

# Antidiabetic Potential and Identification of Phytochemicals from *Tinospora cordifolia*

Varsha V Sonkamble and Laxmikant H Kamble\*

School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, 431606, India

## ABSTRACT

**Objective:** *Tinospora cordifolia* an ayurvedic herb has different classes of phytochemicals with medicinal significance in diabetes management. The hypothesis and possible mode of action of these phytochemicals used as antidiabetic drug has been already reported. So, we focused on identification of the *T. cordifolia* phytochemicals as well as the compounds responsible for antidiabetic activities in context of  $\alpha$ -amylase inhibition.

**Methods:** Total phenol estimation and Thin layer chromatography (TLC) of *T. cordifolia* extracts and assay of  $\alpha$ -amylase inhibition was done. Positively responding extracts were analyzed by liquid chromatography mass spectrometry (LCMS). Obtained LCMS data was processed using online databases like MassBank, ChemSpider and Phenol Explorer for characterization of compounds.

**Results:** Total Phenolic content of *T. cordifolia* extracts showed significant variations in their concentrations with highest phenolic content in ethanol extract, while highest  $\alpha$ -amylase inhibition was showed by ethyl acetate extract. Extracts with more than 40% inhibitory activity were subjected to LCMS; analyzed by MassBank, ChemSpider resource and phenol explorer database for compound identification. Identified compounds were searched in the literature for reported antidiabetic activity and we found seven; Cyanidin 3-O-sambubiosyl 5-O-glucoside, Hesperetin 7-Rhamnoglucoside, quercetin 3-O- $\beta$ -xylopyranosyl-(1 $\rightarrow$ 2)-O- $\beta$ -galactopyranoside, Blumenol C malonylglycosyl galacturonide [M+H]<sup>+</sup>, Verbascoside, Quercetin-3-glucuronide, and Catechin/Epicatechin-(epi) gallocatechin dimer.

**Conclusion:** The phytochemical profiling of *T. cordifolia* presented in this study revealed a diverse range of bioactive phenolics. Also it can be predicted that the potent antidiabetic activity of *T. cordifolia* is due to presence of compounds inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.

**Keywords:** *Tinospora cordifolia*, Phytochemicals, Antidiabetic activity, TLC, LCMS.

## Address for Correspondence

School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, 431606, India.

### E-mail:

[lhkamble@gmail.com](mailto:lhkamble@gmail.com)

## INTRODUCTION

Diabetes mellitus (DM) is characterized by hyperglycemia which is induced by decreased cellular glucose uptake and metabolism. Patients with this kind of issues trying to control in its early treatment suffers a very critical conditions<sup>1</sup>. One of the therapeutic approach is to prevent absorption of carbohydrates after food uptake which induces for reducing postprandial hyperglycemia in patients with DM. Only monosaccharides, such as glucose and fructose, can be transported out of the intestinal lumen into the bloodstream except complex starches, oligosaccharides, and disaccharides. Before absorption in the duodenum and upper jejunum, they must be broken down into individual monosaccharides. This digestion is facilitated by enteric enzymes, like pancreatic  $\alpha$ -amylase and  $\alpha$ -glucosidases which are attached to the brush border of the intestinal cells<sup>2</sup>. Commonly practiced treatments include oral hypoglycemic agents or insulin injections which have certain limitations. To manage diabetes without any side effect is still a challenge to the workers so, research is been switched towards the natural resources like plants and herbs which possess antidiabetic activities with low or no side effects<sup>3</sup>.

In traditional practices medicinal plants were used to control diabetes mellitus in many countries. The ethnobotanical information reports about 800 plants worldwide which have been documented as beneficial in the treatment of diabetes<sup>4</sup>. The majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve blood glucose control. However, a few comprehensive studies of traditional antidiabetic plants have been carried out<sup>1</sup>. This has caused an increase in the number of experimental and clinical investigations directed toward the validation of the anti-

diabetic properties, which are empirically attributed to these remedies<sup>5</sup>. The antidiabetic activity of several plants has been confirmed along with their studies of mechanisms of hypoglycemic activity. Chemical studies directed to the isolation, purification and identification of the substances responsible for the hypoglycemic activity are being conducted. One of the examples is *Tinospora cordifolia* which is widely used in veterinary folk medicine/ayurvedic system of medicine for its general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties<sup>6</sup>. The plant mainly contains alkaloids, glycosides, steroids, sesquiterpenoid, aliphatic compound, essential oils, mixture of fatty acids and polysaccharides. The alkaloids include berberine, bitter gilonin, non-glycoside gilonin gilosterol<sup>7</sup>. Glycosyl composition of a polysaccharide shown terminal-glucose, 4-xylose, 4-glucose, 4, 6-glucose and 2, 3, 4, 6-glucose<sup>8</sup>. These chemical composition studies were done earlier but their relation with antidiabetic activity was not studied. Earlier literature also states the aqueous, alcoholic, and chloroform extracts of the leaves of *T. cordifolia* in doses of 50, 100, 200 mg/kg body weight to normal and alloxan-diabetic induced rabbits exerted significant hypoglycaemic effect<sup>9</sup>. Histological studies of pancreas did not reveal any evidence of regeneration of  $\beta$ -cells of islets of Langerhance. The possible mode of action of the drug has been discussed projecting a hypothesis related to control of glucose metabolism. So, our study is based on identification of the chemical composition of *T. cordifolia* as well as compounds responsible for antidiabetic activities resembling  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition.

## MATERIALS AND METHODS

### Chemicals

Folin-Ciocalteu reagent, Sodium Carbonate, Gallic acid, Starch, Potassium Dihydrogen Phosphate, Dipotassium Hydrogen Phosphate,  $\alpha$ -amylase, and Gram's Iodine was purchased from Hi-Media Laboratories, Mumbai, India. Solvents like petroleum ether, ethyl acetate, chloroform, acetone, ethanol, cyclohexane, formic acid, acetic acid, toluene, butanol and methanol were from Qualigens. TLC Silica gel 60 F254 plates were purchased from Merck.

### Plant material

Dried powder of *T. cordifolia* plant was purchased from the local market of Nanded city, India which was used for the further study.

### Preparation of plant extracts

20 gm of sample was successively extracted in an order of non-polar to polar solvent based on increasing degree of polarity. The different extracts obtained sequentially were with petroleum ether, ethyl acetate, chloroform, acetone, ethanol and distilled water, respectively. Extractions were performed using soxhlet apparatus. The temperature maintained in each extraction was 10- 20 °C lower than the melting point of the solvents used and the time period of each extraction was fixed i.e., six hours. The extracts obtained were then filtered and concentrated to a volume of 5ml by boiling and were stored in a refrigerator until further use.

### Total phenolic content

The total amount of phenol in each extracts was determined by Folin-Ciocalteu reagent method with some modifications. 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 7.5% solution of sodium carbonate was added to 10 $\mu$ l of plant extract. The

resulting mixture was incubated for 15 minutes at room temperature. The absorbance of the sample was measured at 750nm. Gallic acid was used as standard (1mg/ml). All the tests were performed in triplicates. The results were determined from the standard curve and were expressed as gallic acid equivalent (GAE mg/g of extracted compound).

### TLC autography

Thin-layer chromatography was performed on the TLC Silica gel 60 F254 plates (Merck KGaA, Germany). The extracts were spotted on the plates using a micropipette and allowed to dry. One dimensional TLC analysis was performed with different solvent systems depending on respective solvent extracts (table 1). Spots were observed under Ultra-Violet light (UV light) at 254 nm and 366 nm. The TLC plates were then incubated in the amylase solution for 30 min for primary reaction between the enzyme and inhibitor. After incubation, the plates were taken out of the amylase solution and incubated in 1 % starch buffer of pH 6.9 for 10-20 min for enzyme-substrate reaction. The plates were then washed with Gram's Iodine solution and observed<sup>10</sup>.

### Inhibition assay for $\alpha$ -amylase activity

A total of 20  $\mu$ l of plant extract and 500  $\mu$ l of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing  $\alpha$ -amylase solution (0.5 mg/ml) were incubated at 25 °C for 10 min. After pre-incubation, 500  $\mu$ l of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube at 5 s intervals. The reaction mixtures were then incubated at 25° C for 10 min. The reaction was stopped with 1.0 ml of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The

reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm<sup>11</sup>.

% Inhibition = [(A<sub>540</sub> Control - A<sub>540</sub> Extract)] x 100/A<sub>540</sub> Control.

### LC-MS analysis

The chemical constituents of extracts showing positive results for  $\alpha$ -amylase inhibition assay were determined using LCMS analysis. The MS instrument was equipped with Turbo-Ionspray (electrospray ionisation) interface. 1.0 ml of sample extracts was diluted five times with chloroform and filtered with 0.2  $\mu$ M nylon filter prior to analyses. Full scan spectra from 100 to 1000 amu in the positive ion mode were recorded. The resolved compounds were then identified using online software i.e., MassBank<sup>12</sup> which is a public repository for sharing mass spectral data. The identification was based on mass and intensity obtained via records.

## RESULTS AND DISCUSSION

The total Phenolic content of six extracts from powdered sample of *T. cordifolia* which was determined by Folin-Ciocalteu method shows variations in their concentrations as recorded in table 2 and presented in fig. 1. Among the six extracts, Ethanol extract had the highest concentration of phenolic content GAE i.e., 0.244 mg/ml followed by water extract 0.2167 mg/ml, ethyl acetate 0.176mg/ml, petroleum ether 0.125mg/ml, acetone extract 0.087mg/ml and chloroform extract 0.042 mg/ml.

For the screening of  $\alpha$ -amylase inhibitors, extracted samples were run on thin layer chromatography and allowed for treatment of autography. Each extract gave various separated molecules with respective solvent system and also showed starch-iodine complex reaction indicating presence of  $\alpha$ -amylase inhibitors. Highest separated

molecules with blue color of starch-iodine complex reaction were observed in Petroleum ether and Ethyl acetate, Chloroform and Acetone (fig. 2a & 2b). Comparatively, ethanol and aqueous extracts failed to show blue color bands.

When, inhibition assay for  $\alpha$ -amylase activity was done, ethyl acetate extract showed highest percentage of  $\alpha$ -amylase inhibition followed by chloroform, petroleum ether, acetone, ethanol and water extract (table 3) which is represented by the given graph (fig. 3). The extracts showing more than 40% inhibition were further considered for LCMS analysis.

Spectrum analysis was done of samples containing petroleum ether, ethyl acetate, chloroform and acetone extracts. Full scan spectra from 100 to 1000 amu in the positive ion mode recorded was obtained as a raw data. The numbers of detected peaks in petroleum ether extracts were three, ethyl acetate extracts were nine, chloroform extracts were seven and acetone extracts were two. This raw data was then processed via online software MassBank where specific parameters were set for metabolite prediction i.e., for peak-substructure relationship, the raw data obtained from LCMS analysis was copied in text format and sent to the software as a query file, later commanded to read the file. The spectrum list appears on the page and then other parameters were set like, cut off value was 50, tolerance was maintained at 0.005 whereas specificity was set up as 0.4 and the ion mode was set as +ve. The values of mass and intensity is provided to the software which it reads and gives results in form of number of matched formulae which includes mass (m/z) values and its chemical formulae. Thus, each peak had shown different number of matched formulae which are represented in table 4. Obtained chemical formulae were then checked via ChemSpider<sup>13</sup> an online chemical

information resource and phenol explorer database<sup>14</sup> to identify the compound name. The compounds were single or attached with the sub-groups. The compounds were then checked for the existing literature showing positive antidiabetic activity. And hence, our finding reveals some compounds like Cyanidin 3-O-sambubiosyl 5-O-glucoside, Hesperetin 7- Rhamnoglucoside, quercetin 3-O- $\beta$ -xylopyranosyl-(1 $\rightarrow$ 2)-O- $\beta$ -galactopyranoside, Blumenol C malonylglycosyl galacturonide [M+H]<sup>+</sup>, Verbascoside, Quercetin-3-glucuronide, and Catechin/Epicatechin-(epi) gallocatechin dimer showing antidiabetic activities.

## CONCLUSION

The phytochemical profiling of *T. cordifolia* presented in this study revealed a diverse range of bioactive phenolics. Some of the identified bioactive phenolics were reported by several authors for antidiabetic activities. So this was an attempt to identify the potent antidiabetic compounds from *T. cordifolia*. Thus from this study, it can also be predicted that the potent antidiabetic activity of *T. cordifolia* is due to presence of compounds inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.

## REFERENCES

1. Gallagher AM, Flatt PR, Duffy G, Abdel-Wahab YHA. The effects of traditional antidiabetic plants on *in vitro* glucose diffusion. *Nutr Res*. 2015 Jan 2; 23 (3): 413–24.
2. Ortiz-Andrade RR, García-Jiménez S, Castillo-España P, Ramírez-Avila G, Villalobos-Molina R, Estrada-Soto S. alpha-Glucosidase inhibitory activity of the methanolic extract from *Tournefortia hartwegiana*: an anti-hyperglycemic agent. *J Ethnopharmacol*. 2007 Jan; 109 (1): 48–53.
3. Kaur J, Kaur S, Mahajan A. Herbal Medicines: Possible Risks and Benefits. *American Journal of Phytomedicine and Clinical Therapeutics*. 2013; 1(2): 226-239.
4. Patil A, Jadhav V, Arvindekar Aand More T. Antidiabetic Activity of *Maesa indica* (Roxb.) Stem Bark in Streptozotocin Induced Diabetic Rats. *American Journal of Phytomedicine and Clinical Therapeutics*. 2014; 2(8): 957-962.
5. Roman-Ramos R, Flores-Saenz JL, Alarcon-Aguilar FJ. Anti-hyperglycemic effect of some edible plants. *J Ethnopharmacol*. 1995; 48 (1995): 25–32.
6. Singh SS, Pandey SC, Srivastava S, Gupta VS, Patro B, Ghosh AC. Chemistry and medicinal properties of *Tinospora cordifolia* (Guduchi). *Indian J Pharmacol*. 2003; 35: 83–91.
7. Devprakash KK, Srinivasan T, Subburaju SG & S Singh. *Tinospora cordifolia*: a review on its ethnobotany, phytochemical & pharmacological profile. *Asian J Biochem Pharm Res*. 2011; 1(4): 291–302.
8. Musliarakathbacker J, Azadi P. Glycosyl composition of polysaccharide from *Tinospora cordifolia*. II. Glycosyl linkages. *Acta Phrma*. 2004; 54: 73–8.
9. Mishra NP, Singh J, Khanuja SPS. *Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications: A Review. 2004; 3(3): 257–270.
10. Sonkamble V, Zore G, Kamble L. A simple method to screen amylase inhibitors using thin layer chromatography. *Sci Res Report*. 2014; 4(1): 85–8.
11. Suthindhiran KR, Jayasri MA, Kannabiran K.  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activity of *Micromonospora* sp. VITSDK3 (EU551238). *International Journal of Integrative Biology*. 2009; 6(3): 115.
12. Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, *et al*. MassBank: a public repository for sharing mass spectral data for life sciences. *J Mass Spectrom*. 2010; 45 (7): 703–14.
13. Hettne KM, Williams AJ, van Mulligen EM, Kleinjans J, Tkachenko V, Kors JA. ChemSpider: An Online Chemical Information Resource. *J Cheminform*. 2010 Jan; 2(1): 3.
14. Rothwell JA, Perez-Jimenez J, Neveu V, Medina-Remón A, M'hiri N, García-Lobato P, *et al*. Phenol-Explorer 3.0: a major update

- of the Phenol-Explorer database to incorporate data on the effects of food processing on polyphenol content. 2013 Jan; 1–8.
15. Sumbul S, Ahmad MA, Asif M, Akhtar M. *Myrtus communis* Linn.- A review. *Indian J Nat Prod Resour.* 2011; 2:395–402.
  16. Olivieri C, Silvestrin C, Garcia C, Gambato G, Denize M, Souza O De, et al. Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. *Food Chem Toxicol* 2013; 52: 137–42.
  17. Akiyama S, Katsumata S, Suzuki K, Nakaya Y, Ishimi Y, Uehara M. Hypoglycemic and hypolipidemic effects of hesperidin and cyclodextrin-clathrated hesperetin in Goto-Kakizaki rats with type 2 diabetes. *Biosci Biotechnol Biochem.* 2009; 73 (12): 2779–82.
  18. Fawzy GA, Abdallah HM, Marzouk MSA, Soliman FM, Sleem AA. Antidiabetic and Antioxidant Activities of Major Flavonoids of *Cynanchum acutum* L. (Asclepiadaceae) Growing in Egypt. *Verlag der Zeitschrift für Naturforschung, Tübingen.* 2008; 240: 658–62.
  19. Mariela Odjakova, Eva Popova MAS and RM, Mironova R. Glycosylation. Petrescu S, editor. *InTech open science. InTech;* 2012.
  20. Su Z. Anthocyanins and Flavonoids of *Vaccinium* L. *Pharm Crop.* 2012; 3: 7–37.
  21. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. *J Ethnopharmacol.* 2014; 155 (2): 857–924.
  22. Huang W, Wu S, Wang Y, Guo Z, Kennelly EJ. Chemical constituents from *Striga asiatica* and its chemotaxonomic study. *Biochem Syst Ecol.* 2013; 48: 100–6.
  23. Omar SH. Oleuropein in olive and its pharmacological effects. *Sci Pharm.* 2010; 78: 133-154.
  24. Boudjelal A, Henchiri C, Siracusa L, Sari M, Ruberto G. Compositional analysis and in vivo anti-diabetic activity of wild Algerian *Marrubium vulgare* L. infusion. *Fitoterapia* 2012; 83 (2): 286–92.
  25. Sulaiman CT, Gopalakrishnan VK, Balachandran I. Reverse Phase Liquid Chromatography Coupled with Quadra pole-Time of Flight Mass Spectrometry for the Characterisation of Phenolics from *Acacia catechu* (L.f.) Willd. *Int J Phytomedicine.* 2012; 4: 403–8.
  26. Mohamed S. Functional foods against metabolic syndrome (obesity, diabetes, hypertension and dyslipidemia) and cardiovascular disease. *Trends Food Sci Technol;* 2013, <http://dx.doi.org/10.1016/j.tifs.2013.11.001>.
  27. Baenas N, Villan D, Moreno DA, Garcı C. Evaluation of Latin-American fruits rich in phytochemicals with biological effects. *J Funct Foods.* 2014; 7: 599–608.

**Table 1.** Optimized TLC solvent systems used for separation of compounds

S. No.	<i>T. cordifolia</i> extracts	Solvent system used	Ratio of solvent system
1	Petroleum ether	Cyclohexane: Ethyl acetate: Formic acid	6:3:1
2	Ethyl acetate	Cyclohexane: Ethyl acetate: Formic acid	6:3:1
3	Chloroform	Toluene: Chloroform: Methanol	4.5:5:0.5
4	Acetone	Acetic acid: Acetone: Water	6:3.5:0.5
5	Ethanol	Toluene: Chloroform: Methanol	5:4:1
6	Distilled water	Butanol: Acetic acid: water	4:1:5

**Table 2.** Total phenol concentration in extracts of *T. cordifolia*

S. No.	Sample extract	Conc. (ppm)	Conc. (mg/ml) GAE
1	Petroleum ether	125.15	0.125
2	Ethyl acetate	175.63	0.175
3	Chloroform	41.93	0.041
4	Acetone	87.5	0.087
5	Ethanol	243.67	0.243
6	Distilled water	216.77	0.216

**Table 3.**  $\alpha$ -amylase inhibition by extracts of *T. cordifolia*

S. No.	Sample Extract	% of inhibition
1	Pet. Ether	42.29
2	Ethyl acetate	64.04
3	Chloroform	57.6
4	Acetone	46.28
5	Ethanol	13.29
6	Water	7.56

**Table 4.** LC-MS data representing tentatively identified phytochemicals in *T. cordifolia* extracts with retention time, mass, molecular formulae and reported antidiabetic activity

Sample extract	Peak	Retention time	Accurate mass	Proposed molecular formula	Phenolic compound	Anti-diabetic Activity	Ref.
Petroleum ether extract	1	7.51-9.86	447.13	C <sub>22</sub> H <sub>23</sub> O <sub>10</sub>	Petunidin 3-O-rhamnoside	-	-
			559.60	-	-	-	-
	2	17.58-20.49	559.80	-	-	-	-
			951.67	-	-	-	-
	3	27.21-28.99	561.87	-	-	-	-
			743.19	C <sub>32</sub> H <sub>39</sub> O <sub>20</sub>	Cyanidin 3-O-sambubiosyl 5-O-glucoside	Present	(14, 18, 26, 27)
Ethyl acetate extract	1	7.09-10.27	373.133	C <sub>20</sub> H <sub>21</sub> O <sub>7</sub>	4-(2, 4-dimethoxy-3, 6-dimethylbenzoyl) oxy-2-hydroxy-3, 6-dimethylbenzoate	-	-
			447.133	C <sub>22</sub> H <sub>23</sub> O <sub>10</sub>	Petunidin 3-O-rhamnoside	-	-
			462.066	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O <sub>11</sub> P	Adenylosuccinate	-	-
			462.266	C <sub>26</sub> H <sub>40</sub> NO <sub>4</sub> S	-	-	-
			479.332	C <sub>24</sub> H <sub>50</sub> NO <sub>6</sub> P	lysoplasménylcholine	-	-
	2	10.48-12.18	109.066	C <sub>5</sub> H <sub>7</sub> N <sub>3</sub>	Imidazole-Pyrazole	-	-
			109.066	C <sub>7</sub> H <sub>9</sub> O	1-cyclohexenylmethanone	-	-
			112.933	HO <sub>3</sub> S <sub>2</sub>	(Hydroxysulfonylthio)oxidanyl	-	-
			113.066	C <sub>5</sub> H <sub>9</sub> N <sub>2</sub> O	(2S)-2-Pyrrolidinecarboximidate	-	-
			295.133	C <sub>14</sub> H <sub>19</sub> N <sub>2</sub> O <sub>5</sub>	(1E)-3-[2-(Dimethoxymethyl) phenyl]-1-ethoxy-1, 3-dihydroxy-1-propene-2-diazonium	-	-
			295.199	C <sub>16</sub> H <sub>27</sub> N <sub>2</sub> O <sub>3</sub>	2-[(3-Amino-4-propoxybenzoyl) oxy] - N, N-diethylethanaminium	-	-
			313.066	C <sub>17</sub> H <sub>13</sub> O <sub>6</sub>	(4-Cinnamoyl-3, 5-dihydroxyphenoxy) acetate	-	-
			341.266	C <sub>20</sub> H <sub>37</sub> O <sub>4</sub>	2-Hydroxy-3-oxoicosanoate	-	-
			343.266	C <sub>23</sub> H <sub>35</sub> O <sub>2</sub>	Cholic acid	-	-
			343.66	H <sub>341</sub>	-	-	-
			349.066	C <sub>20</sub> H <sub>13</sub> O <sub>6</sub>	2-Hydroxy-5, 10-dioxo-4-phenyl-3, 4, 5, 10-tetrahydro-2H-benzo[g]chromene-2-carboxylate	-	-
			351.199	C <sub>23</sub> H <sub>27</sub> O <sub>3</sub>	4-ethoxy-2-(4-isopentyloxyphenyl)-6-methyl-chromene	-	-
			611.199	C <sub>28</sub> H <sub>35</sub> O <sub>15</sub>	Hesperetin 7-rhamnoglucoside	Present	(15-17)
			821.399	C <sub>42</sub> H <sub>61</sub> O <sub>16</sub>	Monopotassium Glycyrrhizinate	-	-
			933.266	C <sub>43</sub> H <sub>49</sub> O <sub>23</sub>	Peonidin 3-caffeoyl-rutinoside 5-glucoside	-	-
	3	15.41-	611.199	C <sub>28</sub> H <sub>35</sub> O <sub>15</sub>	Hesperetin 7-rhamnoglucoside	Present	(15-17)

		16.25	810.133	$C_{23}H_{39}N_7O_{17}P_3S$	-	-	-
	4	16.87-17.48	595.133	$C_{26}H_{27}O_{16}$	quercetin 3-O- $\beta$ -xylopyranosyl-(1 $\rightarrow$ 2)-O- $\beta$ -galactopyranoside	Present	(18-20)
			595.199	$C_{28}H_{35}O_{14}$	Blumenol C malonylglycosyl galacturonide [M+H] <sup>+</sup>	Present	(21, 22)
			810.133	$C_{23}H_{39}N_7O_{17}P_3S$	-	-	-
	5	19.06-20.85	810.133	$C_{23}H_{39}N_7O_{17}P_3S$	-	-	-
	6	21.87-22.77	810.133	$C_{23}H_{39}N_7O_{17}P_3S$	-	-	-
	7	26.26-27.22	810.133	$C_{23}H_{39}N_7O_{17}P_3S$	-	-	-
	8	27.53-29.33	274.866	$C_8H_5Br_2O$	-	-	-
			604.066	$C_{16}H_{24}N_5O_{16}P_2$	GDP-L-galactose		
			623.199	$C_{29}H_{35}O_{15}$	Verbascoside	Present	(22-24)
			810.133	$C_{23}H_{39}N_7O_{17}P_3S$	-	-	-
			933.266	$C_{43}H_{49}O_{23}$	petanin	-	-
	9	32.19-33.15	798.53	-	-	-	-
Chloroform extract	1	4.33-5.26	973.73	-	-	-	-
	2	8.26-9.88	438.266	$C_{20}H_{41}NO_7P$	-	-	-
			477.066	$C_{17}H_{21}N_2O_{10}S_2$	4-methoxyglucobrassicin	-	-
			477.066	$C_{21}H_{17}O_{13}$	Quercetin 3-glucuronide	Present	(20, 25)
	3	15.14-16.38	291.133	$C_{10}H_{19}N_4O_6$	N-(L-Arginino) succinate5'	-	(12)
			291.199	$C_{18}H_{27}O_3$	(15Z)-12-Oxophyto-10, 15-dienoate	-	-
			335.066	$C_{11}H_{16}N_2O_8P$	3-Carbamoyl-1-(5-O-phosphono- $\beta$ -D-ribofuranosyl) pyridinium	-	-
			593.133	$C_{30}H_{25}O_{13}$	Catechin/Epicatechin-(epi) gallocatechin dimer	Present	(20, 21, 26)
			595.133	$C_{26}H_{27}O_{16}$	quercetin 3-O- $\beta$ -xylopyranosyl-(1 $\rightarrow$ 2)-O- $\beta$ -galactopyranoside	Present	(17-19)
			595.199	$C_{28}H_{35}O_{14}$	Blumenol C malonylglycosyl galacturonide [M+H] <sup>+</sup>	Present	(21, 22)
			651.799	$C_{15}H_{13}I_3NO_4$	Triiodothyronine	-	-
	933.266	$C_{43}H_{49}O_{23}$	Petunidin-3-O-coumaroylrutinoside-5-O-glucoside	-	-		
	4	17.57-	663.87	-	-	-	-

		20.44					
	5	25.17-27.17	623.199	C <sub>29</sub> H <sub>35</sub> O <sub>15</sub>	Verbascoside	Present	(22-24)
	6	27.54-28.98	274.866	C <sub>8</sub> H <sub>5</sub> Br <sub>2</sub> O	-	-	-
			274.99	C <sub>10</sub> H <sub>9</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub>	3, 5-dichloro-4-morpholin-4-ylpyridine-2-carboxylate		
			275.066	C <sub>18</sub> H <sub>11</sub> O <sub>3</sub>	3-(1-Naphthoyl) benzoate	-	-
			275.199	C <sub>18</sub> H <sub>27</sub> O <sub>2</sub>	6, 9, 12, 15-Octadecatetraenoatato		
			623.199	C <sub>29</sub> H <sub>35</sub> O <sub>15</sub>	Verbascoside	Present	(22-24)
	7	29.98-31.48	274.866	C <sub>8</sub> H <sub>5</sub> Br <sub>2</sub> O	-	-	-
			274.99	C <sub>10</sub> H <sub>9</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>3</sub>	3, 5-dichloro-4-morpholin-4-ylpyridine-2-carboxylate		
			275.066	C <sub>18</sub> H <sub>11</sub> O <sub>3</sub>	3-(1-Naphthoyl) benzoate	-	-
			275.199	C <sub>18</sub> H <sub>27</sub> O <sub>2</sub>	6, 9, 12, 15-Octadecatetraenoatato	-	-
			345.133	C <sub>19</sub> H <sub>21</sub> O <sub>6</sub>	3, 3-Bis (3, 4-dimethoxyphenyl) propanoate	-	-
			477.066	C <sub>17</sub> H <sub>21</sub> N <sub>2</sub> O <sub>10</sub> S <sub>2</sub>	4-methoxyglucobrassicin	-	-
			477.066	C <sub>21</sub> H <sub>17</sub> O <sub>13</sub>	Quercetin 3-glucuronide	Present	(20, 25)
		623.199	C <sub>29</sub> H <sub>35</sub> O <sub>15</sub>	Verbascoside	Present	(22-24)	
Acetone extract	1	16.11-20.97	529	-	-	-	
			663.87	-	-	-	
	2	33.97-39.53	557.13	-	-	-	

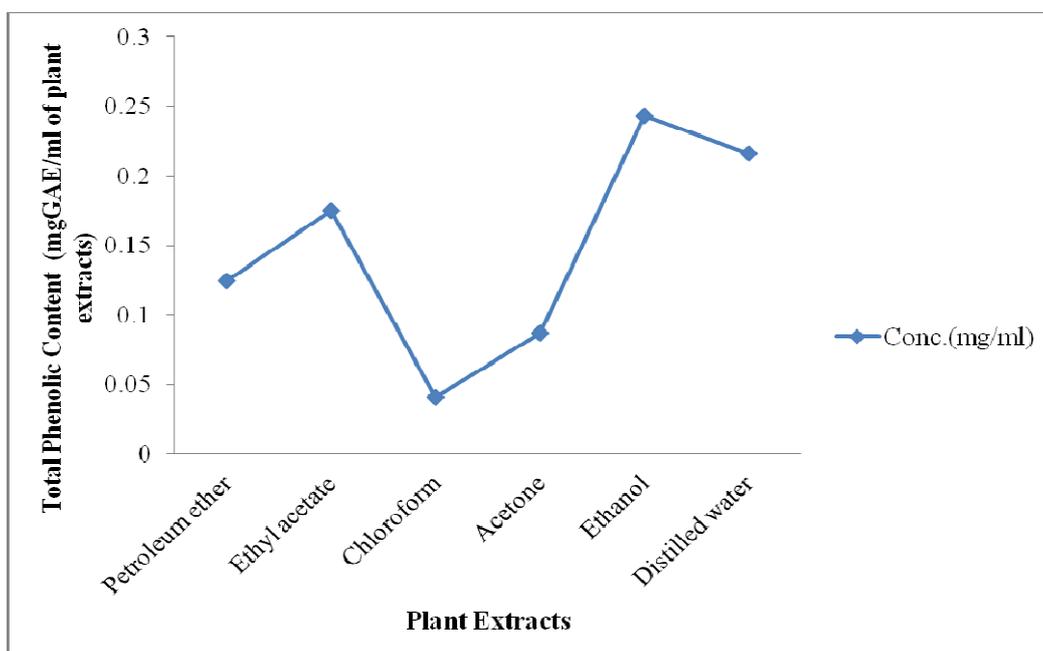


Figure 1. Total phenol concentration in extracts of *T. cordifolia*

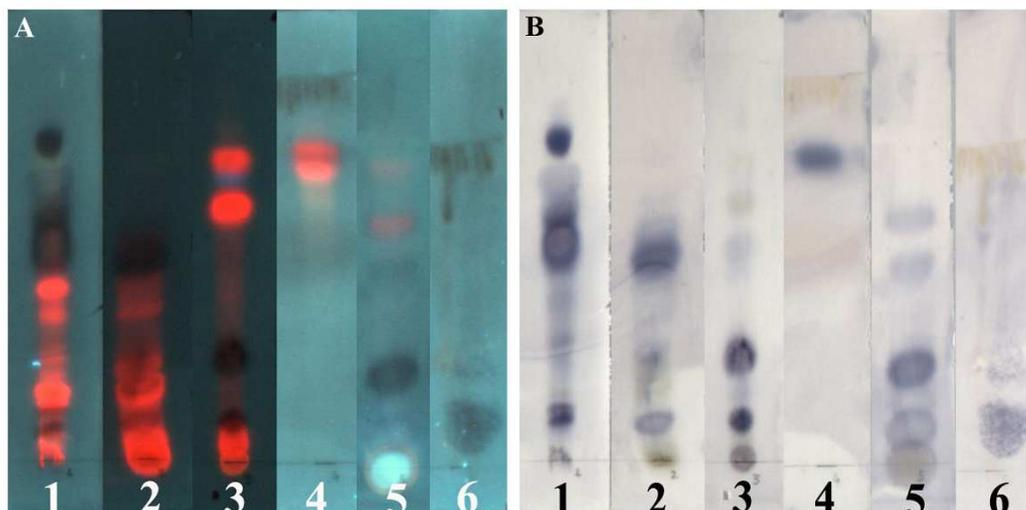


Figure 2. TLC plates A. Separated bands present in the respective solvent extracts under ultra-violet (254 nm) light source, B. Separated bands indicating presence of  $\alpha$ -amylase inhibitors (blue stains) in normal light source

1= petroleum ether extract, 2= ethyl acetate extract, 3=chloroform extract, 4= acetone extract, 5= ethanol extract and 6= water extract.

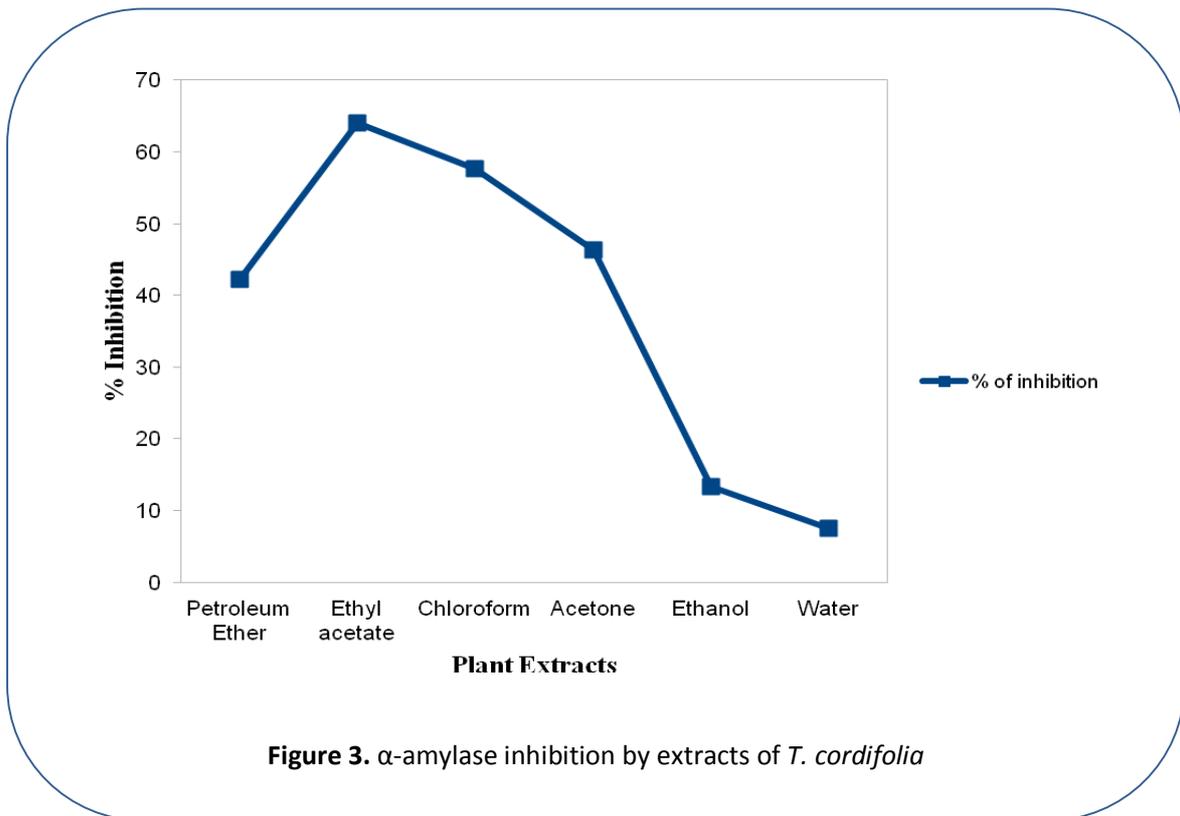


Figure 3.  $\alpha$ -amylase inhibition by extracts of *T. cordifolia*

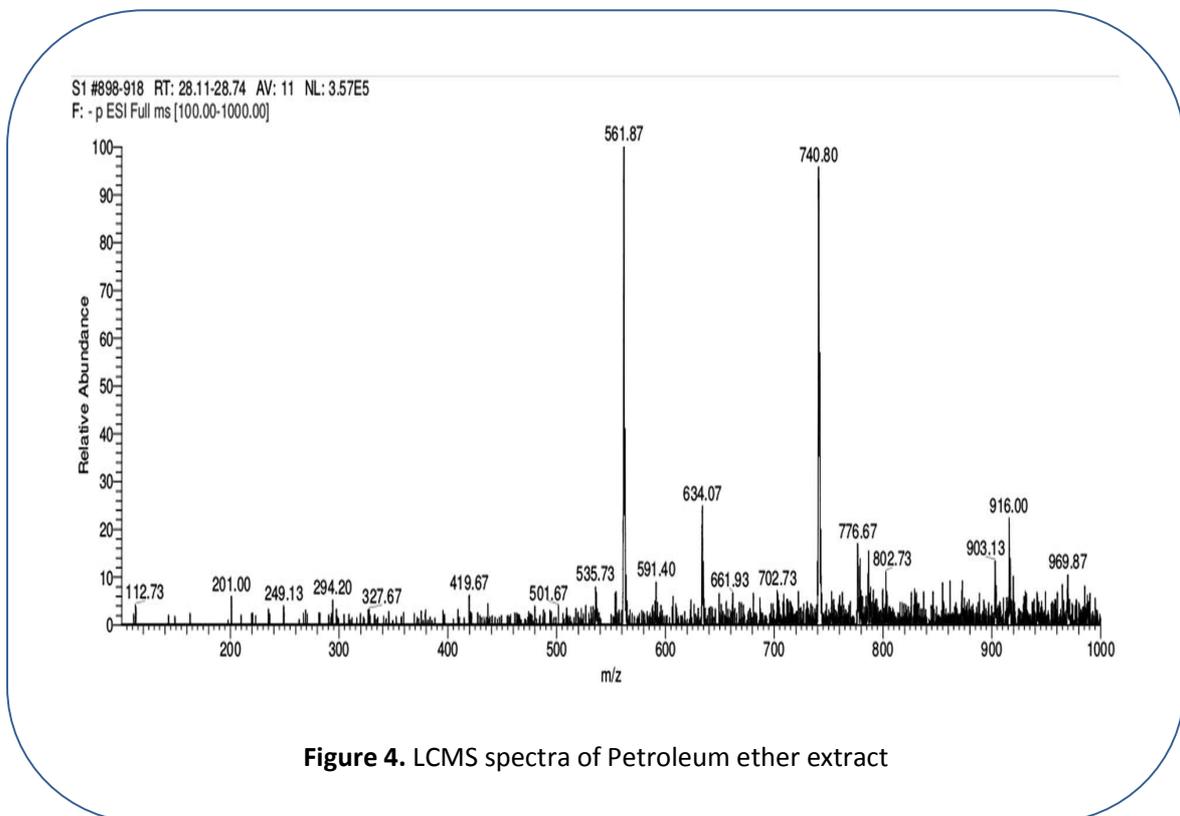
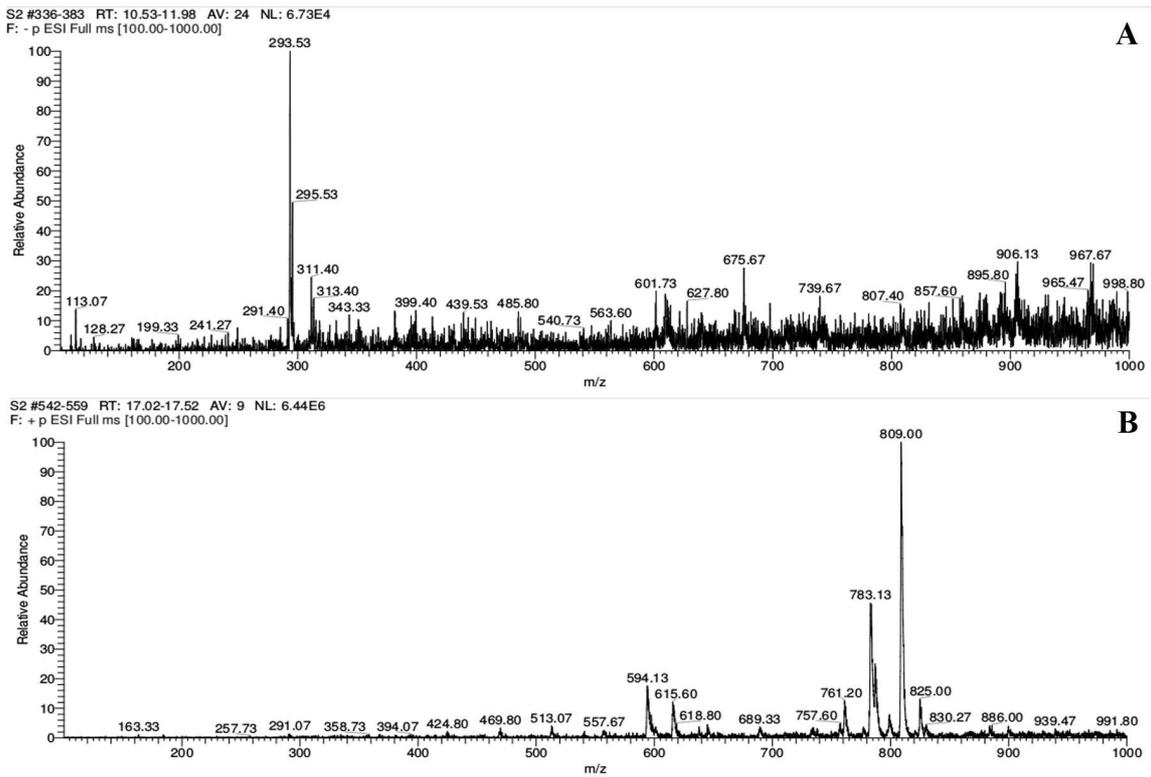


Figure 4. LCMS spectra of Petroleum ether extract



**Figure 5.** LCMS spectra of Ethyl acetate extract

A. Spectra checked at retention time 10.48-12.18, B. Spectra checked at retention time 16.87-17.48 (Detailed analysis is described in table 4).

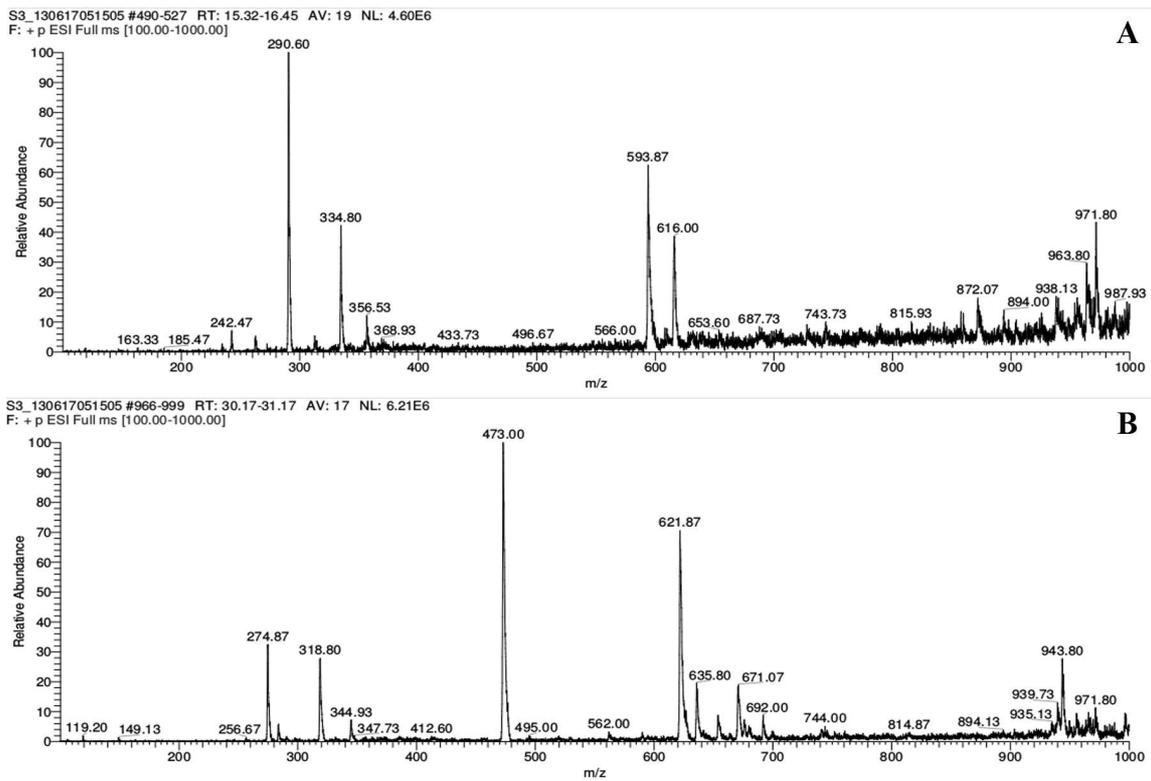


Figure 6. LCMS spectra of chloroform extract

A. Spectra checked at retention time 15.14-16.38, B. Spectra checked at retention time 29.98-31.48 (Detailed analysis is described in table 4).