Research Article

Preliminary Phytochemical Screening and GC-MS Analysis of Ethanolic Stem Extract of the Ethnomedicinal Plant- *Tinospora crispa* (L.) Miers [Menispermaceae]

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ABSTRACT

Tinospora crispa (L.) Miers belongs to the family Menispermaceae and is used as a traditional medicine in China, Malaysia, Philippines, etc. It is one of the most popular local medicinal plants. It is also called Makabühai, which means "to give life". The GCMS analysis revealed the presence of 20 compounds in the stem extract of *Tinospora crispa*. Some of the compounds are 1,2,3-Propanetriol, diacetate (CAS) Diacetin, 3-Tetradecene, (Z)- (CAS), 4-Penten-1-ol, trimethylsilyl ether, Diethyl Phthalate 1,2-benzene dicarboxylic acid, diethyl ester, 9-Eicosene, (E)- (CAS), Benzyl Benzoate, 9-Octadecenoic acid (Z)- (CAS) Oleic acid, Phosphonic acid, dioctadecyl ester (CAS) Di(n-octadecyl) phosphite, 9,12-Octadecadienoic acid (Z,Z)-, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(CAS) Linolenic acid, 3-furylmethanol, 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate. This plant contains compounds such as alkaloids, saponins, glycosides, tannins, and flavonoids. These compounds have antipyretic, antiparasitic, antifungal, antidiabetic and antitumor properties.

Keywords: GC-MS Analysis, Phytoconstituents, Medicinal compounds, *Tinospora crispa*, Ethanolic stem extract, Ethnomedicinal plant.

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INTRODUCTION

Medicinal plants are used by ancient people as they possess rich resources for various diseases.[1] These plants play a vital role in the development of drugs around the whole world. Moreover, some plants are considered an important source of nutrition and are recommended for their therapeutic values.[2]

The genus *Tinospora* has 34 species, in which most of the herbs were used as traditional medicines by ancient people throughout the tropical and subtropical parts of Asia, Africa, and Australia.[3] The literature survey revealed that the *Tinospora* species is a group of important medicinal plants used for the ethnomedicinal treatment of cold, headache, rheumatoid arthritis, fever, oral ulcer, pharyngitis, diabetes, diarrhoea and digestive disorder.[5]

Indian ethnopharmacological data points to the therapeutic values of the *T*inospora species for the treatment of diabetic mellitus. Ethnopharmacological history of *Tinospora* species exhibit, anti-inflammation, antioxidation, immunostimulation, antitumor, antidiabetic, antiosteoporosis, and antimicrobial activities.[6]

Tinospora crispa (L.) Hook. F. and Thomson is a medicinal plant that belongs to the family Menispermaceae. It has been used in conventional medicine to treat numerous ailments in Malaysia, Africa, Thailand, South East Asia, and Indonesia. The purpose of this study is to know the phyto components present in the plant by GCMS.

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MATERIALS AND METHODS

Collection and Processing of Plant Materials

The fresh and healthy stem of *Tinospora crispa* was collected from Ettumanoor, Kerala. The Plant material was identified and authenticated by the Botanical Survey of India, Coimbatore.

The stem was cleaned with fresh running tap water to remove all the unwanted debris, air-dried in shade, and powdered using a mixer grinder. The powder was further passed through a 2 mm sieve to obtain fine particles. The powdered samples were stored in a container for future use.[7]

Preparation of Plant Extracts

20 g of powdered stem material of *Tinospora crispa*., was dispersed in 150 ml of solvents such as hexane, ethanol, and water and subjected to soxhlet extraction for 6-8 hrs. The mixture was evaporated in a rotary flash evaporator and stored in a refrigerator.[8] The condensed extracts were made to 1mg/ml dilution and were used for further phytochemical and pharmacological studies.

Qualitative Phytochemical Assay

Qualitative phytochemical screening of the stem was carried out with three different solvents as Hexane, Ethanol, and water in order to analyze the class of organic compounds. The extracts of the *Tinospora crispa* stem were analyzed by standard chemical tests[9] to determine steroids, triterpenoids, alkaloids, carbohydrates, proteins, flavonoids, glycosides, amino acids, and tannins.

GC-MS Analysis

GC-MS plays a key role in the analysis of phytocompounds present in the origin of plant. 1µl of ethanolic stem extract of *Tinospora crispa* was employed for the analysis of various compounds. GC-MS analysis was performed by using the model: SHIMADZU GCMS-QP-2010plus and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Rtx-5MS Capillary Standard Non - Polar Column For GC/MS detection. Helium (5.5 lb/in2) Flow - 41.0 ML/Min was used as the carrier gas with a constant flow rate 1 ml/min and 2 ml was injected (Split ratio of 10:1), the temperature of the injector was 270°C; ion-source temperature was 200°C. The oven temperature was 70°C which is then raised to 260°C at 6°C /minute. The total running time of GC was 50 min. The relative percentage of each component was calculated by comparing its average peak area to the total areas. The software used to handle mass spectra and chromatograms was Turbo mass.[10]

Identification of Phytocomponents

Identification of chemical constituents was determined based on GC retention time on Rtx-5MS Column and matching of the spectra with computer software data of standards compared with NIST and WILEY Library. The GC-MS analysis was determined at Yaazh genomics, Coimbatore. The name, molecular formula, molecular weight, area under the peak of the components of the test materials, retention time (minutes), nature of the compound, and chemical structure of the constituents were ascertained.[11]

RESULTS

The phytochemical investigation of the ethanolic stem extract of *Tinospora crispa* revealed the presence of carbohydrates, proteins, lipids, glycosides, alkaloids, tannins, steroids, flavonoids, phenols, saponins, coumarins, anthocyanins, leuco anthocyanin, phlobatannin, quinone, resins except for anthraquinones, and terpenoids. Most of the primary and secondary phytochemicals were present in the ethanolic extract and hence ethanolic extract was taken for further studies (Table 1).

The GC-MS analysis of ethanolic stem extract of *Tinospora crispa* revealed the presence of 20 phyto-

Table 1: Details of Phytoconstituents in the stem extract of *Tinospora crispa*.

Phytoconstituents	Stem		
	Hexane	Ethanol	Water
Alkaloids	-	++	+
Flavonoids	-	+	+
Glycosides	+	+++	++
Phenols	-	+	-
Steroids	-	+	-
Terpenoids	+	-	-
Tannins	-	++	+
Saponins	-	+	+
Coumarin	-	+++	+++
Anthocyanin	-	+	-
Leuco anthocyanin	-	+	-
Phlobatannin	-	+	+
Quinones	-	+	+
Anthroquinone	-	-	+
Resin	+	+	+
Carbohydrates	+	+++	++
Proteins	-	+++	+
Lipids	+	+	-

constituents (Figure 1) that could contribute to the medicinal properties. The phytochemical compounds were identified and confirmed based on their peak area, molecular formula and retention time. The active principles with their peak area, molecular weight (MW), retention time (RT), and area percentage are presented in (Table 2).

The GC-MS study of the *Tinospora crispa* stem extract shows about 20 peaks as shown in Figure 1. At the retention time of 10.088, only one major peak was identified, which indicates the presence of Diethyl phthalate with a molecular weight of 222.24 possesses anti-microbial, acetylcholinesterase, and neurotoxic activities. Similarly, between the retention time period of 5.446 to 22.912 fourteen minor peaks were observed. Five moderate peaks were obtained 1,2,3-Propanetriol, diacetate (CAS) Diacetin (5.604), 9-Octadecenoic acid (Z)- (CAS) Oleic acid(13.977), 9,12-Octadecadienoic acid (Z,Z)-(15.839), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (CAS) Linolenic acid(15.913), 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-Ethylhexyl) phthalate(22.912) which possess anti-inflammatory, antibacterial, antioxidant, hypocholesterolemic, hepato-protective, antioxidant, neurodegenerative disorder and anticancer activities. The biological activity and chemical nature of the identified compounds of extract *Tinospora crispa* were given in Table 3. The remaining peaks that were identified from the spectra include, 3-Tetradecene, (Z)- (CAS) (7.218) possess antimicrobial, cytotoxicity, antipyretic, antidiabetic, anti-inflammatory activity, 3-Hexadecene, (Z)-(9.737) possess anticancer, antimicrobial, antioxidant, and antihemolytic activities, 1,2-benzene dicarboxylic acid, diethyl ester (11.087) possess Anti-microbial, acetylcholinesterase, and neurotoxic activities, 9-Eicosene (12.085) possess Anti-microbial and cytotoxic properties.

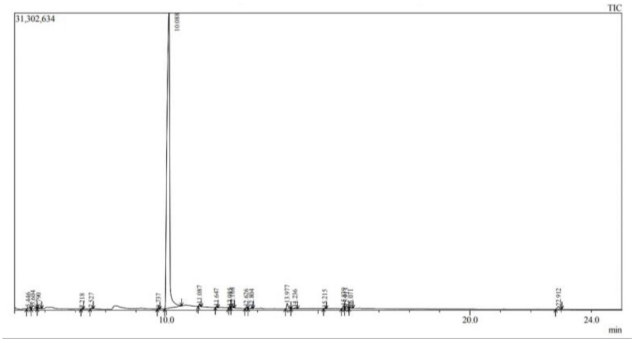

Figure 1: The GC-MS analysis of ethanol extract of *Tinospora crispa*.

Table 2: Phyto-compounds present in Ethanolic extract of *Tinospora crispa* using GC-MS Profiling.

Sl. No	Rt time	Area %	Mol. Weight	Mol. Formula	Name
1.	5.446	0.45	126.20	$C_8H_{14}O$	4-Hepten-3-one, 4-methyl- (CAS) 4-METHYL-4-HEPTEN-3-ONE
2.	5.604	1.03	176.17	$C_7H_{12}O_5$	1,2,3-Propanetriol, diacetate (CAS) Diacetin
3.	5.790	0.23	172.34	$C_9H_{20}OSi$	Allyl(2-butoxy)dimethylsilane
4.	7.218	0.05	198.39	$C_{14}H_{30}$	3-Tetradecene, (Z)- (CAS)
5.	7.527	0.13	86.13	$C_5H_{10}O$	4-Penten-1-ol, trimethylsilyl ether
6.	9.737	0.08	224.4253	$C_{16}H_{32}$	3-Hexadecene, (Z)- (CAS)
7.	10.088	93.43	222.24	$C_{12}H_{14}O_4$	Diethyl Phthalate
8.	11.087	0.29	222.24	$C_{12}H_{14}O_4$	1,2-BENZENE DICARBOXYLIC ACID, DIETHYL ESTER
9.	11.647	0.12	282.36	$C_{12}H_{18}N4_{02}S$	ETHYL 4-[(E)-1-AZEPANYLDIAZENYL]BENZOATE
10.	12.085	0.12	280.5	$C_{20}H_{40}$	9-Eicosene, (E)- (CAS)
11.	12.168	0.28	212.24	$C_{14}H_{12}O_2$	Benzyl Benzoate
12.	12.626	0.16	252.5	$C_{18}H_{36}$	1-Octadecyne
13.	12.804	0.16	282.36	$C_{12}H_{18}N_{40}2S$	ETHYL 4-[(E)-1-AZEPANYLDIAZENYL]BENZOATE
14.	13.977	1.03	282.5	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)- (CAS) Oleic acid
15.	14.236	0.30	238.5	$C_{17}H_{34}$	1-Heptadecene
16.	15.215	0.09	80.988	H_2O_3P+	Phosphonic acid, dioctadecyl ester (CAS) Di(n-octadecyl) phosphite
17.	15.839	0.53	280.4	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-
18.	15.913	0.53	278.4	$C_{18}H_{30}O_2$	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(CAS) Linolenic acid
19.	16.071	0.22	98.10	$C_5H_6O_2$	3-FURYLMETHANOL
20.	22.912	0.76	390.6	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate

Table 3: Biological Activity of Phyto-compounds present in Ethanolic extract of *Tinospora crispa*.

Sl. No	Chemical name	Compound Nature	Biologival Activity
1	4-Hepten-3-one, 4-methyl- (CAS) 4-METHYL-4-HEPTEN-3-ONE	Fatty alcohols	No activity
2	1,2,3-Propanetriol, diacetate (CAS) Diacetin	Glycerol	Fragrance agent, cellular narcotic
3	Allyl(2-butoxy)dimethylsilane	Alkane	No activity reported
4	3-Tetradecene, (Z)- (CAS)	Unsaturated aliphatic hydrocarbons	Antimicrobial, cytotoxicity, Antipyretic,, Anthelmintic, Tumour, Bronchitis, Asthma, Tuberculosis, Dyspepsia, Constipation, Anemia, Throat diseases, Elephantiasis, Antidiabatic, Anti-inflammatory, Antidiarrhoeal lactafuge, larvicide, Laxative, emollient, Expectorant, Enterostimulant, Ectoparasiticide
5	4-Penten-1-ol, trimethylsilyl ether	Ester	No activity reported
6	3-Hexadecene, (Z)- (CAS)	Saturated fatty acid	anticancer, antimicrobial, antioxidant, and antihemolytic activity
7	Diethyl Phthalate	Ester	Anti-microbial, acetylcholinesterase, and neurotoxic activity
8	1,2-Benzenedicarboxylic Acid, Diethyl Ester	Ester	Anti-microbial, acetylcholinesterase, and neurotoxic activity
9	Ethyl 4-[(E)-1-Azepanyldiazenyl]Benzoate	Aromatic carboxylic acid	Antimicrobial preservative
10	9-Eicosene, (E)- (CAS)	Alkane	Anti-microbial and cytotoxic properties. Antibacterial, antitumor, antifungal, cytotoxic
11	Benzyl Benzoate	Carboxylic acid	Anti-microbial activity
12	1-Octadecyne	Alkane	No activity reported
13	Ethyl 4-[(E)-1-Azepanyldiazenyl]Benzoate	Aromatic carboxylic acid	Antimicrobial preservative
14	9-Octadecenoic acid (Z)- (CAS) Oleic acid	Fatty acid	Antioxidant, anti cancer, Anti-inflammatory, Anti-androgenic Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
15	1-Heptadecene	Alkene	Flavoring, fragrance component and perfuming agent
16	Phosphonic acid, dioctadecyl ester (CAS) Di(n-octadecyl) phosphite	Organic Acid	No Activity
17	9,12-Octadecadienoic acid (Z,Z)-	Fatty Acid	Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic, Nematicide, Antihistaminic
18	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-(CAS) Linolenic acid	Fatty Acid	Antibacterial, Antioxident, Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, Antioxidant and hypocholesterolemic
19	3-Furylmethanol	Alcohol	No activity reported
20	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate	Ester	Neurodegenerative disorder and Anticancer

DISCUSSION

Phytochemical analysis of *Tinospora cordifolia* plant revealed the presence of Phyto constituents that shows medicinal properties as well as their physiological activities.[12] Analysis of this plant extract shows the presence of phytochemical components such as saponins, steroids, terpenoids, glycosides, phenols, tannins, flavonoids, and alkaloids.[13]

The qualitative analysis of phytochemicals present in the extracts of ethanol, n-hexane, aqueous and diethyl ether, of *Feretia apodanthera* showed the presence of carbohydrates, triterpines, unsaturated sterols, and reducing sugars. Flavonoids and tannins were present in the ethanol, diethyl ether, and aqueous extracts; saponin and reducing sugars were present in the aqueous and ethanol extracts only.[14]

The phytochemical analysis of the seed kernel extract of *Mangifera indica* indicated the presence of phytochemical constituents such as alkaloids, gums, flavanoids, phenols, saponins, steroids, tannins and xanthoproteins.[15]

The phytochemical evaluation of the Leaves of *Aegle marmelos* which is used as a traditional medicine revealed the presence of phytochemicals with the highest quantity of alkaloids, flavonoids, and phenols in Pant Aparna variety.[16]

The identified compounds with major peaks possess some the important biological potentials for future drug development. The phytochemical compounds and their biological activities were correlated. Similar to this study, eighteen phytochemical constituents have been identified from the ethanolic leaf extract of *Desmodium gyran* by GC-MS.[17]

Similarly, the GC-MS analysis of the *Nigella sativa* plant extract using ethanolic extract was identified with the presence of 5 compounds which includes 9-Octadecenoic acid, 9,12-Octadecadienoic acid, ethyl ester methyl ester, (E); linoleic acid; Diisooctyl phthalate; 9,12- Octadecadienoic acid (Z,Z)-, and 2,3-dihydroxypropyl ester. Some of these possesses antioxidant, antimicrobial, antiinflammatory and anticancer properties.[18]

GC-MS analysis of methanolic leaf extract of Hibiscus aspera shows the presence of 9,12- Octadecadienoic acid, methyl ester which possess antiandrogenic, hypocholesterolemic, nematicide, anti-inflammatory, anti-arthritic, anticoronary, insectifuge, antieczemic, antiacne, hepatoprotective, 5-alpha-reductase inhibitor, antihistaminic properties.[19]

Similarly, the ethanolic extract of *Tylophora pauciflora* shows the presence of n-Hexadecanoic acid which has 5- alpha-reductase inhibitors, anti-fibrinolytic, hemolytic, antioxidant, hypocholesterolemic nematicide, pesticide, antimicrobial activity, antiandrogenic flavour, and hemolytic properties. The compound 9, 12, 15-Octadecatrienoic acid, methyl ester (Z, Z, Z) has cancer-preventive, antioxidant, cancer preventive, hepatoprotective, antioxidant, anti-inflammatory, and hypocholesterolemic properties.[20]

CONCLUSION

About half a million plants around the world are medicinal plants, they have a promising future because of their medicinal properties.Their medicinal activities could be a source for drug development and in the treatment of present or future studies. *Tinospora crispa* is used in traditional systems of medicine to treat various conditions like rheumatism, fever, and headache. GC-MS analysis of the stem of *Tinospora crispa* ethanolic stem extract showed the presence of 20 compounds which includes phenolic compounds, alkaloids, amino acids, saponins, carbohydrates, proteins, tannins, and flavonoids. These compounds exhibit antipyretic, antitumor, analgesic, antiparasitic, antiseptic, antidiabetic, and antifungal properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

GC-MS: Gas Chromatography-Mass Spectrometry.

SUMMARY

The present study summarises that the *Tinospora crispa* stem extract shows the presence of secondary metabolites such as Alkaloids, glycosides, flavonoids, tannins, terpenoids, saponins, etc. GC-MS analysis shows the presence of about 20 phytocompounds which possess a lot of medicinal values and hence further studies on this plant exhibit that it can be used as a source for drug development in the future.

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