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Research Article

Phytochemical Screening, FT-IR and Gas Chromatography Mass Spectrometry Analysis of *Tinospora cordifolia* (Thunb.) Miers

Papitha R[1], Lokesh R[2], Kaviyarasi R[2], Selvaraj C I[1*]

[1]*Department of Biotechnology, School of Biosciences and Technology, SBST, VIT University, Vellore-632014, India*
[2]*Department of Biomedical Sciences, School of Biosciences and Technology, VIT University, Vellore-632014, India.*

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ABSTRACT
The purpose of the current study is to monitor the phytochemical constituents in *Tinospora cordifolia* stem extract. Phytochemical screening of the sequential extracts were used for analysis which showed the presence of bioactive compounds like alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and carbohydrates. Ultra violet visible spectroscopy was used to recognize quantitative determination of different analytes by using wavelength and absorbance values. FT-IR analysis was used to identify the functional groups of the compounds. Four functional groups were identified such as aromatics (C-H), secondary amines and amides (N-H), α, β- unsaturated aldehydes and ketones (C=O) and alkyl halides (C-H). GC-MS analysis for methanolic stem extract was done. The compound 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol reported to possess analgesic, anti-inflammatory and antipyretic activities, 9- Eicosene, (e) has anticancer activity, Hexamethyl-Cyclotrisiloxane, has antibacterial activity.

Keywords: *Tinospora cordifolia,* phytochemical screening, UV-Vis spectroscopy, FT-IR, GC-MS analysis.

INTRODUCTION

Medicinal plants are being used as natural medicines. Newly, there has been growing interest in the use of medicinal plants. The use of medicinal plants in current medicine suffers from the fact that though hundreds of plants are used in the biosphere to prevent or to cure diseases, but scientific information in terms of modern medicine is lacking in most cases. However, nowadays it is necessary to deliver scientific proof as whether to justify the use of plant or its dynamic principles[1]. Throughout the biosphere many traditional plant usages for diabetes treatment do exist. Indian traditional medicine is one of the richest medicinal organizations between those obtainable around the world. *Tinospora cordifolia is* also called Giloe, which belongs to the family of *Menispermaceae*. It is a well-known traditional medicinal plant in India. It needs fair humidity level and can be grown in extensive varieties of soil, ranging from acid to alkaline. In Ayurveda system of medicine *Tinospora cordifolia is also known as* "Guduchi" "Amrutha" (Sanskrit) and Gurcha in Hindi. It is used in adaptogenic and immuno-modulatory activity in fighting infections[2]. It is a large, deciduous widely spreading hiking shrub with elongated twining branches. Leaves simple, alternate, ex-stipulate, long petioles up to 15 cm long, roundish, pulvinate, both at the base and apex with the basal one longer and twisted partially and half way around. *Tinospora cordifolia* possess juicy inner stem which is white grey or creamy in colour with a thin bark. The blossoming season extends over summers and winters[3]. The florae are yellow in colour with long stalked racemes. Usually flowers can be seen during June and fruits during November. Mostly *Guduchi* can be found in dry forests throughout India. The oil extracted from plant is used to effectively reduce pain, edema (in gout). Rock candy is mixed with fresh juice of guduchi, which speed up the reclamation in hepatitis patients. The good mixture of guduchi and sunthi (*Zingiber officinale)* decoction can be used to treat rheumatic disorders and gout. Cow's milk when taken with guduchi juice is more effective in combating leucorrhea. Guduchi roots can be strong emetic, which is used to treat bowel obstruction. Henceforth, this plant can be selected as a basis for the drug development for numerous diseases[4].

MATERIALS AND METHODS

Collection of plant materials

The plant materials were collected from the (FRLHT) Foundation of Revitalization of Local Health Traditions, Bangalore. Then the plants were taxonomically authenticated by the botanist NM Ganesh Babu PhD, FRLHT, Bangalore.

Extraction and phytochemical screening

Plant samples (stem with bark) were dried and powdered using a mixer blender to make fine powder. Then two grams of the powdered sample was added to 250 mL of solvent was eluted sequentially based on the polarity index of the solvents. Then the extracts were subjected for rotary evaporator and kept at fridge for future uses.

Preliminary qualitative analysis of phytochemical screening was performed with shade dried and powdered stem of the plant. The presence and absence of derivative

**Author for Correspondence: immmer@gmail.com*

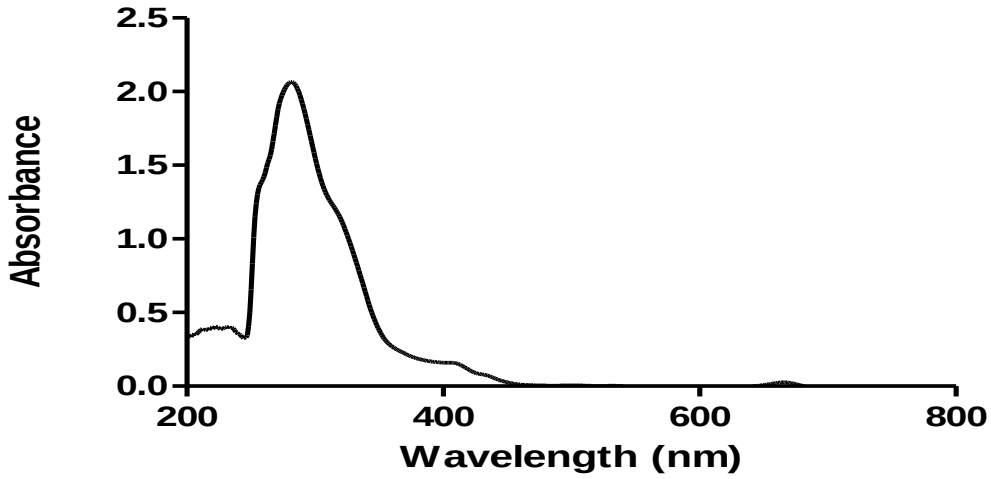

Figure 1: Ultra violet -Visible spectroscopy of *T.cordifolia* stem.

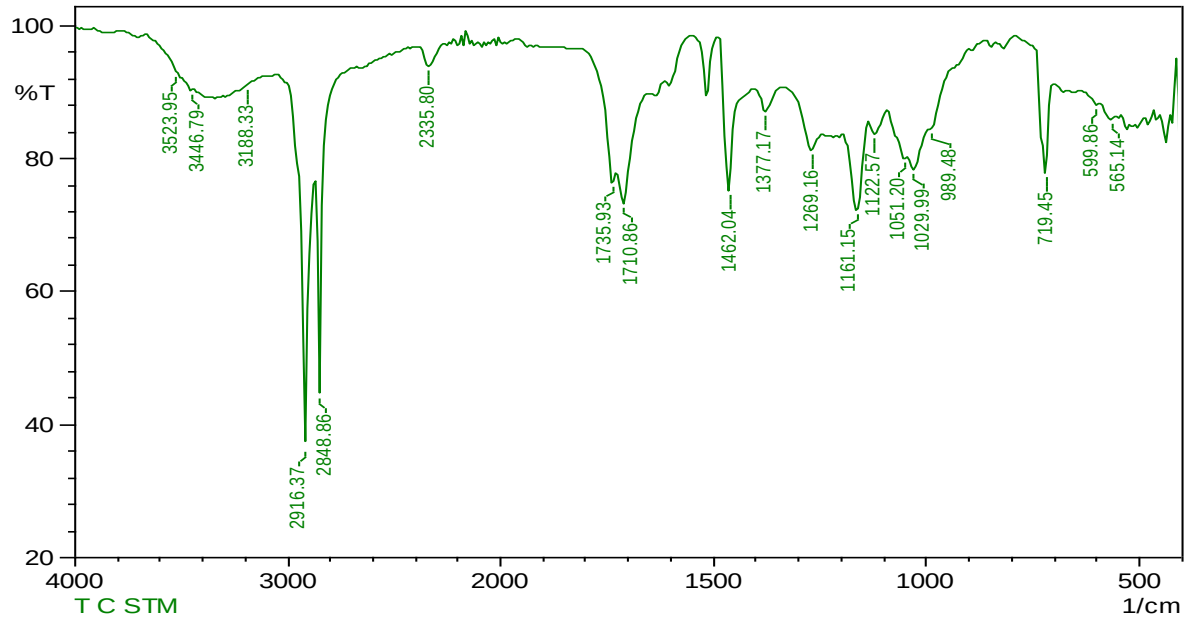

Figure 2: FT-IR functional group peaks analyse – *Tinospora cordifolia*.

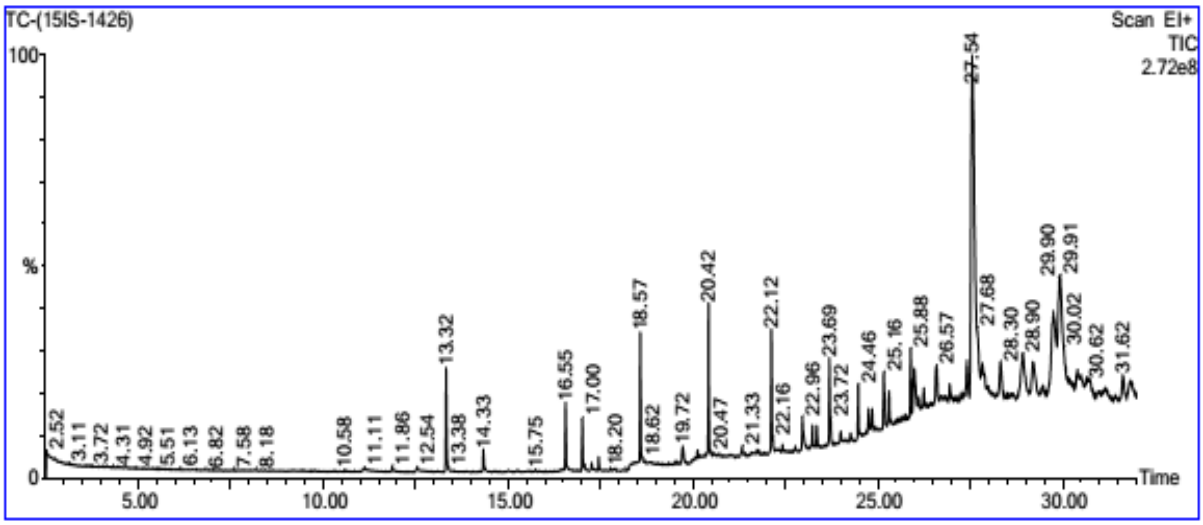

Figure 3: Peak area percentage of (GC-MS) Gas column mass spectrometry in *Tinospora cordifolia*.

compounds like alkaloids, flavonoids, phenolic, tannins, saponins, and terpenoids were confirmed by phytochemical screening using standard protocols[5].

UV-Visible spectroscopy

UV-Visible spectroscopy analysis for the samples was performed by diluting one gram of the extracted powder with 10ml of the identical solvent[6,7]. The extracts were scanned in the wavelength extending from 200-800 nm using (Shimadzu UVd-1800 PC, Japan) and the individual peaks were noticed.

FT-IR

FTIR analysis was achieved using Perkin Elmer Spectrophotometer system, which was used to notice the typical peaks and their functional groups. FT-IR (Fourier Transform Infrared spectrophometry is conceivably the most controlling tools for recognizing the kinds of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is distinguishing of the chemical bond can be seen in the annotated spectrum. The infrared absorption of spectrum can be inferring using chemical bonds in a molecule can be resolute. The plant constituents of dried powder sample of methanol extract was used for FTIR investigation[8]. One hundred Milligrams of the dried powder extract was condensed in KBr pellet, in order to prepare translucent sample discs. In FTIR spectroscopy powdered sample of plant specimen as loaded with a scan range from 400 to 4000 cm^{-1} with a resolution of $4cm^{-1}$.

GC-MS

The analysis of unidentified constituents of GC-MS plays major role in plant origin. The crude methanol (3 µl) extract containing different compounds of *Tinospora cordifolia* was subjected for (GC-MS) analysis. Instruments and chromatographic circumstances GC-MS examination was carried out on a GC clarus 500 Perkin Elmer system containing a AOC-20i auto analyst and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument retaining the subsequent conditions; column Elite-1 attached silica capillary column (30 ×0.25 mm × ID x 1µm of capillary column, composed of (100% Dimethyl poly siloxane), operational in electron impact mode at 70 eV; helium (99.99%) was used as transporter gas at a persistent flow of 1ml/minute and an injection capacity of 0.5 EI was employed (split ratio of 10:1) inject or temperature 250°C; ion-source temperature280°C. The oven temperature was programmed from 110°C (isothermal for 2min), with an increase of 10°C/minutes, to200°C/minutes, then 5°C/minutes to 280°C/min, finish with a 9 minutes isothermal at 280°C. Mass spectra were occupied at 70 eV; a scan intermission of 0.5 seconds and fragments from 45 to 450Da. The eluted constituent is identified in the mass detector. The spectrum of the unidentified constituent is matched with the spectrum of the recognized constituents stored in NIST library and concludes the name and molecular weight.

RESULTS AND DISCUSSION

Phytochemical screening of sequential extracted crude extracts were analysed for the presence of carbohydrates, alkaloids, flavonoids, phenol, tannins, saponins, and terpenoids (Table 1). Carbohydrates, alkaloids and phenol were present in all the four solvents of crude extracts. Tannin was absent in chloroform and methanol extract. Flavonoid was absent in methanol extract.

Two types of metabolites were present in plant cells. They are primary and secondary metabolites. Growth and metabolism of plants were directly linked with primary metabolites (carbohydrates, lipids, vitamins, proteins, crude fibre and fats)[9]. Secondary metabolites were considered as products of primary metabolites and are usually not involved in metabolic activity (Phenol, alkaloids, terpenoids, sterols, flavonoid, lignins and tannins etc,). The major uses of secondary metabolites are food seasoning, perfumes, pharmaceuticals, and pesticides[10]. Reducing sugars and alkaloids, terpenoids and flavonoids in *Morus alba* has anti-diuretic, anti-cancer, anti-viral, antianalgesic, antimalarial and antibacterial activities, due to the occurrence of secondary metabolites[11]. The well-known alkaloids have antimicrobial[12] and antidiabetic[13] activities. Steroidal alkaloids are used as medicine.

UV- visible spectroscopy interpretations of *T. cordifolia* has one major peak. The UV spectrum peaks show the peak value of 290nm with the absorption value of 2.1 mentioned in the fig.1

FTIR

FTIR was used to analyze the functional groups of compounds. It showed four major peaks, first major peak showing (C-H) aromatic ring with a peak value of 2916, followed by primary, secondary amines and amides (N-H) with a peak value of 2484. α, β- unsaturated aldehydes and ketones (C = O) with a peak value at 1710. The final peak value indicated the presence alkyl halides (C-H) with a peak value of 1122 (Fig 2). The major peaks and functional of dynamic compounds groups were

Table 1: Phytochemical screening of stem extracts in *Tinospora cordifolia*

S. No	Phyto-compounds	Petroleum Ether	Chloroform	Ethyl acetate	Methanol
1	Carbohydrate	++	++	++	++
2	Alkaloids	++	++	++	++
3	Flavonoids	++	++	++	--
4	Phenol	++	++	++	++
5	Tannins	++	--	++	--
6	Saponins	++	++	--	++
7	Terpenoids	--	++	++	++

(++) = Positive, (--) = Negative.

Table 2: Chemical composition of *Tinospora cordifolia* stem

Peak	RT	Peak name	Chemical formula
1.	13.33	Phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$
2	16.55	E-15-Hepta-decenal	$C_{17}H_{32}O$
3	17.01	3,7,11,15-Tetra methyl-2-Hexa-decen-1-ol	$C_{20}H_{40}O$
4	20.42	Hepta-Decyl-Trifluoro-Acetate	$C_{19}H_{35}O$
5	22.12	Trifluoro-acetoxy hexadecane	$C_{18}H_{33}O$
6	23.69	9-Eicosene, (e)-	$C_{20}H_{40}$
7	24.47	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$
8	25.16	Cyclohexane,1,1'-(2-tridecyl-1,3-Propanediyl)bis-	$C_{28}H_{54}$
9	25.98	1,4-didecyl- Cyclohexane	$C_{26}H_{52}$
10	27.40	Di-n-Decylsulfone	$C_{20}H_{42}O$
11	28.91	Hexamethyl-Cyclotrisiloxane,	$C_6H_{18}O_2$

analysed and results were compared with standard Infrared chart[14].

GC-MS analysis

The analysis of methanolic stem extract of *Tinospora cordifolia* indicated the presence of eleven compounds. Phenol, 2, 4-bis (1, 1-dimethyl-ethyl) is known for UV stabilizing activity and an antioxidant for hydrocarbon-based products, antimalarial, antibacterial and antioxidant activities[15]. The compound 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol is reported as an analgesic, anti-inflammatory and has antipyretic activities[16]. 9- Eicosene, (e)- was found to possess anticancer activity[17], Hexamethyl-Cyclotrisiloxane has antibacterial activity[18]. The GC-MS analysis of the sample was listed (Table 2).

CONCLUSION

This preliminary analysis of crude extract from *Tinospora cordifolia* proves that the plant contains various bioactive phytochemical constituents that contribute towards the biological applications of this plant that are identified in previous reports. Separation of individual phytochemical constituents and studying its individual biological activity will certainly give successful results. In conclusion, *T. cordifolia* is suggested as a plant of phyto pharmaceutical significance.

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