1, 2, 3, 4-tetrahydrospiro [1,3] dioxolar extracted in water, cyclopentanecarboxamide, 2-[1-(2-butenyl)-3- in ethanol and 9,19-cyclo-9.betalanostane-3.beta,25-diol and stigmasterol in methanol. Hexadecanoic acid methyl ester, ethyl tridecanoate and heptacosanol were common in aqueous and methanolic extracts of *T. cordifolia*, 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-dio was common in aqueous and ethanolic extracts, 1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl and 1,4-Methanocycloocta[d] pyridazine, were common in ethanolic and methanolic extracts of *T. cordifolia.* Androstan present in all three extracts, is known for hypocholesterolemic and nematicidal properties, whereas hexadecanoic present in aqueous extract and methanolic extracts is reported for antioxidant, hypocholesterolemic and pesticide properties, ethyl tridecanoate is known for antibacterial and antifungal properties (Arora and Saini, 2017), hepatocosanol for skin cancer, antioxidant, anticancer and antimalarial properties, whereas 2,6,8 trimethylbicyclo[4,2,0]oct-2-ene-1,8-dio present in aqueous extract and ethanolic extracts is known for healing properties as reported by Bowler *et al*. (2001). In our earlier work, we have identified that aqueous extract of *T. cordifolia* showed highest antidiabetic property as compared to other extract (Dubey and Srivastava, 2017). Therefore,1-Vinyl-1, 2, 3, 4-tetrahydrospiro [1,3] dioxolar present only in aqueous extract along with beta amyrin may be responsible for the antidiabetic properties. Other compounds present in aqueous extracts are reported to have antibacterial, antifungal, anticancer, antioxidant, anti-inflammatory, hyperthyroidism, absorption of calcium from the stomach, antimuscarinic, antiproliferative, antidiabetic, immune-stimulatory and hypoglycemic properties as well as being used in the synthesis of dyes, perfumes, and other organic compounds as reported by several workers earlier. Remaining compounds present in ethanolic extract are reported to have anti-inflammatory, antibacterial, antimicrobial, antiallergic, antidepressant, antioxidant, prevent heartache, serotonin activity, cell cycle, apoptosis, obesity, fatty acid synthesis, antiprotozoal, free radical scavenging activity, enhancing sexual activity, *etc*. (Kanthal *et al*., 2014). The compounds present in methanolic extracts showed antimicrobial, antioxidant, anti-inflammatory, pesticide, hypocholesterolemic, antispasmodic, immunomodulatory, emulsifying agent, soaps, paint, hemolytic, antiandrogenic, antitubercular agent, aggregation behavior, proliferation, blurred vision, asthma, antiascaris, hepatoprotective, fat absorption and memory enhancing properties. Presence of a large number of compounds showing various activities in all the three extracts has been listed in Table 4. The medicinal properties of the compounds present in all the three extracts of *T. cordifolia* stem have been compiled from the reported information and listed in Table 5.

The stem of *T. cordifolia* contains many important phytochemical components such as alkaloids, carbohydrates, flavonoids, *etc*. In FTIR analysis, aqueous and ethanolic extracts contained 11 peaks while methanolic extract showed 10 peaks, confirming the presence of compounds with at least 11 and 10 types of functional groups, respectively. In GC-MS analysis, the methanolic extract is found to be the richest in active components as maximum number of phytoconstituents have been extracted in this solvent. Compounds with reported antidiabetic and hepatoprotective effects have been isolated from all the three extracts of *T. cordifolia*, however in our earlier report, aqueous extract showed highest antidiabetic and hepatoprotective properties compared with other two extracts (Dubey and Srivastava, 2017). Knowledge of chemical constituents of plant is important and desirable because such information will be useful for synthesis of new chemical compounds with greater therapeutic efficacy.

5. Conclusion

Results of the present study have shown the presence of various phytocomponents in all the extracts prepared in water, ethanol and methanol. Methanol seems to be the best solvent for extraction of such components as highest number of compounds have been extracted in this solvent. The antidiabetic and antioxidant compounds has been found widely distributed in all the three extracts with water being slightly superior solvent which is of benefit due to its safety. Further, validation of isolated compounds for their antihyperglycemic potential is mandatory for their use as antidiabetic agent for humans. Further, investigations and testing of the isolated compounds from *T. cordifolia* extracts is required which may become a part of standard drug designing and treatment protocols for diabetes and hence a promising and powerful weapon for diabetes treatment.

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Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and agreed to submit findings for publication.

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