Research Article

Phyto-Chemical Studies of Methanol Extract of *Tinospora cordifolia* Leaf by GC-MS

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ABSTRACT

Plants are the almost exclusive source of drugs for the majority of the world population. The present study was carried out to investigate the phytoconstiuent of the *Tinospora cordifolia* leaf which contain alkaloids, flavanoids, steroidal glycoside, terpenoids and sterol are confirmed by preliminary phyochemical studies. GC-MS analysis of methanol extract of the plant *Tinospora cordifolia* leaf showed the presence of 15 bio active compounds. They are 4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl,1,2,3-propanetriol,2-methoxy-4-vinylphenol, Benzene, 1-(2-chloroethyl)-2-(trifluoromethyl),3-Hydroxy-4-methoxybenzoic acid, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester,9,12-Octadecadienoic acid (Z,Z)-,methyl ester, Oleic Acid, Linoleic acid ethyl ester,9-octadecenoic acid (z)-, ethyl ester, octadecanoic acid, ethyl ester,2,4-imidazolidinedione, 5,5- diphenyl-, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester.

Keywords: *Tinospora cordifolia,* phyto-constituent, GC-MS analysis, bioactive compound.

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INTRODUCTION

The *Tinospora cordifolia* has been subjected to chemical investigation extensively and number of chemical constituents belonging to the different groups viz terpenoid, alkaloids, lignans, steroids have been analysed.

Herbal plants produce and contain a variety of chemical substances with varied physiological effects. They are huge reservoir of various chemical substances with potential therapeutic properties[1]. Herbal plants are being increasingly utilized to treat a wide variety of clinical diseases [2]. Herbs have been used by all cultures throughout history and thus, herbal medicine is the oldest form of health care known to mankind. It was an integral part of the development of modern civilization. Many drugs commonly used today are of herbal origin. Higher plants as source of medicinal compound continue to play a dominant role in maintenance of human health since antiquities [3].

MATERIALS AND METHODS

Collection of samples

The leaf *Tinospora cardifolia* was collected in Dindigul district of Tamilnadu during the first week end of December. This plant was authenticated by the Department of the Botany, American College, Madurai.

Extracted plant material powder by maceration method.

The shade dried material (250gms) was extracted with petroleum ether (60°C-80°C) in soxhlet apparatus. After the completion of the extraction, the solvent was removed. The marc left petroleum ether extraction was dried and extracted with chloroform in soxhlet apparatus. The solvent was removed after the completion of extraction. The marc left after the chloroform extraction was dried and then extracted with ethanol in soxhlet apparatus. The solvent was removed after the extraction was completed, the solvent was taken out and concentrated.

Preparation of sample for phytochemical screening

The plant parts (leaf) were cleaned, dried and powdered with the help of mixer grinder separately. Methanol extracts were prepared and concentrated using rotary evaporator and stored at 4ºC in air tight containers.

Preparation of extract for Gas Chromatography Mass Spectroscopy (GC-MS) Analysis

15 grams of dried leaf powder of *Tinospora cordifolia* was taken and soaked in 150 methanol and it was kept in room temperature for 72 hours with constant shaking. After incubation, solutions were filtered with whattman filter paper no. 1 and filtrate were kept at room temperature for drying. After drying, the weight of extract was measured and according to weight solvent was added and maintained the concentration of extract as 25 mg/ml.

Methodology for phytochemical screening[4-7]

Chemical tests were carried out on the extract and on the powdered specimens using standard procedures based on the protocols of Edeoga et al,2005; Harborne,1973and Sofowara,1993;to identify the various constituents present.

Test for Alkaloids

Test solution (1 ml) was taken in test tube and few drops of Mayer's reagent (Potassium mercuric iodine solution) were added into it and then cream color precipitate was observed. To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added by the side of the test tube. A prominent red precipitate indicates test as positive.

Test for Tannins

To test solution added 10 ml distilled water, then filtered, in the filtrate 2 ml FeCl3 (10%) was added blue-black or green precipitate formed, indicate the presence of tannins.

Test for Cardiac Glycosides

5 ml of each extracts was treated with 2 ml of glacial acetic acid containing onedrop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Flavonoid

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H2SO4. A yellow coloration observed in each extract indicated the presence of flavonoids. The yellow coloration disappeared on standing. Test for Terpenoids to the test solution, added 2 ml of chloroform and 1 ml H2SO4, reddish brown color at interface, indicate the presence of terpenoids.

Test for Saponins

2 gm of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken thoroughly, then observed for the formation of emulsion thereafter in plant sample (filterate/powder).

Test for Steroids

2 ml of acetic anhydride was added to 0.5 gm ethanolic extract of each sample with 2 ml H2SO4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

RESULT AND DISCUSSION

Phytochemical Screening

Qualitative estimation

In the present study, preliminary phytochemical screening of the extract of leaves of experimental plant *T.cordifolia*, revealed the presence of various bioactive components such as alkaloids, saponins, tannins, cardiac glycosides, steroids, flavonoids, terpenoids etc were identified.

Table 1: Phytochemical screening of leaf parts of *Tinospora cordifolia*

S. No	Tannins	Saponins	Steroids	Terpenoids	Flavonoids	Alkaloids	Cardiac glycosides
+ or -	+	+	+	+	+	+	+

Gas Chromatography –Mass Spectroscopy (GC-MS) Analysis[8]

For GC-MS analysis, methanolic extract of the plant sample (leaf) was sent to Bishop heber college, Herbal analytical instrument facility, Trichy. The results of GC-MS analysis of *Tinospora cordifolia* extracts were very interesting. GC-MS of the *T.Cordifolia* extract revealed the presence of 15 components having different pharmacological importance.

GC Programme [GCMS-QP2020]

Ion Source Temp. : 200.00C

Interface Temp. : 250.00C

Solvent Cut Time : 3.50min

Detector GainMode : Relative to the Tuning Result

Detector Gain : +0.00kV

Threshold : 1000

Oven temperature Programme

S.no	Rate	Temperature (°C)	Hold Time (min)
1	-	50.0	0.00
2	6.00	280.0	2.00

MS Programme

Start time : 4.00 min

End time : 40.33 min

ACQ mode : Scan

Event time : 0.3 sec

Scan speed : 1666

Start m/z : 50.00

End m/z : 500.00

Sample inlet unit : GC

Table 2: Components identified in *Tinospora Cardifolia* **[GC MS study]**

No.	RT	Name of the compound	Molecular Formulae	MW	Peak Area %
1.	10.394	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	144	0.62
2.	11.358	1,2,3-propanetriol	C3H8O3	92	1.1
3.	14.191	2-methoxy-4-vinylphenol	C9H10O2	150	2.05
4.	15.39	benzene, 1-(2-chloroethyl)-2-(trifluoromethyl)-	C9H8C1F3	208	0.58
5.	19.96	3-hydroxy-4-methoxybenzoic acid	C8H8O4	168	0.65
6.	20.153	3-hydroxy-4-methoxybenzoic acid	C8H8O4	168	1.56
7.	21.178	benzoic acid, 3,4-dimethoxy-	C9H10O4	182	0.56
8.	26.803	n-hexadecanoic acid	C16H32O2	256	3.97
9.	27.175	hexadecanoic acid, ethyl ester	C18H36O2	284	4.7
10.	28.73	9,12-octadecadienoic acid (z,z)-,methyl ester	C19H34O2	294	0.98
11.	29.563	9,12-octadecadienoic acid (z,z)-	C18H32O2	280	6.92
12.	29.634	oleic acid	C18H34O2	282	6.05
13.	29.797	linoleic acid ethyl ester	C20H36O2	308	13.06
14.	29.887	9-octadecenoic acid (z)-, ethyl ester	C20H38O2	310	10.04
15.	30.259	octadecanoic acid, ethyl ester	C20H0O2	312	1.05
16.	33.281	2,4-imidazolidinedione, 5,5- diphenyl-	C15H12N2O2	252	1.08
17.	34.677	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H38O4	330	0.91
18.	37.051	e,z-1,3,12-nonadecatriene	C19H34	262	10.8
19.	37.587	-1-benzopyran-2-one, 7-hydroxy-3- (4-methoxyphenyl)-	C16H12O4	268	11.49
20.	37.805	2-(4-dicyanomethyl-2,3,5,6-tetrafluorophenyl) malononitrile	C12H2F4N4	278	0.66
21.	37.963	h-furo[3,2-g][1]benzopyran-7-one, 2,3,5-trimethyl-	C14H12O3	228	2.59
22.	38.089	n,o-dimethylstephine	C20H25NO6	375	2.41
23.	38.223	(+)-isostephodeline	C21H27NO5	373	4.6
24.	38.387	1,3,8-trimethoxy-6-methylanthracen-9-ol	C18H18O4	298	0.74
25.	38.48	2-[(1 hydroxy)hexyl]indol-3-acetic acid	C16H21NO3	275	1.62
26.	38.61	phenol, 3-[2- (dimethylamino)ethyl]-6- methoxy-2-[2-(4- methoxyphenyl)ethenyl]-, (e)-	C20H25NO3	327	0.61
27.	38.76	3-hydroxy-4,7,8-trimethoxy-17-methyl-7,8- didehydrohasubanan-6-one #	C20H25NO5	359	0.72
28.	33.876	dibenz[a,c]cycloheptan-7-amine, 1,2,3-trimethoxy-	C18H21NO3	299	1.22
29.	39.101	(7as)-11-methoxy-6,7,7a,8- tetrahydro-5h-1,3]benzodioxolo[6,5,4-de]benzo[g]quinolin-12-ol	C18H17NO4	311	0.53
30.	39.766	benzofuran, 6-methoxy-2-(2,4,6-trimethoxyphenyl)-	C18H18O5	314	6.1

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HEBER ANALYTICAL INSTRUMENTATION FACILITY

Sample Information

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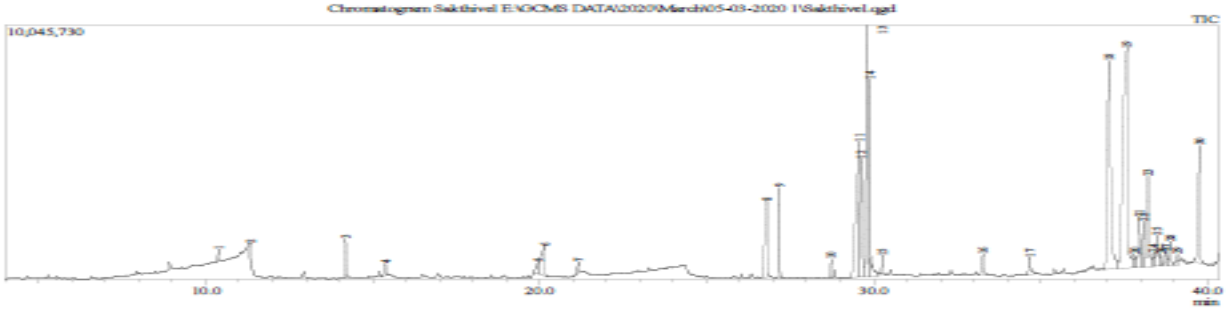
Analyzed	: 3/5/2020 8:23:12 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: Sakthivel
Sample ID	: Sakthivel
Vial #	: 5
Injection Volume	: 1.00
Method File	: E:\GCMS METHOD\New Method 40 Minutes.qgm
Report File	: E:\GCMS REPORT\BISHOP HEBER 1.qgr
Tuning File	: E:\TUNING\2019\29-01-2019.qgt

Chromatogram Sakthivel E:\GCMS DATA\2020\March\05-03-2020 1\Sakthivel.qgd



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Hit#:2 Entry:21081 Library:NIST14.lib

SI:93 Formula:C6H8O4 CAS:28564-83-2 MolWeight:144 RetIndex:1269

CompName:4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-

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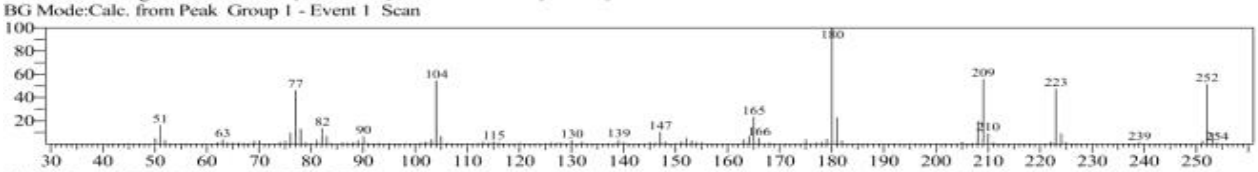

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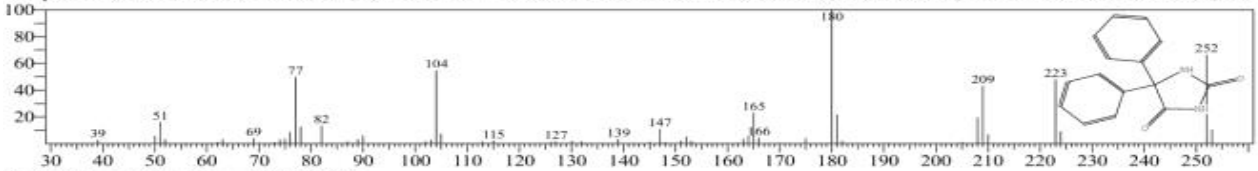
CompName:2,4-IMIDAZOLIDINEDIONE, 5,5-DIPHENYL- $$ 5,5-DIPHENYLIMIDAZOLIDINE-2,4-DIONE $$ 2,4-IMIDAZOLIDINEDIONE, 1,3-DI



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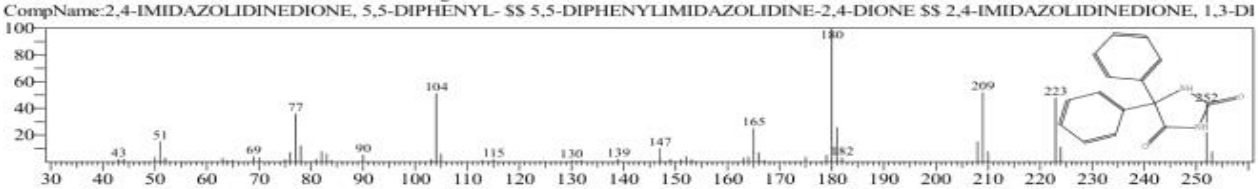

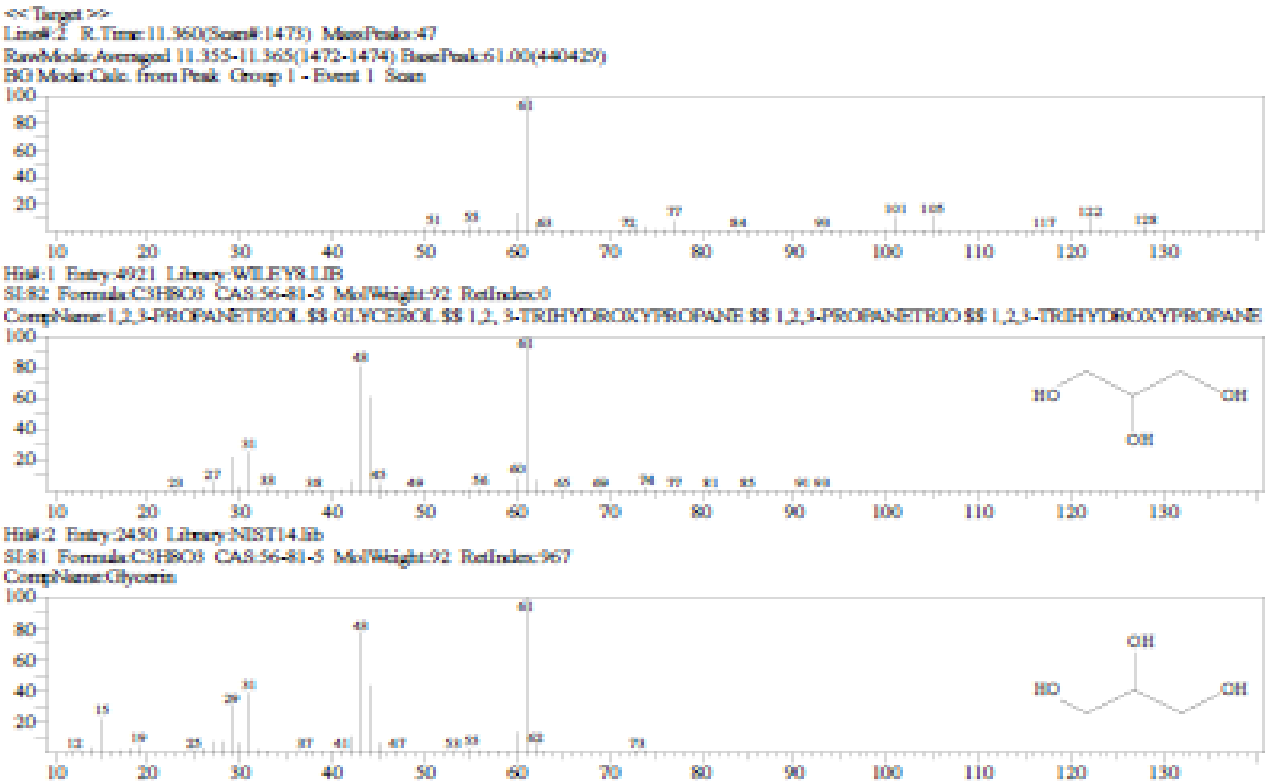

RESULTS AND DISCUSSION

Fifteen components in leaves were identified in *Tinospora cordifolia* by Gas Chromatogram-Mass spectrometry (GC-MS) analyzed. GC MS Studies of *Tinospora cordifolia* indicates that the prevailing components were the presence of various bioactive components justifies the use of the whole plant for various ailments by traditional practitioners. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented. The prevailing components were The GC-MS analysis revealed the presence of various components like 4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl,1,2,3-propanetriol,2-methoxy-4-vinylphenol,Benzene, 1-(2-chloroethyl)-2-(trifluoromethyl),3-Hydroxy-4-methoxybenzoic acid, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester,9,12-Octadecadienoic acid (Z,Z)-,methyl ester, Oleic Acid, Linoleic acid ethyl ester,9-octadecenoic acid (z)-, ethyl ester, octadecanoic acid, ethyl ester,2,4-imidazolidinedione,5,5-diphenyl-,hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester.(Table2) This study explores the goodness of the leaf of the plant *Tinospora cordifolia* which has a commendable sense of purpose and can be advised as a plant of phyto pharmaceutical importance. GC-MS figure for 4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6-methyl,2,4-imidazolidinedione, 5,5- diphenyl-, hexadecanoic acid, 1,2,3-propanetriol,2-methoxy-4-vinylphenol ,(Figure 1-3). This study explores the goodness of the leaf of the plant *Tinospora cordifolia* which has a commendable sense of purpose and can be advised as a plant of phyto pharmaceutical importance.

CONCLUSION

Knowledge of chemical constituents of plants is important and desirable because such information will be important for synthesis of chemical substances. It could be well qualified for application in pharmaceutical industry. The GC-MS analysis of methanolic extract of experimental plant showed the presence of pharmacologically active compounds such as antioxidant and antihyperlipidemic. This plant can be saved through biotechnological approaches and its quality can be improved through secondary metabolites production and thus it can be used as a source for developing new drugs and commercialization.

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