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Preliminary Phytochemical Analysis and *In Vitro* Antidiabetic Potential of *Tinospora cordifolia*

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Abstract

Tinospora cordifolia (Willd.) Miers, a traditional medicinal plant in Ayurveda and Siddha, is widely recognized for its therapeutic potential in metabolic disorders, including diabetes mellitus. This study investigated the preliminary phytochemical profile and in vitro antidiabetic activity of ethanol and aqueous extracts of *T. cordifolia* stem, leaf, and root. Extraction yields were higher in ethanol than aqueous extracts, reflecting greater recovery of semi-polar metabolites. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, steroids, and reducing sugars, with variations across plant parts and solvents. The antidiabetic potential was evaluated using α-amylase and α-glucosidase inhibition assays. Ethanol extracts, particularly leaf extract, exhibited potent inhibitory activity in a concentration-dependent manner, with IC_{50} values significantly lower than aqueous extracts. The findings highlight *T. cordifolia* as a promising source of bioactive constituents with α-amylase and α-glucosidase inhibitory potential, supporting its ethnomedicinal use in managing postprandial hyperglycemia. Further purification and mechanistic studies are warranted.

Keywords: *Tinospora cordifolia*, phytochemicals, α-amylase, α-glucosidase, antidiabetic

Introduction

Diabetes mellitus is a global health crisis characterized by impaired insulin secretion leading to chronic hyperglycemia and associated complications such as cardiovascular disease, neuropathy, and nephropathy (American Diabetes Association, 2023). The International Diabetes Federation estimates that 537 million adults were living with diabetes in 2021, a number projected to rise to 643 million by 2030 (IDF, 2021). Type 2 diabetes mellitus accounts for more than 90% of cases and is closely linked to obesity, sedentary lifestyle, and aging populations (Cho et al., 2018). Conventional treatments include insulin therapy, oral hypoglycemic agents, and lifestyle interventions. However, synthetic drugs such as sulfonylureas, biguanides, and α-glucosidase

inhibitors often present limitations including gastrointestinal side effects, hypoglycemia risk, and high cost (Patel et al., 2012). Hence, the search for natural alternatives with improved safety and efficacy profiles has intensified.

Medicinal plants have historically played a central role in diabetes management, providing bioactive compounds with hypoglycemic, antioxidant, and enzyme-inhibitory properties (Kooti et al., 2016). Among them, *Tinospora cordifolia* (Willd.) Miers, (Tamil name: Seenthi or Shindilakodi) commonly known as Guduchi or Amruth, is highly esteemed in Ayurveda, Siddha, and other traditional systems for its rejuvenating and immunomodulatory properties (Saha & Ghosh, 2012). The plant is a large deciduous climbing shrub distributed widely across tropical Asia. Phytochemical investigations have revealed the presence of diverse secondary metabolites including alkaloids, diterpenoid lactones, flavonoids, steroids, and polysaccharides (Bansal et al., 2020). Pharmacological studies have demonstrated immunomodulatory, hepatoprotective, antioxidant, antimicrobial, and anti-inflammatory activities (Kapil & Sharma, 2020).

In the context of diabetes, *T. cordifolia* has attracted interest due to its reported hypoglycemic and antihyperglycemic effects in animal and human studies. Its potential mechanisms include modulation of glucose metabolism, enhancement of insulin secretion, and inhibition of carbohydrate-hydrolyzing enzymes (Singh et al., 2020). Targeting digestive enzymes such as α-amylase and α-glucosidase is particularly relevant, as these enzymes catalyze the breakdown of complex carbohydrates into absorbable monosaccharides, contributing to postprandial hyperglycemia (Shobana et al., 2009). Inhibitors of these enzymes, such as acarbose, are clinically employed to control blood glucose spikes but are often associated with gastrointestinal discomfort (Chiasson et al., 2002). Thus, natural enzyme inhibitors from plants offer an attractive alternative.

Previous studies have primarily focused on the stem of *T. cordifolia*, while systematic comparisons of phytochemical composition and antidiabetic potential across different plant parts remain limited. Moreover, solvent polarity significantly influences phytochemical yield and biological activity (Anjum et al., 2023). This study was therefore undertaken to evaluate and compare the phytochemical profile and in vitro enzyme inhibitory potential of ethanol and aqueous extracts of *T. cordifolia* stem, leaf, and root. The objectives were: (i) to qualitatively identify major phytochemical classes; (ii) to determine extraction yields for different plant parts and solvents; and (iii) to assess α-amylase and α-glucosidase inhibitory activities, benchmarked against acarbose. The findings aim to provide preliminary evidence supporting the traditional use of *T. cordifolia* in diabetes management and guide future studies on bioactive compound isolation.

Materials and Methods

Collection of plant sample

Fresh *Tinospora cordifolia* stems, leaves and roots were collected from a cultivated population near Government Siddha Medical College, Chennai. Plant parts were washed, shade-dried at room temperature, and ground to a coarse powder using a mechanical grinder. All reagents were of analytical grade.

Preparation of extracts

For each plant part (stem, leaf, root), two solvent extracts were prepared: 70% ethanol (ethanol extract) and distilled water (aqueous extract). For each extract, 50 g of powdered material was macerated with 500 mL solvent for 48 h at room temperature with occasional stirring. Extracts were filtered through Whatman No.1 filter paper and concentrated: ethanol extracts under reduced pressure (rotary evaporator) and aqueous extracts by freeze-drying to obtain dried extracts, which were weighed to determine percent yield (w/w). Dried extracts were stored at 4 °C until use (Anjum et al., 2023).

Phytochemical analysis

The classical qualitative assays were performed on each extract following standard procedures (Harborne, 1998; Trease & Evans, 2002). The assays included: (1) Dragendorff's test for alkaloids; (2) Shinoda test for flavonoids; (3) Ferric chloride test for phenolics/tannins; (4) Froth test for saponins; (5) Salkowski test for terpenoids (and Liebermann–Burchard for steroids); (6) Keller–Killiani test for cardiac glycosides; and (7) Benedict's test for reducing sugars (presence of glycosidic sugars). Tests were performed in triplicate and recorded as absent (−), present (+) or strongly present (++). Positive and negative controls were included for each assay.

In vitro α-amylase inhibitory activity

The *in vitro* α-amylase inhibitory activity of ethanol and aqueous extracts of *Tinospora cordifolia* stem, leaf, and root was assessed using the dinitrosalicylic acid (DNSA) method with slight modifications (Miller, 1959; Sharma et al., 2021). Porcine pancreatic α-amylase (1 U/mL) prepared in 20 mM phosphate buffer (pH 6.9) was used as the enzyme, while a 1% soluble starch solution served as the substrate. Extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer to final test concentrations of 100, 250, 500, and 1000 µg/mL, ensuring that the DMSO concentration did not exceed 1%. In the assay, 500 µL of extract solution was preincubated with 500 µL of α-amylase at 37 °C for 10 minutes, after which 500 µL of starch solution was added and incubated for another 10 minutes. The reaction was terminated by adding 1 mL of DNSA reagent, followed by boiling for 5 minutes, cooling, and dilution with 10 mL distilled water. Absorbance was measured at 540 nm using a UV–Vis spectrophotometer. Acarbose (1 mg/mL) was used as the positive control, and all experiments were performed in triplicate. Percentage inhibition was calculated relative to the control (enzyme + substrate without extract), and IC_{50} values were determined from concentration–response curves using nonlinear regression analysis with GraphPad Prism v.9.

In vitro α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of ethanol and aqueous extracts of *Tinospora cordifolia* stem, leaf, and root was determined following the method of Sharma et al. (2021) with modifications. α-Glucosidase enzyme from *Saccharomyces cerevisiae* (0.5 U/mL) was prepared in 100 mM phosphate buffer (pH 6.8), while p-nitrophenyl-α-D-glucopyranoside (pNPG, 5 mM) served as the substrate. Extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer to final concentrations of 100, 250, 500, and 1000 µg/mL. In the assay, 50 µL of extract solution was preincubated with 100 µL of enzyme solution at 37 °C for 10 minutes. The reaction was initiated by adding 50 µL of pNPG solution and incubated for 20 minutes at 37 °C. The reaction was stopped by adding 1 mL of 0.1 N sodium carbonate, and the absorbance was recorded at 405 nm using a UV–Vis spectrophotometer. Acarbose (1 mg/mL) was used as the positive control. All tests were conducted in triplicate. The percentage inhibition was calculated relative to control wells (enzyme + substrate without extract), and IC_{50} values were obtained from concentration–response curves using nonlinear regression analysis in GraphPad Prism v.9.

Data analysis

All assays were performed in triplicate and results presented as mean ± standard deviation (SD). IC_{50} values were calculated by nonlinear regression (GraphPad Prism). One-way ANOVA followed by Tukey's post hoc test compared IC_{50}s among extracts; $p < 0.05$ was considered significant (Altman, 1991).

Results

Extraction yields. Yields (w/w on dry material) differed by plant part and solvent: leaf ethanol 14.8%, leaf aqueous 10.6%; stem ethanol 11.5%, stem aqueous 8.9%; root ethanol 9.2%, root aqueous 7.5%. Ethanol generally gave higher yields for semi-polar constituents.

Qualitative phytochemical screening

Table 1 summarizes the results of eight qualitative assays performed for ethanol and aqueous extracts of leaf, stem and root. The ethanol extracts of all three parts tested positive for flavonoids, terpenoids and glycosides; leaf ethanol extract showed strongest responses (++ for flavonoids). Aqueous extracts were richer in tannins and saponins, particularly in leaf and stem. Alkaloids were present (+) in stem and root ethanol extracts but weak or absent in leaf aqueous extract. Steroids were indicated in ethanol extracts of stem and root but were absent in aqueous extracts. Reducing sugars were detected in aqueous extracts of all parts.

Table 1. Qualitative phytochemical screening of *T. cordifolia* extracts

Phytochemical test	Leaf		Stem		Root	
	EtOH	Aqueous	EtOH	Aqueous	EtOH	Aqueous
Alkaloids	+	−	+	+	+	+
Flavonoids	++	+	+	+	+	−
Tannins	+	++	+	++	+	+
Saponins	+	++	−	+	−	+
Terpenoids	+	−	+	−	+	−
Glycosides	+	+	+	+	+	+
Steroids	+	−	+	−	+	−
Reducing sugars	+	++	+	++	+	++

Note: (EtOH = 70% ethanol; Aq = aqueous) (− absent; + present; ++ strongly present).

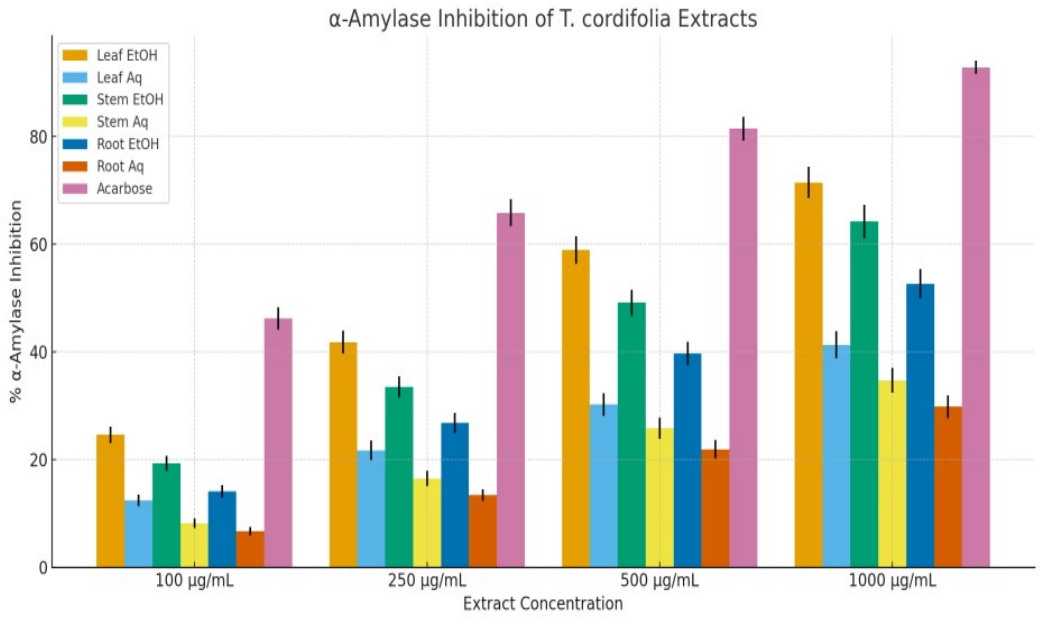
α-Amylase Inhibition Assay

The α-amylase inhibitory effect of ethanol and aqueous extracts of *Tinospora cordifolia* stem, leaf, and root was evaluated at concentrations of 100, 250, 500, and 1000 µg/mL. All extracts showed a concentration-dependent increase in inhibitory activity, with ethanol extracts generally more potent than aqueous extracts (Table 2 and Fig 1).

The ethanol leaf extract exhibited the strongest inhibition, reaching 71.4 ± 2.9% at 1000 µg/mL, with an IC_{50} value of 162.9 ± 8.4 µg/mL. Stem ethanol extract showed moderate activity (64.2 ± 3.1% at 1000 µg/mL; IC_{50} = 205.1 ± 9.6 µg/mL), while root ethanol extract showed weaker inhibition (52.6 ± 2.7% at 1000 µg/mL; IC_{50} = 275.8 ± 11.9 µg/mL). Aqueous extracts demonstrated lower activity, with inhibition ranging from 18.5–41.3% at 1000 µg/mL, and IC_{50} values exceeding 300 µg/mL. Acarbose, the positive control, showed potent activity with 92.8 ± 1.2% inhibition at 1000 µg/mL and an IC_{50} of 34.5 ± 1.8 µg/mL.

Table 2: α-Amylase Inhibition (%) of *T. cordifolia* extracts

Plant sample	Extract	100 µg/mL (%)	250 µg/mL (%)	500 µg/mL (%)	1000 µg/mL (%)	IC_{50} (µg/mL)
Leaf	EtOH	24.6 ± 1.5	41.8 ± 2.1	58.9 ± 2.6	71.4 ± 2.9	162.9 ± 8.4
	Aq	12.4 ± 1.1	21.7 ± 1.8	30.2 ± 2.1	41.3 ± 2.5	318.3 ± 12.7
Stem	EtOH	19.3 ± 1.4	33.5 ± 2.0	49.1 ± 2.4	64.2 ± 3.1	205.1 ± 9.6
	Aq	8.2 ± 0.9	16.5 ± 1.4	25.8 ± 2.0	34.7 ± 2.3	>500
Root	EtOH	14.1 ± 1.2	26.8 ± 1.9	39.7 ± 2.2	52.6 ± 2.7	275.8 ± 11.9
	Aq	6.7 ± 0.8	13.4 ± 1.1	21.9 ± 1.7	29.8 ± 2.1	>500
Acarbose (Std)		46.2 ± 2.1	65.8 ± 2.5	81.4 ± 2.2	92.8 ± 1.2	34.5 ± 1.8

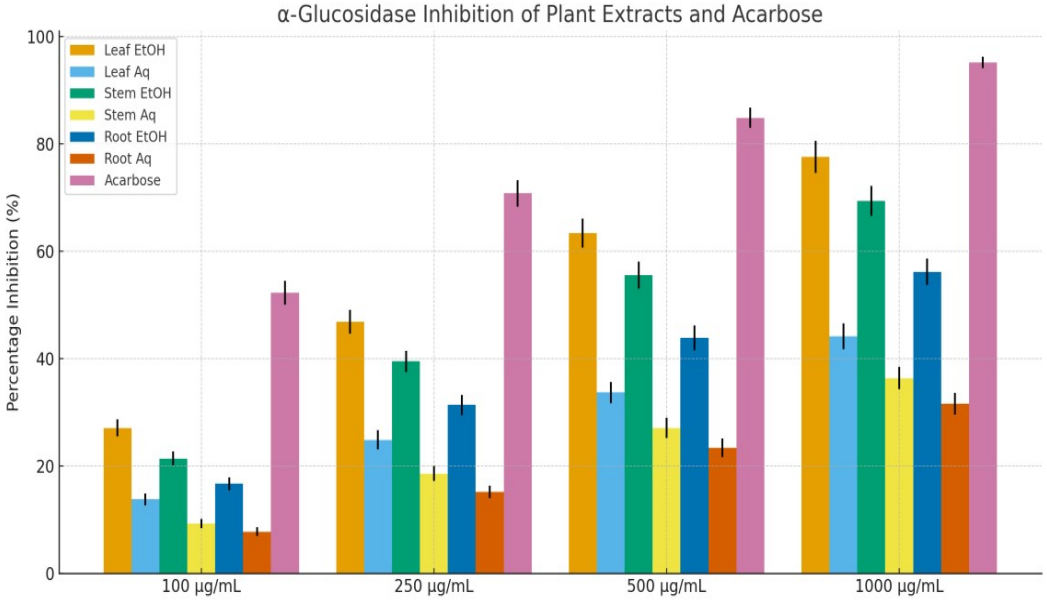


In vitro α-Glucosidase Inhibition

All extracts of *Tinospora cordifolia* demonstrated concentration-dependent inhibition of α-glucosidase, with ethanol extracts showing greater potency than aqueous extracts ((Table 3 and Fig 2). The ethanol leaf extract showed the highest inhibition (77.6 ± 3.0% at 1000 µg/mL) with an IC_{50} of 141.5 ± 7.8 µg/mL, followed by ethanol stem extract (69.4 ± 2.8%; IC_{50} = 192.7 ± 9.1 µg/mL). Root ethanol extract showed moderate activity (56.2 ± 2.5% at 1000 µg/mL; IC_{50} = 243.6 ± 10.2 µg/mL). Aqueous extracts were weaker inhibitors, with maximum inhibition ranging from 25–44% at 1000 µg/mL and IC_{50} values above 300 µg/mL. Acarbose, the positive control, produced strong inhibition with 95.2 ± 1.1% at 1000 µg/mL and an IC_{50} of 28.7 ± 1.5 µg/mL.

Table 3: α-Glucosidase Inhibition (%) of *T. cordifolia* extracts

Plant sample	Extract	100 µg/mL (%)	250 µg/mL (%)	500 µg/mL (%)	1000 µg/mL (%)	IC_{50} (µg/mL)
Leaf	EtOH	27.1 ± 1.6	46.9 ± 2.2	63.4 ± 2.7	77.6 ± 3.0	141.5 ± 7.8
	Aq	13.8 ± 1.1	24.9 ± 1.8	33.7 ± 2.0	44.2 ± 2.4	327.5 ± 13.4
Stem	EtOH	21.4 ± 1.3	39.5 ± 2.0	55.6 ± 2.5	69.4 ± 2.8	192.7 ± 9.1
	Aq	9.3 ± 0.9	18.6 ± 1.4	27.1 ± 1.9	36.4 ± 2.1	>500
Root	EtOH	16.7 ± 1.2	31.4 ± 1.9	43.9 ± 2.3	56.2 ± 2.5	243.6 ± 10.2
	Aq	7.8 ± 0.8	15.2 ± 1.2	23.4 ± 1.7	31.6 ± 2.0	>500
Acarbose (Std)		52.3 ± 2.2	70.8 ± 2.5	84.9 ± 1.9	95.2 ± 1.1	28.7 ± 1.5



Discussion

The results of this study provide novel insights into the phytochemical composition and *in vitro* antidiabetic activity of *Tinospora cordifolia* extracts. Extraction yields were higher for ethanol compared to aqueous solvents, consistent with the ability of hydroethanol to solubilize a broader range of semi-polar metabolites including flavonoids, alkaloids, and terpenoids (Anjum et al., 2023). The relatively lower yield of aqueous extracts suggests selective recovery of polar constituents such as tannins, saponins, and reducing sugars, which was supported by the phytochemical screening results. Similar solvent-dependent differences have been reported in *T. cordifolia* and other antidiabetic plants (Bansal et al., 2020).

Qualitative phytochemical assays confirmed the presence of diverse bioactive classes across plant parts, with notable differences. Ethanol leaf extracts contained abundant flavonoids, which are well-known for their antioxidant and enzyme-inhibitory activities (Shahidi & Ambigaipalan, 2015). The presence of alkaloids and terpenoids in stem and root extracts further indicates potential bioactivity. Aqueous extracts were richer in tannins and saponins, compounds with reported antidiabetic effects through inhibition of intestinal glucose absorption and modulation of insulin signaling (Kumar et al., 2021). These phytochemical differences likely contributed to the observed variability in enzyme inhibition.

In the α-amylase inhibition assay, ethanol extracts particularly from leaves exhibited superior activity, with IC_{50} values substantially lower than aqueous extracts. This suggests that semi-polar metabolites such as flavonoids and terpenoids may play a key role in modulating α-amylase activity. The findings align with earlier studies reporting that flavonoid-rich extracts inhibit α-amylase by binding to its active site and interfering with starch hydrolysis (Shobana et al., 2009, Ramanathan et al., 2014 and Selvi and Yogananth, 2016). While root and stem ethanol extracts also showed activity, their potency was comparatively weaker, indicating differential distribution of bioactive metabolites across plant parts.

The α-glucosidase inhibition results were consistent with α-amylase findings. Ethanol leaf extract again showed the strongest inhibition, with an IC_{50} of 141.5 µg/mL, close to reported values for other potent plant-derived inhibitors (Sharma et al., 2021). Aqueous extracts showed modest

inhibition, supporting the view that non-polar to semi-polar compounds contribute predominantly to α-glucosidase inhibition. The potent activity of ethanol leaf extracts suggests that *T. cordifolia* leaves, though less studied than stems, may represent a valuable source of enzyme inhibitors.

Comparisons with acarbose, the positive control, highlight the relative potency of the extracts. Although acarbose showed much lower IC_{50} values, the plant extracts demonstrated significant activity, underscoring their potential as natural alternatives or adjuncts. Importantly, plant-derived inhibitors may exert fewer gastrointestinal side effects due to milder inhibitory action and the presence of synergistic phytochemicals (Chiasson et al., 2002).

Conclusion

This study demonstrates that *Tinospora cordifolia* possesses significant in vitro antidiabetic potential, with ethanol leaf extract showing the strongest α-amylase and α-glucosidase inhibitory activity. Preliminary phytochemical screening confirmed the presence of multiple bioactive classes that may contribute synergistically to enzyme inhibition. These findings validate traditional claims regarding the antidiabetic use of *T. cordifolia* and highlight the need for further research on bioactive compound isolation and in vivo validation.

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