

# Phyto-chemical studies of methanol extracts of *Tinospora cordifolia* growing with different supporting trees by GC-MS

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**Abstract-** Plants are the basis of life on the planet earth as plants and their products directly or indirectly support the life processes. Specially, wild plants constitute an integral part of customs and cultures of different ethnic communities. 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, 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. In the present investigation *Tinospora cordifolia*, independent climber and climbers on different supporting trees has been identified for phytochemistry by screening and quantitative estimation. In the proposed work three categories of experimental plant has been identified: (i) *Tinospora cordifolia* independent climber, (TC); (ii) Climber on Aam or *Mangifera indica*, TC (A) and (iii) Climber on Neem or *Azadirachta indica*, TC (N). Present study was carried out to investigate the phytoconstituents 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 a variety of bioactive compounds. Some of them are of great interest as they are either anti-cancer or anti-oxidant or both

Key words: 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. 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.<sup>1</sup> This plant is of great interest to researchers across the globe because of its wideranging medicinal properties like anti-diabetic, antiperiodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, antimalarial, hepatoprotective, immunomodulatory and antineoplastic activities.<sup>3-4</sup> Therefore, the plant *T. cordifolia* can be chosen as a source for the development of industrial products for treatment of various diseases.<sup>5</sup> In the present study *Tinospora cordifolia* is subjected to phytochemical screening and GC-MS analysis in order to screen and confirm the presence of various pharmaceuticals compounds.

# **MATERIALS & METHODS**

**Collection of samples:** Plant material *Tinospora cordifolia* (independent climber) and climbers on Aam and Neem trees was collected from the campus of Arogya Bhawan, Ranchi. Jharkhand, India.

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# 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°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 Whatman 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)<sup>6</sup>, Harborne (1973)<sup>7</sup> and Sofowara (1993)<sup>8</sup> to identify the various constituents present.

# **Phytochemical Screening:**

In the present study, preliminary phytochemical screening of the extract of leaves and stem of experimental plant T. cordifolia, TCE (A) and TCE (N) revealed the presence of various bioactive components such as alkaloids, saponins, tannins, Phlobatannins, cardiac glycosides, steroids, flavonoids, terpenoids etc. were identified in appreciable, moderate or in trace amount. Terpenoids, steroids, alkaloids and Phlobatannins were found in moderate amount in T. cordifolia stem extract whereas other bioactive compounds such as saponins, tannins, flavonoids and cardiac glycosides were trace in amount. Phlobatannins was absent in case of TCE leaf, TCE (A) stem and leaf. In case of TCE (N), both stem and leaf contained flavonoids in appreciable amount, however it was in moderate amount in TCE leaf, TCE (A) stem and leaf. Other components were in trace amount (Table 1).

Chemistry and medicinal properties of *Tinospora cordifolia* were also reported by many workers.<sup>6,9</sup> These phytochemicals have been attributed to the medicinal properties of the experimental plant.<sup>10</sup> Screening active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer.<sup>11</sup>

#### **RESULT & DISCUSSION**

 Table 1- Phytochemical screening of secondary metabolites of different parts of Tinospora cordifolia grown with different supporting trees

Sl. No.	Plant Part	Saponin	Tannins	Steroids	Terpenoides	Phlobatanins	Flavanoids	Cardiac Glycosides	Alkaloids	
1.	TC (stem)	+	+	++	++	++	+	+	++	
2.	TC (Leaf)	+	+	+	+	-	++	+	+	
3.	TC (A) stem	+	+	+	+	-	++	+	+	
4.	TC (A) leaf	+	+	+	+	-	++	++	+	
5.	TC (N) stem	+	+	+	+	++	+++	+	++	
6.	TC (N) leaf	+	+	+	+	+	+++	+	+	
ТС	: Tinospora cordifolia				TC (A) :	Tinospora cordifolia on Aam (Mangifera indica)				

:

:

:

TCImasport conditionalTC (N)TC (N)Inospora cordifolia on Neem(+++)(Azadirachta indica)(++)(+):Trace(-)

**Quantitative estimation- 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. 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.<sup>12</sup> Analysis of bioactive compounds by GC-MS has been reported by different workers.<sup>13-15</sup> The results of GC-MS analysis of *Tinospora cordifolia* extracts were very interesting. GC-MS of the *T. cordifolia* extract revealed the presence of 55 to 75 components having different pharmacological importance along with antioxidant and/ or anticancer potential.

Appreciable amount

Moderate amount

Absent

n-Hexadecanoic acid or Palmitic acid, Heptadecanoic acid or margaric acid, Stigmasterol, STIGMAST-5-EN-3-

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OL, (3.BETA.)- and 1-Heptacosanol were present in TCE (A) and TCE (N) and anticancer in nature. Oleic Acid and Calcitriol was found to be present in TCE (A) and TCE (N) and absent in T. cordifolia extract (independent climber). Tetradecanoic acid or Myristic acid was antioxidant and anticancer and Pentadecanoic acid was found to be antioxidant in nature. These two bioactive compounds were present only in TCE (A) and TCE (N). However, there were some compounds which were present only in TCE and anticancer in nature such as pentanoic acid, propyl ester or valproic acid, phytol or 2-hexadecen-1-ol and 9,12-octadecadienoic acid (Z, Z)-. 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 2). 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 normal or independent climber as compared to TCE (A) and TCE (N) in which area per cent were 9.67% and 7.15% respectively.

In our investigation, GC-MS analysis of methanolic extract of the stem of experimental plant showed presence of bioactive compounds which are of pharmacological importances. There are 16-17 compounds showing antioxidant and/or anticancer activity of the experimental plant (Table 2).

Table 2- Anticancer and antioxidant compounds obtained from GC-MS analysis of Tinospora cordifolia, TCE, TCE (A) and TCE (N)

Name of compound	Common name	Chemica l formula	Mol. wt.	TCE (A)	TCE (N)	TCE	Activity	
Pentanoic acid, Propyl ester	Valproic acid	$C_8H_{16}O_2$	144	-	_	+	Anticancer <sup>16</sup>	
Calcitriol		C27H44O3	416	+	+	—	Anti-proliferative, Pro-apoplotic, Anticarcinogenic and tumor inhibitory <sup>17</sup>	
n-Hexadecanoic acid	Palmitic acid	C16H32O2	256	+	+	+	Antioxidant and anticancer <sup>18</sup>	
Heptadecanoic acid	Margaric acid	C17H34O2	270	+	+	+	Anticancer <sup>19</sup>	
Stigmasterol		C29H48O	412	+	+	+	Anticancer inhibit tumor promotion <sup>20</sup>	
STIGMAST-5-EN-3- OL, (3. BETA)	Beta sitosterol	C29H50O	414	+	+	+	Anticancer and antioxidant <sup>15</sup>	
1-Heptacosanol		C27H56O	396	+	+	+	Anticancer and antioxidant <sup>20</sup>	
Oleic acid		C18H34O2	282	+	+	—	Cancer preventive <sup>19</sup>	
Octadecanoic acid	Stearic acid	C18H36O2	284	+	+	+	Antioxidant and cancer preventive <sup>21</sup>	
Benzene propanoic acid, 2,5-dimethoxy-		$C_{11}H_{14}O_4$	210	+	-	-	Antioxidant <sup>20</sup>	
Phytol	2-Hexadecen-1-ol	C20H40O6	296	-	-	+	Anticancer <sup>20</sup>	
9,12-Octadecadienoic acid (Z,Z)-				_	_		Cancer preventive <sup>19</sup>	
Hexadecanoic acid, methyl ester	Palmitic acid methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	+	+	+	Antioxidant <sup>19</sup>	
Tetradecanoic acid	Myristic acid	C14H28O2	228	+	_	_	Antioxidant and cancer preventive <sup>19</sup>	
Pentadecanoic acid		C15H30O2	242	+	I	_	Antioxidant <sup>18</sup>	
Icosanoic acid						+	Antitumor <sup>18</sup>	
Cholest-4-en-3-one	Cholesterol			+	+	+	Antioxidant <sup>21</sup>	

TCE

Extract of Tinospora cordifolia independent climber,

TCE (A) Extract of Tinospora cordifolia climbing on Aam (Mangifera indica), :

TCE (N) · Extract of Tinospora cordifolia climbing on Neem (Azadirachta indica)

# **SUMMARY & CONCLUSION**

Knowledge of chemical constituents of a plant 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. Phytochemical screening showed the presence of Alkaloids, Flavonoids, Phlobatannins, Saponins, Steroids and Tannins. A comparative analysis of phyto-chemicals and GC-MS of Tinospora cordifolia which grows independently and

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growing on two different supporting trees *Azadirachta indica* and *Mangifera indica*, established the superiority of the one which grows independently. However, both the antioxidant potential and alkaloid content was higher in TCE (N). Presence of different phytochemicals in the experimental plant suggests its profuse therapeutic efficacy. Quality of this plant 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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