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Research Article

Separation and Identification of Anti-diabetic compounds in *Tinospora cordifolia* extract and Ayurvedic formulation *Guduchi Satva* by GCMS and FTIR study with Subsequent Evaluation of *in-vitro* Hypoglycemic Potential

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ABSTRACT

Tinospora cordifolia stems (*T. cordifolia*), commonly known as Guduchi, is an effective Ayurveda drug used for diabetes management and various other disorders and ailments. *Guduchi satva* is classical *Ayurvedic* medicine being used for the treatment of diabetes and a variety of other disorders. The present study was conceded to determine the aqueous extract of *T. cordifolia* and *Guduchi satva* formulation for their relative identification of secondary metabolites using physicochemical parameters, fourier-transform infrared spectroscopy (FTIR) fingerprint, and GCMS profiling. *In vitro* enzyme inhibition assay on α amylase and α glucosidase were also evaluated for both the extracts, in which *Guduchi satva* possess enhanced inhibition of enzymatic activity over the aqueous extract of *T. cordifolia*. The phytochemical and FTIR investigations were confirmed for the presence of alkaloids, tannins, carbohydrates, flavonoids, steroids, aromatic, hydroxy, and nitrogen-containing compounds. The gas chromatography mass spectrometry (GCMS) analysis revealed the existence of stigmasterol (1.2%), stigmastane3, 6 dione,-5a (2.2%), betulin (8.4%), eicosanoic acid (1.09%), glycerol 1 palmitate (20.72%), ascorbyl palmitate (0.92%) and triamcinolone acetonide (0.89%) in both the extracts and effective in diabetes management.

Introduction

Besides basic needs, healthy and ailment-free life is the foremost need for humans, and natural products play a key role in managing a healthy life. Bharat has a unique blessing in biodiversity, and vast knowledge provides a base of the traditional system of medicine which is still widely practiced. Indian Materia medica gives immense information on traditional uses and folklore practices of natural products with significant therapeutic value.[1] The use of the plant in the *Ayurvedic* system of medicine was dating from 1000 BC. According to the World Health Organization, about 80% of the world's population in developing countries depend on herbal therapy and medicines to meet their basic health needs.[2]

Diabetes is a clinical condition characterized by risen blood sugar levels, varied lipid, carbohydrate and protein metabolism.[3] Diabetes mellitus (DM) type-2 (Insulin independent), the most common type of diabetes, accounts for over 90% of total cases in developing and developed countries.[4] Symptoms include elevated blood sugar level, frequent urination, excessive thirst; extreme hunger with loss of weight; extreme weakness and tiredness, mood changes, etc.[5]

T. cordifolia (Willd.) Miers stems, usually known as Guduchi, are used in several formulations of Ayurveda and modern medicine. The stem of *T. cordifolia* was reported to reconcile its anti-diabetic effect through reducing oxidative stress, regulating blood glucose by improving insulin secretion and also stopping gluconeogenesis and

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glycogenolysis. Various secondary metabolites of *Guduchi* have been reported to possess anti-diabetic activity.[6] Palmatine, jatrorrhizine, and magnoflorine (isoquinoline alkaloid) isolated from the stem have been identified both *in vitro* and *in vivo* for insulin-mimicking and insulin-secreting effect. [7]

The present experiments were initiated to compare the presence of secondary metabolites in *T. cordifolia* and *Guduchi satva* by gas chromatography-mass spectrometry (GCMS) and FTIR with subsequent evaluation of hypoglycaemic potential by *in vitro* enzyme inhibition assay.

Material and Methods

Collection, Identification and Extraction *T. Cordifolia* Stem

The plant material was collected from the forest region of Jhallana, Jaipur (Raj.) and identified from the Department of Botany, Rajasthan University, Jaipur via letter no. Bot./2016/5080 with authentication no. "RUBL21163" as *T. cordifolia*, Family-Menispermaceae. Long and thick stems were thoroughly washed, cut into small pieces, shade dried and reduced to a coarse powder and finally passed through mesh size 14#. The powdered drug was subjected to cold maceration extraction using de-mineralized water. The extract was concentrated under reduced temperature (50°C) and pressure (10 mbar) to avoid loss of secondary metabolites.[8]

Chemical and Reagents

All the chemical reagents and enzymes were procured from Sigma Aldrich, Merck, JT baker, and TCI to maintain a high level of accuracy and precision during analysis.

Formulation of *Guduchi satva*

Guduchi satva was prepared to process fresh T. cordifolia stems that were thoroughly washed with potable water, chopped into small pieces of 1–2 inches, pounded completely into course slimy mass, and soaked in water overnight. The material was thoroughly macerated the next day and filtered through four folded muslin cloths. The filtrate or extracted liquid was kept for sedimentation up to 5 hours, then supernatant liquid decanted carefully, and the starchy material left into the bottom was scrapped into the tray. This starchy material was then air-dried using a hot air oven with blowing air at 40°C, which took 4–5 hour and finally obtained white color crystalline powder which was stored properly in an airtight jar for further evaluation.[9]

Preliminary Phytochemical Studies

Qualitative chemical tests for alkaloids, saponins, flavonoids, tannins, steroids, carbohydrates, proteins, and glycosides were carried out to ensure the existence and type of secondary metabolites in the aqueous extract of *T. cordifolia* and *Guduchi satva*.[10]

Physio-chemical Studies

Quality control tests were performed on *T. cordifolia* raw herb powder and *Guduchi satva* for the standardization. The selected parameters were total ash, acid insoluble ash, loss on drying, water-soluble ash, water and alcohol soluble extractive performed in triplicate.[11]

Infra-red Fingerprint Analysis

Fourier transform infrared spectrophotometer (FTIR) is a powerful technique for identifying the types of functional groups present in chemical compounds. Dried extract of *T. cordifolia* and *Guduchi satva* were analyzed using FTIR fingerprinting. The spectrum was generated by using Thermo Scientific, Nicolet iS 10 FTIR spectrometer. The iTR basic technique, also known as attenuated total reflectance (ATR), was used to mount around 5 mg of powdered extract mass over the zinc selenium (ZnS) crystal. Thermo Insight software was used to acquire the IR spectrum of the extract, which was scanned over a range of 4000 to 400 cm^{-1}.[12]

Gas Chromatography and Mass Spectrometry Analysis

Aqueous extract of *T. cordifolia* and *Guduchi satva* were dissolved in a small amount of dimethyl sulphoxide and makeup with methanol in 1 mg/mL concentration. The solution were filtered by 0.5 µm pore size syringe filter, and analysis was performed on GCMS technique. The GCMS instrument was of Thermo Scientific with Trace 1300 GC coupled with a TSQ 8000 equipped with fused silica capillary column DB-5MS (Length 30m; Thickness 0.25 µm and Internal diameter 0.25 mm;). 1 µL of filtered solution injected with 99.9999% pure Helium carrier gas in a flow rate of 1 mL/min. The temperature of the injector kept at 250°C; ion-source at 280°C. The temperature of oven was maintained at 70°C (isothermal for 5 minutes), with an increase of 10°C/min, to 200°C and hold for 10 minutes, then 5°C per min to 280°C, and hold for 12 minutes. The MS was operated on positive electronic ionization with 70eV energy. The solvent delay was programmed at 2.5 minutes with a scan-interval of 0.5 seconds and fragments from m/z 40 to 500 Da. The relative percentage amount of each compound was considered by comparing its average peak area in proportion to the total areas. Separated compounds were identified by using the NIST (National Institute Standard and Technology) 2.0 library database.[13]

In vitro Inhibition of α-amylase Activity

The α-amylase inhibition assay was determined by preparing 1 U/mL of α-amylase mixed in the buffer of 20 mM sodium phosphate with 6.7 mM sodium chloride (pH 6.9) and boiling in a water bath for 15 minutes. The same amount of 96 mM 3, 5-dinitro salicylic acid (DNS), and sodium-potassium tartrate tetrahydrate solution were used to prepare the colorimetric solution. 1000 μL starch solution (0.5% w/v) was mixed with incremental concentration of standard Acarbose (10–100 μg/mL),

aqueous extract of *T. cordifolia* stem (50–300 µg/mL) and *Guduchi satva* (25–250 µg/mL). The α-amylase solution 1000 µL was added to each tube and incubated at 25°C for 3 minutes. The incubated enzyme mixture was added with 1000 µL of DNS reagent and followed to heat for 15 minutes on a boiling water bath. Make up the volume with double distilled water and absorbance was determined at 540 nm using a UV-Visible spectrophotometer. The inhibitory concentration at 50% (IC_{50}) was calculated using the linear curve equation obtained by the calibration graph of concentration versus percentage inhibition. The percentage inhibition was calculated using the below formula.[14]

% Inhibition (at each concentration) = (Control absorbance – Test absorbance) × 100 / Control absorbance

In vitro Inhibition of α-glucosidase Activity

The enzyme inhibition assay was measured by using a mixture of α-glucosidase enzyme (1 U/mL) 100 µL of solution with 100 µL of phosphate buffer (pH 7.0). Above solution was added with incremental concentration, 100 µL of Acarbose (0.5–8 µg/mL), aqueous extract of *T. cordifolia* (30–80 µg/mL) and *Guduchi satva* (20–60 µg/mL). The mixture was dissolved and incubated at 37°C for 60 minutes in maltose solution. The α-glucosidase action on maltose was stopped by kept the reaction mixture in boiling water for 2 minutes and cooled. The amount of glucose released was measured by the addition of 2 mL of glucose reagent in the reaction mixture and absorbance was measured at 540 nm using a UV-visible spectrophotometer. The inhibitory concentration at 50% (IC_{50}) was calculated using the linear curve equation obtained by the calibration graph of concentration versus percentage inhibition. The percentage inhibition was calculated using the below formula.[14]

% Inhibition (at each concentration) = (Control absorbance – Test absorbance) x 100 / Control absorbance

Table 1: Phytochemical analysis of aqueous extract of *T. cordifolia* and *Guduchi satva*

Chemical group	*Aqueous extract of T. cordifolia*	*Guduchi satva*
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	-
Glycosides	+	-
Steroids	-	-
Carbohydrates	+	+
Proteins	-	-

'+' Present, '-' Absent

Table 2: Physicochemical parameters for *T. cordifolia* raw powder and *Guduchi satva*

Parameters	*T. cordifolia raw powder % w/w*	*Guduchi satva % w/w*
Loss on drying	7.46 ±0.128	5.409±0.091
Total ash	7.54 ±0.018	2.27±0.061
Acid insoluble ash	1.65±0.011	1.82±0.039
Water soluble ash	1.52±0.011	0.91±0.039
Water soluble extractive	9.64±0.030	2.939±0.030
Alcohol soluble extractive	5.72±0.366	3.861±0.366

Each value represents the mean ± SEM, N=3, unit- % w/w

Results and Discussion

Phytochemical Screening

Preliminary qualitative phytochemical tests were performed on aqueous extract of *T. cordifolia* and *Guduchi satva*. Qualitative observations in negative and positive were shown in Table 1.

Physicochemical Parameters

Various physicochemical parameters were determined for *T. cordifolia* raw powder and *Guduchi satva*, the result is summarized in Table 2.

GCMS analysis

Aqueous extract of *T. cordifolia* and *Guduchi satva* were subjected for GCMS scanning and separation of chemical compounds presented in Tables 3 and 4.

Identification and Separation of Chemical Compounds in Aqueous Extract of *T. cordifolia* and *Guduchi satva*

The compounds with their retention time (RT), area percentages, molecular formula, and molecular weight are presented in Tables 3 and 4, with separation patterns in chromatograms in Figs 1 and 2. Fourteen and seventeen

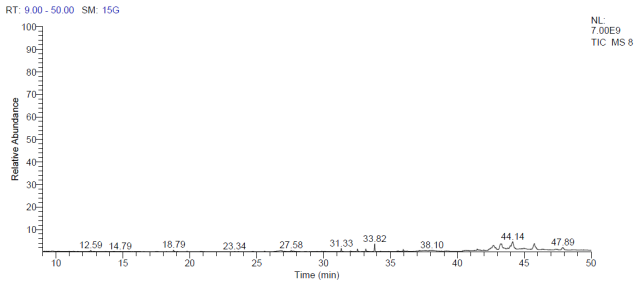

Fig. 1: GCMS scan chromatogram of aqueous extract of *T. cordifolia*

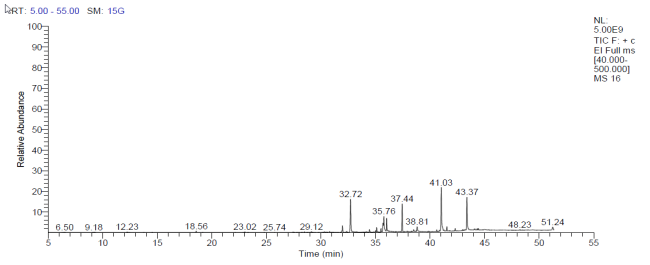

Fig. 2: GCMS scan chromatogram of *Guduchi satva*

Table 3: Identification and separation of chemical compounds in aqueous extract of *T. cordifolia*-

S. No.	*R. time*	*Name of compound*	*Molecular formula*	*Molecular weight*	*Peak area (%)*	*Reported bio-activity*
1.	3.08	Methoxyacetic acid, pentyl ester	$C_9H_{18}O_3$	174	2.3	Antimicrobial[15]
2.	26.73	Melezitose	$C_{18}H_{32}O_{16}$	504	18.4	-
3.	26.82	d Mannose	$C_6H_{12}O_6$	180	24.4	-
4.	31.33	Nootkaton11,12 epoxide	$C_{15}H_{22}O_2$	234	1.4	-
5.	32.54	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1	Antimicrobial and Antiinflammatory[16,17]
6.	33.16	Estafiatin	$C_{15}H_{18}O_3$	246	1.3	Immuno-modulatory[16]
7.	33.82	2,6,8Trimethylbicyclo[4.2.0]oct2ene1,8diol	$C_{15}H_{26}O$	222	3.4	-
8.	38.11	Ethyl isoallocholate	$C_{26}H_{44}O_5$	436	1.5	Antimicrobial[18]
9.	40.63	Stigmasterol	$C_{29}H_{48}O$	412	1.2	Antiperoxidative and hypoglycemic effects [19]
10.	41.49	Sarreroside	$C_{30}H_{42}O_{10}$	562	1.1	-
11.	42.70	Betulin	$C_{30}H_{50}O_2$	442	8.4	Antimalarial, anti-inflammatory and anti viral[20,21]
12.	43.27	çSitosterol	$C_{29}H_{50}O$	414	8.7	-
13.	43.97	Stigmastane3,6dione, (5à)	$C_{29}H_{48}O_2$	428	2.2	Antidiabetic, antioxidant anticancer [22,23]
14.	47.89	1Heptatriacotanol	$C_{37}H_{76}O$	536	3.5	antioxidant, Anti-inflammatory Hypocholesterolemic, antimicrobial, and anticancer Properties [24,25]

compounds were separated and identified in aqueous extract of *T. cordifolia* and *Guduchi satva,* respectively. The major fourteen components present in the aqueous extract of *T. cordifolia* stem were identified from the literature, mostly compounds are antimicrobial and anti-inflammatory and very few compounds identified as treatment of diabetes.

While in the case of *Guduchi satva,* out of seventeen compounds, five compounds exhibited antimicrobial and anti-inflammatory properties, four possessed anti-diabetic action and involved their application in the treatment of post diabetes problems such as neuropathy, macular edema, and hypertension associated. The literature revealed that other compounds possess immune-modulatory, anticancer, antioxidant, and anti-thyroid potential. A study of literature and compound identification by GCMS analysis revealed the greater potential of Guduchi satva for hypoglycemic action and other diabetes associated problems, and aqueous *T. cordifolia* relatively has a moderate or low effect on diabetes.

FTIR Analysis

In both extracts, functional groups were identified. The presence of C-H and H-O stretch indicated the presence of alcohol group attached to a carbon chain in aqueous extract of *T. cordifolia* found with low-intensity valleys observed at 2956 and 2825 cm^{-1}. Strong valleys confirm C-C stretch and N-O stretch of aromatic cyclic groups at 1566, 1401, and 1277cm^{-1}. Other valleys at 827, 664 and 559cm^{-1} were identified in the fingerprint region, which indicates the compounds of long aliphatic chain and aldehyde groups. In the second sample of *Guduchi satva*, a valley at 3307 and 2931cm^{-1} indicated the presence of O-H and C-H stretch in aliphatic chain of compounds, medium intensity valley 1664cm^{-1} was observed, thus confirmed the presence of C=O ketone group attached with carbon chain and 1337, 1149 and 1076 cm^{-1} found and indicated the presence of aromatic C-O, C-C, C-H bending and N-O stretch. 996, 926, 860, 763, and 706cm^{-1} indicated the presence of long aliphatic C-C and C=C stretch. The observed valleys identified as the presence of sugar, nitro-based aromatic chain, aliphatic, keto, and aldehyde sugar compounds in aqueous extract of *T. cordifolia,* while *Guduchi satva* confirmed the presence of long-chain unsaturated and saturated aliphatic compounds, aromatic and ketone groups mainly for keto groups and aromatic ketones. A strong fingerprint region in both of samples indicated the availability of aliphatic, nitro, and keto group-based compounds. FTIR spectrum of each extract given in Figs 3 and 4.

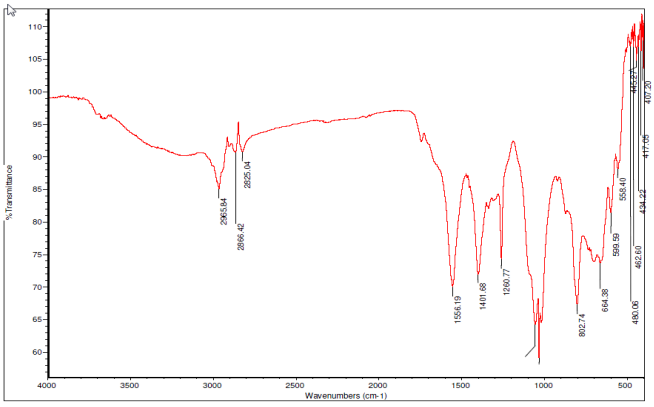

Fig. 3: FTIR spectrum of aqueous extract of *T. cordifolia* stem

Table 4: Separation and identification of chemical compounds in *Guduchi satva*-

S. No.	*R. Time*	*Name of compound*	*Molecular formula*	*Molecular Weight*	*Peak area (%)*	*Reported bio-activity*
1.	32.72	n Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.73	Anti-inflammatory, Antioxidant, hypocholesterolemic[17]
2.	34.16	9 Hexadecenoic Acid	$C_{16}H_{30}O_2$	254	0.39	-
3.	34.45	Eicosanoic acid	$C_{20}H_{40}O_2$	312	1.09	Pathobiology of hypertension and type 1 and type 2 DM. [26]
4.	35.10	9 Octadecenoic acid (Z), methyl ester	$C_{19}H_{36}O_2$	296	1.51	Antibacterial [17]
5.	35.69	9,12 Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	3.89	Antibacterial [17]
6.	35.76	Oleic Acid	$C_{18}H_{34}O_2$	282	8.18 + 2.65	Antibacterial [17]
7.	36.02	Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.57	-
8.	37.44	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	402	8.54	Insecticidal[27]
9.	38.49	Z5Methyl6heneicosen11one	$C_{22}H_{42}O$	322	1.14	-
10.	40.61	Heptanoic acid, docosyl ester	$C_{29}H_{58}O_2$	438	0.56	-
11.	41.03	Glycerol 1 palmitate	$C_{19}H_{38}O_4$	330	20.72	Effective against adepocyte insulin resistance[28]
12.	41.28	Tricosanoic acid	$C_{23}H_{46}O_2$	354	0.47	-
13.	42.30	Ascorbyl Palmitate (L Ascorbic acid 6 palmitate)	$C_{22}H_{38}O_7$	414	0.92	Inhibition of oxidative stress associated with diabetic neuropathy[28]
14.	43.37	Octadecanoic acid, 2,3dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	14.43	-
15.	44.09	Triamcinolone Acetonide	$C_{24}H_{31}FO_6$	434	0.89	Inflammatory in diabetic macular edema [29]
16.	44.33	Docosanoic acid, 1,2,3 propanetriyl Ester	$C_{69}H_{134}O_6$	1058	1.48	Anti-inflammatory, hypercholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistamine, anti-eczemic, anti-acne, 5reductase inhibitor, antiandrogenic, antianti-coronary. [30]
17.	51.24	Campesterol	$C_{28}H_{48}O$	400	1.84	Antioxidant[31]

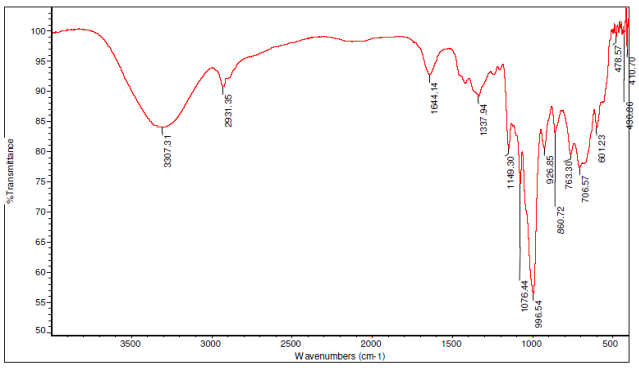

Fig. 4: FTIR spectrum of *Guduchi satva*

In vitro α Amylase and α Glucosidase Inhibitory Activity

T. cordifolia is a *Rasayana* drug in Ayurveda that maintains a balance of *Vata*, *Pitta* and *Kapha* altogether and is considered as a highly potent drug for incurable diseases and ailments. A series of biological evaluations of plant extracts and their classical formulations provided practically authenticated data against diabetes and its associated problems. Plant extracts have been used directly or indirectly for the preparation of many modern medicines. The *T. cordifolia* stem ethyl acetate, chloroform, dichloromethane, and hexane extracts were reported to inhibit the salivary amylase, pancreatic amylase, and glucosidase. Thus decreasing the postprandial blood glucose concentration and finds important application for DM treatment.[32] *In vitro* enzyme inhibition assays were developed to inhibit enzyme action on the peripheral breakdown of higher sugar molecules to reducing sugars (glucose and fructose); this mechanism leads to treatment of non-insulin-dependent diabetes. Several compounds have been reported for their enzyme inhibition potential, and *T. cordifolia* and *Guduchi satva* were already claimed to have hypoglycaemic properties.

Guduchi satva was identified to be more effective than aqueous extract of *T. cordifolia* in α-amylase and α-glucosidase enzyme inhibition, activity correlated with bioactive compounds identified through GCMS.

Table 5: α-Amylase inhibitory study of aqueous extract of *T. cordifolia* and *Guduchi satva*-

Sample	*Conc. (µg/mL)*	*Average ± SEM*	*IC_{50} (µg/mL)*
Acarbose	100	76.87 ± 0.87	59.43
	80	63.15 ± 0.86	
	60	51.38 ± 0.67	
	40	38.57 ± 0.41	
	20	21.24 ± 0.67	
	10	17.53 ± 1.17	
Aqueous extract of *T. cordifolia*	300	55.18 ± 0.51	275.39
	250	47.70 ± 0.96	
	200	32.23 ± 0.55	
	150	23.17 ± 0.85	
	100	16.63 ± 0.79	
	50	6.99 ± 0.11	
Guduchi satva	250	67.00 ± 1.28	183.26
	200	52.25 ± 0.50	
	150	43.19 ± 1.32	
	100	29.27 ± 0.58	
	50	18.41 ± 0.45	
	25	11.33 ± 0.78	

Each value represents the mean ± SEM, N=3

Table 6: Alpha-glucosidase inhibitory study of aqueous extract of *T. cordifolia* stem and *Guduchi satva*-

Sample	*Conc. (µg/mL)*	*Average ± SEM*	*IC_{50} (µg/mL)*
Acarbose	8	81.48 ± 0.69	4.01
	6	65.38 ± 0.70	
	4	53.51 ± 0.57	
	2	42.77 ± 0.46	
	1	23.83 ± 0.77	
	0.5	11.47 ± 0.47	
Aqueous extract of *T. cordifolia*	80	57.39 ± 0.67	73.65
	70	45.84 ± 0.26	
	60	36.41 ± 0.69	
	50	23.17 ±0.85	
	40	17.32 ±0.96	
	30	5.79 ± 0.18	
Guduchi satva	60	75.18 ± 0.81	42.52
	50	61.33 ± 0.53	
	40	47.46 ± 0.71	
	30	35.43 ± 0.55	
	20	25.71 ± 0.50	
	10	14.71 ± 0.50	

Each value represents the mean ± SEM, N=3

The reported claims of *Guduchi satva* as an anti-diabetic formulation support the obtained results in the present investigation.[33]

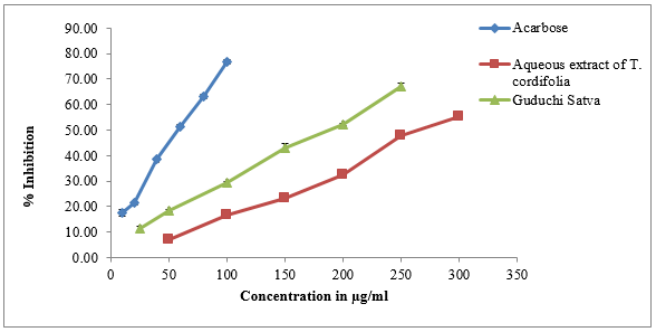

Fig. 5: Graphical presentation of alpha amylase inhibitory activity of standard, aqueous extract of *T. cordifolia* stem and *Guduchi satva*.

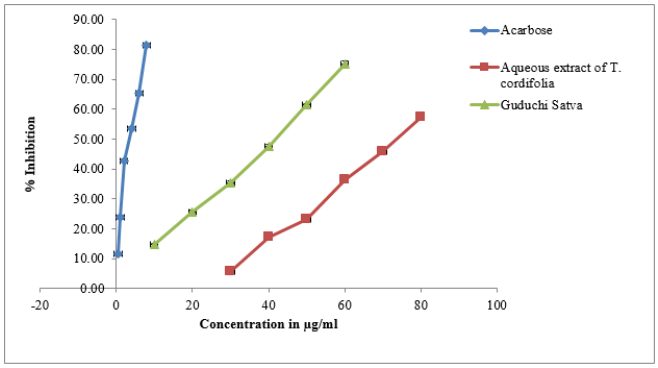

Fig. 6: Graphical presentation of alpha glucosidase inhibitory activity of standard, *T. cordifolia* and *Guduchi satva*.

The GCMS analysis revealed water-soluble compounds were present in aqueous extract *T. cordifolia;* this may attribute for their low potency in enzyme inhibition. *Guduchi satva* formulation is a fraction of water-insoluble, thick and starchy material consisting of non-polar constituents, which may contribute by inhibiting the enzyme activity and resulted in potent inhibition concentration with lesser IC_{50} concentrations than aqueous extract of *T. cordifolia*. Results and graphical data of activity are presented in Tables 5 and 6 with Figs 5 and 6.

Conclusion

The present study was assessed to confirm the traditional indication of anti-diabetic and diabetes management claims of *T. cordifolia* and *Guduchi satva* along with the GCMS and FTIR characterization of bioactive compounds. *T. cordifolia* was found to be effective for the management of diabetes. Hence, it finds its place in developing new chemical entities as antidiabetics. To summarise the biological and chemical findings, the analysis will serve as a baseline for further research into the drug, including clinical trials, toxicity tests, and the discovery of newer and safer compounds with much better anti-diabetic efficacy.

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