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PHYTO-CHEMICAL STUDIES OF METHANOL EXTRACTS OF *TINOSPORA CORDIFOLIA* STEM BY GC-MS

[1]Anita Sinha*, [2]H. P. Sharma, [3]Binod Singh and [4]Amit Patnaik

[1]Department of Microbiology, Ranchi College, Ranchi.
[2,4]University Department of Botany, Ranchi University, Ranchi.
[3]K.C.B. College, Bero, Ranchi.

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***Corresponding Author'**
Dr. Anita Sinha
Department of Microbiology, Ranchi College, Ranchi.

ABSTRACT

Plants are the almost exclusive source of drugs for majority of the world population. The phyto components present *in Tinospora cordifolia*, locally known as Gilloy, Guduchi or Amrita, is used in the treatment of various ailments in the long-established medicinal system in the state of Jharkhand, India and is also an anti-oxidant and immuno-modulator. Present study was carried out to investigate the phyto-constituenst of the *Tinospora cordifolia* which contain terpenoids, steroids, glycosides, flavonoids and phlobatannins etc. are confirmed by preliminary phyto-chemical studies. GC-MS analysis of methanol extract of the plant showed the presence of 64 bioactive compounds. Some of them are of great interest as they are either anti- cancer or anti-oxidant or both, such as pentanoic acid, propyl ester or valproic acid, phytol or 2-hexadecen-1-ol and 9, 12-octadecadienoic acid (Z, Z), benzenepropanoic acid, 2, 5-dimethoxy-, hexadecanoic acid, methyl Ester, octadecanoic acid, n-hexadecanoic acid or palmitic acid. Among all these reported anti-cancer compounds, n-hexadecanoic acid or palmitic acid was present in highest proportion (area 23.12%) in the GC-MS chromatogram.

KEYWORDS: *Tinospora cordifolia*, Phyto-constituent, GC-MS analysis, Anti-cancer, Bioactive compounds.

INTRODUCTION

Tinospora cordifolia belongs to family Menispermaceae is a large deciduous climbing shrub found throughout tropical Indian subcontinent. It consists of about 70 genera and 450 species.

The Genus *Tinospora* has about 32 species, out of which a few important are *Tinospora malabarica, Tinospora crispa, Tinospora uliginosa, Tinospora sinensis, Tinospora glabra*. This plant is known for its variety of uses in *Ayurveda*. Some of the active components present in the plant are alkaloids, steroids, diterpenoid lactones, aliphatics and glycosides (Upadhyaya *et al*, 2010). This plant is of great interest to researchers across the globe because of its wide ranging medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, antiinflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities (Parthipan *et al*,2011; Tambekar *et al*,2009 and saha *et al*,2012). Medicinal plants are an important source of compounds for the pharmaceutical industries and traditional medicine. Plants of economic interest in general and medicinal plants in particular are disappearing at an alarming rate due to various developmental activities. The scientists realize that the effective life span of any antibiotic is limited so new sources especially plant sources have to be investigated. Therefore, the plant *T. cordifolia* can be chosen as a source for the development of industrial products for treatment of various diseases (Preeti, 2011). In the present study *Tinospora cordifolia* is subjected to phytochemical screening and GC- MS analysis in order to screen and conferm the presence of various pharmaceuticals compounds.

MATERIALS AND METHODS

Collection of samples

Plant material *Tinospora cordifolia* was collected from the campus of Botany Department of Ranchi University and also from Arogya Bhawan, Ranchi. Jharkhand, India.

Preparation of sample for phytochemical screening

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

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

15 grams of dried stem 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. It was

sent to Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi, India.

Methodology for phytochemical screening.

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, 1973 and 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 $FeCl_3$ (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 one drop 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 H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Terpenoids

To the test solution, added 2 ml of chloroform and 1 ml H_2SO_4, 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 H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for Phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

RESULT AND DISCUSSION

Phytochemical Screening

Qualitative estimation

In the present study, preliminary phytochemical screening of the extract of leaves and stem of experimental plant *T. cordifolia*, revealed the presence of various bioactive components such as alkaloids, saponins, tannins, phlobatanins, cardiac glycosides, steroids, flavonoids, terpenoids etc were identified. Some of the bioconstituents such as, terpenoids steroids, alkaloids and phlobatanins were found in moderate amount in stem extract of *T. cordifolia* whereas bioactive saponins, tannins, flavonoids and cardiac glycosides were present in trace amount. Phlobatanins was absent in case of TCE leaf. (Table 1).

Table 1: Phytochemical screening of secondary metabolites of different parts of *Tinospora Cordifolia.*

Sl. No.	Plant part	Saponin	Tanins	Steroids	Terpenoids	Phlobatanin	Flavanoidds	Cardiac Glycosides	Alkaloids
1	TC (stem)	+	+	++	++	++	+	+	++
2	TC (leaf)	+	+	+	+	–	++	+	+

(++) : Moderate amount

(+) : Trace

(–) : Absent

Gas Chromatography – Mass Spectroscopy (GC-MS) Analysis

For GC-MS analysis, methanolic extract of the plant sample (stem) was sent to Advance Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi.

The results of GC-MS analysis of *Tinospora cordifolia* extracts were very interesting. GC-MS of the *T. cordifolia* extract revealed the presence of 64components having different pharmacological importance along with antioxidant and/or anticancer potential.

Table 2:Peak report of GC-MS analysis of methanolic stem extract of *Tinospora cordifolia.*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	3.858	936912	0.72	3,5-DIMETHYL-1,3,4-HEXANETRIOL
2	4.048	526853	0.41	PENTANOIC ACID, PROPYL ESTER
3	4.335	1331520	1.03	1,3-Dioxane, 4-methyl-
4	5.103	1794973	1.39	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
5	5.368	933875	0.72	OCTANOIC ACID
6	6.429	905542	0.70	BUTANOIC ACID, HEXYL ESTER
7	6.783	1039109	0.80	2-DECENAL, (E)-
8	7.625	1258416	0.97	5-Hexen-2-ol, 5-methyl-
9	8.051	326276	0.25	Ethanone, 1-(2,2-dimethylcyclopentyl)-
10	8.286	571014	0.44	2-Undecenal
11	8.612	2970042	2.30	2-Pentylcyclopentanone
12	8.915	522600	0.40	4-Hydroxy-2-methoxybenaldehyde
13	9.562	422813	0.33	1-CHLOROOCTADECANE
14	10.742	191402	0.15	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-
15	10.892	1518354	1.17	
16	11.142	252503	0.20	Tridecane, 3-methylene-
17	11.220	1753166	1.36	1-HEXADECENE
18	11.917	1685708	1.30	
19	12.205	1550016	1.20	8-Pentadecanone
20	12.913	573698	0.44	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
21	13.198	1738978	1.35	9-OCTADECENOIC ACID (Z)-
22	13.500	999385	0.77	1-Nonadecene
23	14.015	621565	0.48	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
24	14.089	1627255	1.26	2-Pentadecanone, 6,10,14-trimethyl-
25	14.256	685407	0.53	9-OCTADECENOIC ACID (Z)-
26	14.392	1483998	1.15	8-Octadecanone
27	14.897	1425965	1.10	Hexadecanoic acid, methyl ester
28	15.015	561497	0.43	
29	15.329	29881409	23.12	n-Hexadecanoic acid
30	15.548	855956	0.66	
31	16.140	570198	0.44	9-OCTADECENOIC ACID (Z)-
32	16.236	1611244	1.25	HEPTADECANOIC ACID
33	16.736	316860	0.25	Phytol
34	16.820	367539	0.28	Octadecanoic acid, methyl ester
35	17.006	2936605	2.27	
36	17.174	11513391	8.91	Octadecanoic acid
37	17.421	1583976	1.23	HEPTADECANOIC ACID, ETHYL ESTER
38	17.728	896305	0.69	9,12-Octadecadienoic acid (Z,Z)-
39	18.193	364867	0.28	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-
40	18.281	674896	0.52	
41	18.575	1832353	1.42	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
42	18.770	2525650	1.95	HAHNFETT
43	18.903	1655909	1.28	ICOSANOIC ACID

Peak#	R.Time	Area	Area%	Name
44	19.240	1565706	1.21	
45	19.717	312826	0.24	Octadecanoic acid
46	19.818	244628	0.19	Andrographolide
47	20.511	2347643	1.82	Octadecanoic acid
48	21.496	529034	0.41	1-Heptacosanol
49	22.127	981408	0.76	Tetracosanoic acid
50	23.406	1981684	1.53	1-Heptacosanol
51	24.383	461100	0.36	
52	24.642	460264	0.36	cis-1-Chloro-9-octadecene
53	24.908	1863889	1.44	Cholesta-4,6-dien-3-ol, (3.beta.)-
54	25.335	1401438	1.08	3.beta.-Acetoxystigmasta-4,6,22-triene
55	26.110	2254988	1.74	1-Heptacosanol
56	26.197	2589332	2.00	Cholesta-4,6-dien-3-ol, (3.beta.)-
57	29.008	2057391	1.59	ERGOST-5-EN-3-OL, (3.BETA.,24R)-
58	29.676	1577025	1.22	Stigmasterol
59	31.044	3226856	2.50	STIGMAST-5-EN-3-OL, (3.BETA.)-
60	31.996	1470650	1.14	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-
61	32.197	3157562	2.44	Cholest-4-en-3-one
62	32.711	1067415	0.83	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-
63	33.038	1994949	1.54	4,22-Stigmastadiene-3-one
64	34.834	9921429	7.68	Cholest-4-en-3-one
		129263217	100.00	

Table 3: Anticancer and antioxidant compounds obtained from GC-MS analysis of *Tinospora cordifolia.*

Sl. No	Name of compound	Common name	Chemical formula	Mol. wt.	Area %	Activity
1.	Pentanoic acid, Propyl ester	Valproic acid	$C_8H_{16}O_2$	144	0.41	Anticancer, Cincarova *et al*,
2.	n-Hexadecanoic acid	Palmitic acid	$C_{16}H_{32}O_2$	256	23.12	Antioxidant and anticancer, Harada *et al*, 2002.
3.	Heptadecanoic acid	Margaric acid	$C_{17}H_{34}O_2$	270	1.25	Anticancer, Basu *et al*,2013
4.	Stigmasterol		$C_{29}H_{48}O$	412	1.22	Anticancer and inhibit tumor promotion, Venkata *et al*, 2012.
5.	STIGMAST-5-EN-3-OL, (3.BETA.)-	Beta sitosterol	$C_{29}H_{50}O$	414	2.50	Anticancer and antioxidant (*Basu et al*, 2013.
6.	9,12-Octadecadienoic acid (Z,Z)-			270	0.69	Cancer preventive, Basu *et al*,2013).
7.	Phytol	2-Hexadecen-1-ol	$C_{20}H_{40}O_6$	296	0.25	Anticancer Venkata *et al*, 2012).
8.	1-Heptacosanol		$C_{27}H_{56}O$	396	1.74	Anticancer and antioxidant ,Venkata *et al*,2012.

9.	Octadecanoic acid	Stearic acid	$C_{18}H_{36}O_2$	284	8.91	Antioxidant and cancer preventive, Dr. Duke's Phytochemical and Ethnobotanical Database.
10.	Hexadecanoic acid, methyl ester	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270	1.10	Antioxidant, Basu *et al*, 2013.
11.	Icosanoic acid				1.28	Antitumor, Harada *et al*, 2002).
12.	Cholest-4-en-3-one	Cholesterol			7.68	Antioxidant, Duke's Phytochemical and Ethnobotanical Database.

Peak report of TCE along with retention time, area and per cent area have been shown in table 2. In the present investigation a variety of compounds has been detected. Some of the compounds present are anticancer in nature such as pentanoic acid, propyl ester or valproic acid, phytol or 2-hexadecen-1-ol, n-hexadecanoic acid or palmitic acid STIGMAST-5-EN-3-OL, (3.BETA.)- and 9,12-octadecadienoic acid (Z,Z)-. Octadecanoic acid is cacer preventive and antioxidant and 9,12-Octadecadienoic acid (Z,Z)- is cancer preventive compounds. Other antioxidant compounds found were benzenepropanoic acid, 2,5-dimethoxy-, hexadecanoic acid, methyl Ester, pntadecanoic acid, octadecanoic acid, n-hexadecanoic acid or palmitic acid(Table 3).Among all these anticancer compounds, n-hexadecanoic acid or palmitic acid was having highest per cent area (23.12%) in the extract of *Tinospora cordifolia* as shown in table 2.

SUMMARY AND 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 anticancerous. 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. Further investigations on preclinical and clinical trials of these extracts could become a part of standard drug designing and treatment protocols for cancer and hence a promising and powerful weapon for cancer treatment.

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