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Evaluation Of Medicinal Properties Of *Tinospora Cordifolia*

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Abstract: Herbal medicines represent one of the most important fields of traditional medicine all over the world. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. *Tinospora cordifolia* is a popular medicinal plant which is used in several traditional medicines to cure various diseases such as jaundice, rheumatism, urinary disorder, skin diseases, diabetes, anemia, inflammation, allergic condition, anti-periodic, radio protective properties, etc. In the present study an attempt has been made to evaluate the phytochemical constituents (phenols, tannins, glycosides, steroids, alkaloids, flavonoids, terpenoids, amino acids, protein and carbohydrates) present in *Tinospora cordifolia* using standard methods. Separation of plant pigments and secondary metabolites was done using the quantitative methods such as TLC and HPTLC. Antioxidant, Anti diabetic, anti-inflammatory activity was checked using different solvents.

Keywords: *Tinospora cordifolia,* phytoconstituents, secondary metabolites, TLC, HPTLC, anti- diabetic, anti- inflammatory.

Introduction: The chemical compositions of herbs can vary due to different factors, leading to varying effects of herbal remedies on individuals. Traditional medicine, deeply rooted in history and cultural beliefs, offers diverse practices used for maintaining health and treating various ailments. The World Health Organization actively contributes to establishing guidelines for researching and evaluating traditional medicine's effectiveness. (Fabio Firenzuoli *et al.,* 2007). Approximately 80% of the global population, particularly in developing nations, relies on herbal medicines for primary healthcare due to their safety, efficacy, cultural acceptance, and minimal side effects. Historical records reveal extensive use of herbal remedies for age-related ailments and conditions where modern medicine may offer limited solutions. (Thillaivanan.*S et al.,* 2014). Despite a decline in usage after the advent of synthetic compounds, medicinal plants remain significant in developing therapeutic agents. Over 1.5 million traditional medicinal practitioners worldwide employ herbal remedies for preventive, promotional, and curative purposes, attracting interest from professionals and scientists across disciplines. This study focuses on *Tinospora cordifolia*, commonly known as Giloy, a vital component of Indian Ayurveda recognized for its immunity-boosting properties during the pandemic. *Tinospora cordifolia* exhibits diverse medicinal effects such as anti-inflammatory, anti-diabetic, antimicrobial, analgesic, and immunomodulatory properties. Phytochemical analysis reveals the presence of alkaloids, flavonoids, and steroids, contributing to its therapeutic potential

(Avnish K. Upadhyays 2010). Chromatographic and spectral fingerprint analysis is crucial for ensuring the quality control of complex herbal medicines. Techniques like Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) provide efficient, rapid, reliable, and reproducible means of identifying phytochemical constituents and detecting marker compounds in plant samples. Standardizing chromatographic procedures for plants like *Tinospora cordifolia* is essential for guaranteeing their quality, safety, and efficacy (Acharya Balkrishna *et al.,* 2016). Antioxidants play a vital role in combating oxidative stress caused by environmental factors, diseases, inflammation, and aging. Various herbs and botanicals, including *Tinospora cordifolia*, contain antioxidants that scavenge free radicals and help prevent cell damage. Moreover, *Tinospora cordifolia's* potential against inflammation-associated anaemia (AI) remains unexplored. Given its traditional use for anti-inflammatory and immunomodulatory purposes, evaluating its effectiveness against AI is crucial for identifying safe and effective therapies.

Material and methods:

Plant materials used: Dried *Tinospora cordifolia* stems were acquired from the Ayurvedic market in Kalyan. The plant material was authenticated at Agharkar Laboratories in Pune.

Extract preparation: The *Tinospora cordifolia* stems were finely powdered and dissolved in various solvents (chloroform, ethanol, methanol, acetone, distilled water) at a ratio of 10 g of sample per 200 ml of solvent. The mixture was stirred at 100 rpm for 8-10 hours. The following day, the solvent was filtered, and the filtrate was used for qualitative and quantitative analysis of the plant materials.

Phytochemical analysis: The qualitative analysis of the phytochemicals in *T. cordifolia* stems revealed a wide array of phytochemicals, including alkaloids, steroids, flavonoids, carbohydrates, phenols, and others (Shwetha R.J *et al.,* 2016).

Tannin test: 1ml gelatin sol is mixed with 1ml of plant sample. Presence of white precipitate indicates the presence of tannins.

Glycoside test: 2ml chloroform, acetic acid, concentrated sulphuric acid is mixed with 2ml of plant sample. Appearance of green layer indicates the presence of glycosides.

Alkaloid test (Wagner test): Mixing 2 drops of Wagner reagent with 2 ml of the extract can indicate the presence of alkaloids if a red colour appears.

Phenol test: Mixing 1 ml of the sample with a few drops of 10% ferric chloride can indicate the presence of phenol if a black colour appears.

Steroid test: Mixing 2 ml of chloroform and 2 ml of concentrated H2SO4 with 2 ml of the plant sample can confirm the presence of steroids if a brown ring forms.

Terpenoids test: Salkowski test: 2ml chloroform, 3ml concentrated sulphuric acid mixed with 5ml of plant sample. Formation of reddish- brown colour in interface indicates the presence of terpenoids.

Flavonoid test: Adding 2-3 drops of sodium hydroxide to 2 ml of the extract and then gradually adding a few drops of diluted HCL can indicate the presence of flavonoids if the colour changes from dark yellow to colourless.

Amino Acid Test (Ninhydrin Test): Mixing 1 ml of the plant sample with a few drops of ninhydrin reagent and observing the colour change in a boiling water bath can reveal the presence of amino acids.

Protein test (Burette test): Adding 2 ml of sodium hydroxide and 5-6 drops of copper sulphate solution to the sample and observing a bluish-violet colour after shaking the tube thoroughly can indicate the presence of protein.

Carbohydrate Test (Molisch Test): Adding 1 ml of concentrated H2SO4 and 1 ml of Molisch reagent to 1 ml of the plant sample can indicate the presence of carbohydrates if a purple ring forms.

Estimation of phenols: The amount of phenols in the plant extract was determined using the Folin-Ciocalteu redox-based colorimetric method. Standard Gallic acid solutions were prepared at concentrations of 1 mg/ml, 0.1 mg/ml, and 0.01 mg/ml. Various amounts of the standard Gallic acid solution were added to different tubes, ranging from 0.2 to 1.0 ml, resulting in concentrations of 0.002, 0.004, 0.006, 0.008, and 0.010. One millilitre of the unknown sample was added to tubes containing methanol, ethanol, and distilled water. The volume of each tube was adjusted to 5 ml, 1 ml of Folin-Ciocalteu reagent was added, and the mixture was shaken well. After 5 minutes of incubation, 7 ml of 7.5% Na2CO3 solution was added and mixed well. The mixture was then placed in the oven for 90 minutes, resulting in a dark blue colour. Approximately 3 ml of distilled water was added, and the absorbance at 660 nm was measured using a suitable calorimeter. The concentration of the unknown sample was calculated using the standard formula, which is based on the acid concentration on the X-axis and the absorbance on the Y-axis. The total phenolic content of the plant extracts is expressed as Gallic acid equivalent (GAE) per 100 g of dry weight others (Shwetha R.J *et al.,* 2016).

Estimation of flavonoids: The total flavonoid content was determined using the colorimetric aluminium chloride assay method. Solutions of quercetin dihydrate were prepared at concentrations of 1 mg/mL, 0.1 mg/mL, and 0.01 mg/mL. Quercetin standard solutions at concentrations of 0.02, 0.04, 0.06, 0.08, and 0.10 were pipetted into 5 separate tubes. Additionally, one millilitre of the unknown sample was pipetted into tubes containing methanol, ethanol, and distilled water. The total volume of each tube was adjusted to 1 ml with distilled water and 0.3 ml of 5% sodium nitrite solution was added. The mixture was then allowed to sit for 5 minutes before adding 0.3 ml of 10% aluminium chloride and 2 ml of 1M sodium hydroxide over a 6-minute period. Finally, 2.4 ml of distilled water was added and thoroughly mixed. The resulting solution appeared orange-yellow in colour. The absorbance at 510 nm was measured using a suitable blank in the calorimeter. A standard graph was prepared with quercetin concentration on the x-axis and absorbance on they-axis. The concentration of the unknown sample was then calculated using the formula. The data on total flavonoid content of polyhedral compositions are expressed in mg quercetin equivalent/100 g dry weight others (Shwetha R.J *et al.,* 2016).

Estimation of tannins: The total tannin content in the plant extract was determined using the Folin-Dennis colour imetric method, which relies on the non-stoichiometric oxidation of molecules containing phenolic hydroxyl groups. Tannic acid solutions at concentrations of 1 mg/ml, 0.1 mg/ml, and 0.01 mg/ml were prepared. Various aliquots of standard tannic acid solution, ranging from 0.2 to 1.0 ml (concentrations 0.002, 0.004, 0.006, 0.008, and 0.010), were pipetted into five different tubes. One millilitre of the unknown sample was pipetted into tubes containing three different solvents: methanol, ethanol, and distilled water, and the volume were adjusted to 1 ml using distilled water. Then, 1 ml of Folin-Denis reagent was added, and the contents were mixed well and incubated for 5 minutes in the dark. After 5 minutes, 7 ml of Na2CO3 was added, and the mixture was incubated for 90 minutes. The absorbance was measured at 660 nm using a suitable calorimeter. A standard chart was created based on tannic acid concentration on the X-axis and absorbance on the Y-axis, and the concentration of the unknown sample was calculated based on this standard chart. The data on the total phenolic content of plant extracts are expressed as mg tannic acid equivalent (TAE)/100 g dry weight others (Shwetha R.J *et al.,* 2016).

Thin Layer Chromatography: The extracted sample is used for TLC separation. Samples are transported to the TLC plate with the help of capillary tube. TLC plates were prepared at three different concentrations including toluene: acetone: formic acid (12:3:1.5 v/v), toluene: acetone: ethyl acetate (6:3:12 v/v), and ethyl Acetate: Methanol: water (7:6:1.5 v/v) ratio at room temperature. Dry the plate, observe the different bands in 366nm UV light and calculate the Rf value (Acharya Balkrishna *et al.,* 2016).

HPTLC fingerprinting: HPTLC fingerprinting of dry stem powder of *T. cordifolia* using the solvent system of toluene: acetone: formic acid (12: 3: 1.5 v/v), toluene: acetone: ethyl acetate (6: 3: 12 v/v) and ethyl acetate: methanol: water (7: 6: 1.5 v/v) using CAMAG HPTLC system consisting of linomat 5 spotting and scanner 3. The 0.2microliter sample was loaded on the TLC plate (coated with thin layer of silica gel F254 of thickness 0.2mm) in the form of bands using the linomat 5 applicator attached to HPTLC system which was

programmed with winCATS software. The chromatogram was developed in CAMAG twin through TLC chamber using the mentioned solvent systems. The developed chromatogram was scanned using CAMAG scanner at 256nm and 366nm. The bands obtained were visualized using the linomat visualizer (Acharya Balkrishna *et al.,* 2016).

Antioxidant:

Hydroxyl radical scavenging assay: The method of evaluating total antioxidant test using UV spectrophotometric method is widely used. The standard use for evaluating total antioxidant tests is ascorbic acid. Acids were added to test tubes at concentrations (0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml). Aliquots of ascorbic acid extract or standard solution. Add 0.4 ml of phosphate buffer (pH 7.4). Add 0.6ml of hydrogen peroxide solution (40mM). Incubate for 10 minutes at room temperature. Reagent blanks were prepared without using plant extract. Absorbance was measured at 230 nm for blank samples after incubation. Ascorbic acid is commonly used (Shwetha R.J *et al.,* 2016).

Phosphomolybdenum assay: Antioxidant capacity using the phosphomplybdate assay can be assessed by the reduction of molybdenum to green molybdenum complexes by antioxidant compounds present in plant extracts. 2. Ascorbic acid extract or standard solution (concentrations 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml) was added to the test tube. 3. Add 1ml of phosphomolybdenum reagent. Incubate in a boiling water bath at 95°C for 90 minutes. 4. A reagent blank was prepared without using plant extract. Absorbance was measured at 765 nm for blank samples. Ascorbic acid is commonly used (Shwetha R.J *et al.,* 2016).

Ferric Reducing Antioxidant Power assay: FRAP antioxidant activity analysis using raw plant extracts. Samples were added to test tubes to prepare extracts or standard solution aliquots with ascorbic acid concentrations (0.02, 0.04, 0.06, 0.08, 0.1 mg/ml). *Tinospora cordifolia* plant extract is taken in various concentrations. Adjust the sample volume of various concentrations to 1 ml using the same solvent used for sample extraction. Add 2.5ml of PBS to all test tubes. Mix the contents of each tube well. Then add 2.5 ml of 1% solution of iron cyanide and potassium cyanide to all samples of different concentrations. Afterwards, each reaction mixture is shaken well in a vortex shaker. Incubate the samples at 50°C for approximately 20 minutes. After incubation time, add 2.5 ml of 10% TCA to each sample. Centrifuge the tube at 3000 rpm for 10 minutes. Collect 2.5 ml of supernatant from the centrifuged sample into a separate tube. Then add 2.5 ml of deionized water to a new, separate tube containing 2.5 ml of supernatant. Then add 0.5 ml of ferric chloride to the same new separate test tube. This forms a bluish colour. Then measure at 700 nm with a colorimeter (Shwetha R.J *et al.,* 2016).

Antidiabetic Activity: Alpha Amylase Method (DNSA Method): Prepare 0.1% starch solution (0.1 g starch dissolved in 100 ml distilled water). 0.5% alpha amylase (0.5 g dissolved in 100 ml distilled water). The control tube (without inhibitor) was prepared with reagents only, and the test sample (with inhibitor) was prepared with 1 ml of 500 µg/ml plant samples (inhibitor) and 0.5 ml of enzyme solution. Incubate both tubes at room temperature for 10 minutes. After incubation, add 1 ml of 1% starch solution and incubate again for 20 minutes at room temperature. Add 1 ml DNSA to stop the reaction. Place both tubes in boiling water and wait until red appears in the control tube. Remove the tube and cool it to room temperature and measure the absorbance.

Formula: Enzyme activity (µmol/min) = µg of maltose released × 1000/Molecular weight of maltose × Incubation time.

Anti- inflammatory:

Protein Denaturation Method: The experimental setup involved mixing 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and 0.02 mL of extract in a reaction mixture (total volume 5 mL). This mixture was thoroughly mixed and then incubated in a water bath at 37 °C for 15 minutes, followed by heating at 70 °C for 5 minutes. After cooling, the turbidity of the solution was measured at 660 nm using a UV/VIS spectrometer. A phosphate buffer solution served as the control. The percentage of protein denaturation inhibition was calculated using a specified formula (Shwetha R.J *et al.,* 2016).

Formula: % Proteinase inhibition = 100 x (1-A2/A1)

Were, A1 = absorption of the control sample, and A2 = absorption of the test sample.

Protein Inhibition Method: Similarly, another reaction solution (total volume 2 mL) was prepared by mixing 0.06 mg trypsin, 1 mL of 20 mM Tris-HCl buffer (pH 7.4), and 1 mL test sample (0.02 mL extract + 0.980 mL methanol). This solution was then incubated at 37 °C for 5 minutes, followed by the addition of 1 mL of 0.8% (w/v) casein and further incubation for 20 minutes. Upon completion of the incubation, 2 mL of 70% perchloric acid was added to stop the reaction. After centrifugation, the absorbance of the supernatant was measured at 210 nm against buffer as a blank. A phosphate buffer solution served as the control. The percentage inhibition of protein denaturation was calculated using a specified formula (Shwetha R.J *et al.,* 2016).

Formula: % Proteinase inhibition = 100 x (1-A2/A1)

Were, A1 = absorption of the control sample, and A2 = absorption of the test sample.

Results:

Preliminary phytochemical screening: Preliminary screening of phytochemicals was carried out on *Tinospora cordifolia* stem revealed the presence of wide range of phytoconstituents including alkaloids, steroids, flavonoids, carbohydrates, phenols etc. shown in **Table 1.**

TEST	OBSERVATION				
	Ethanol	**Methanol**	**Acetone**	**Chloroform**	**D/w**
TANNINS Gelatin test:	Present	Present	Present	Absent	Absent
GLYCOSIDES Liebermann's test:	Present	Present	Present	Absent	Present
ALKALOIDS Wagner's test:	Absent	Absent	Absent	Absent	Absent
STEROIDS Salkowski test:	Absent	Present	Present	Absent	Present
TERPENOIDS Salkowski test:	Present	Present	Present	Present	Present
FLAVONOIDS Sodium Hydroxide test:	Absent	Present	Present	Present	Present
PHENOLS Ferric Chloride test:	Absent	Absent	Absent	Absent	Absent
PROTEINS Biuret test:	Present	Present	Absent	Present	Absent
CARBOHYDRATES Molisch Test:	Present	Present	Present	Absent	Present
AMINO ACIDS Ninhydrin test:	Present	Present	Absent	Present	Absent

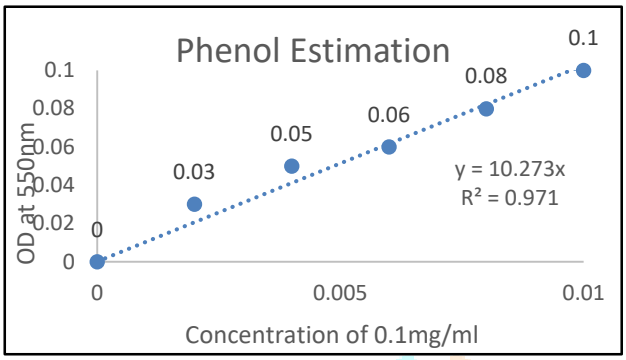
Table 1: qualitative analysis of stem of *Tinospora cordifolia*

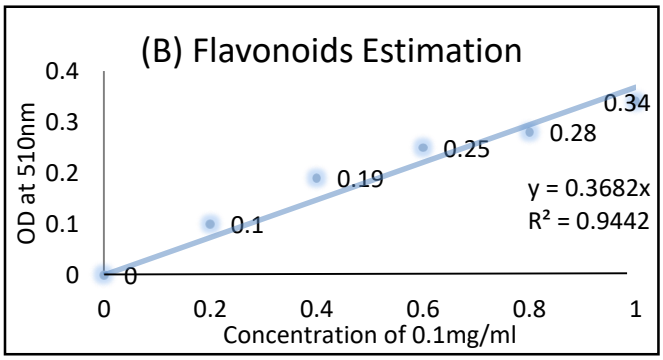
Discussion: Table 1 displays the phytochemical analysis findings. The sample from Rishikesh Herbal Collection Centre, Kalyan showed tannin content in ethanol, methanol, and acetone, while absent in chloroform and distilled water. Liebermann's test revealed glycosides in ethanol, methanol, distilled water, and acetone. Terpenoids were detected in all solvents via the Salkowski test. Flavonoids were present in all solvents except ethanol. Alkaloids and phenols were absent across all solvents. Protein was found in ethanol, methanol, and chloroform. Carbohydrates were present in all solvents except chloroform. Amino acids were detected in ethanol, methanol, and chloroform.

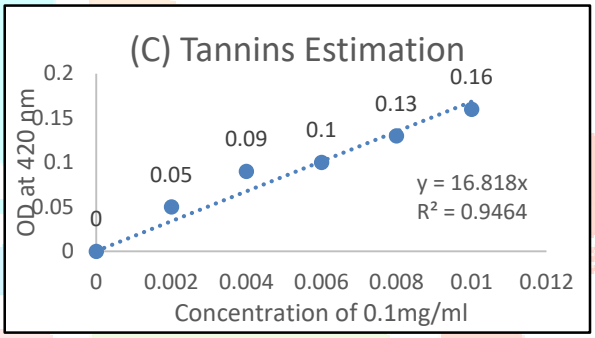
Quantitative estimation:

Table 2: Quantitative estimation of total phenols, flavonoids and tannins content

Sample	Total phenols (mg GAE/g)	Total tannins (mg TAE/g)	Total flavonoids (mg QE/g)
T. cordifolia	**8.75 mg/g**	**2.9 mg/g**	**56mg/g**







Graph 1: (A) Standard Gallic acid curve graph of phenol estimation. **(B)** Standard quercetins curve graph of flavonoid estimation. **(C)** Standard tannic acid curve graph for tannins estimation.

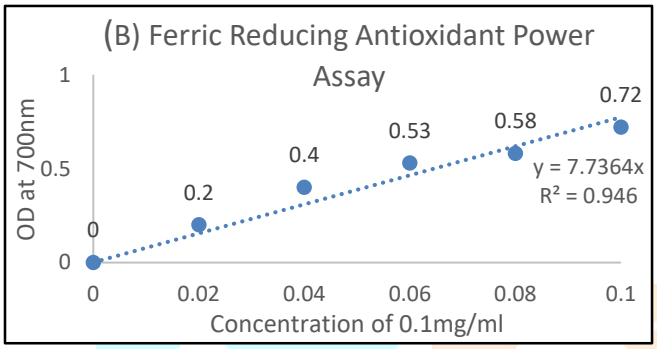
Discussion: Quantitative analysis of phenolic phytoconstituents in *Tinospora cordifolia* employed Gallic acid as the standard, with various solvents used for extraction. The methanolic extract exhibited a phenolic content of 8.75 mg/g, confirmed by comparing optical density (OD) with the standard. The OD range aligned with the standard, indicating phenolic presence and suggesting medicinal potential. Similarly, flavonoid analysis utilized Quercetin dihydrate as the standard, with methanolic extraction yielding a phenolic content of 56 mg/g. The absorbance range matched the standard, affirming phenolic presence in *T. cordifolia*. Quantitative analysis using tannic acid standard also confirmed phenolic presence, with a content of 2.9 mg/g in the methanolic extract. The OD range paralleled the standard, emphasizing phenolic compounds' health benefits in *T. cordifolia* extracts. **(Table 2)**

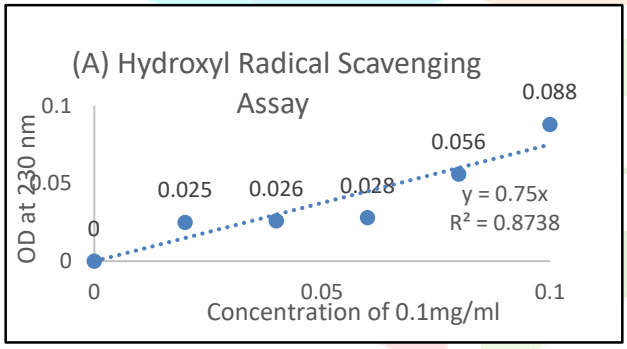
Antioxidant:

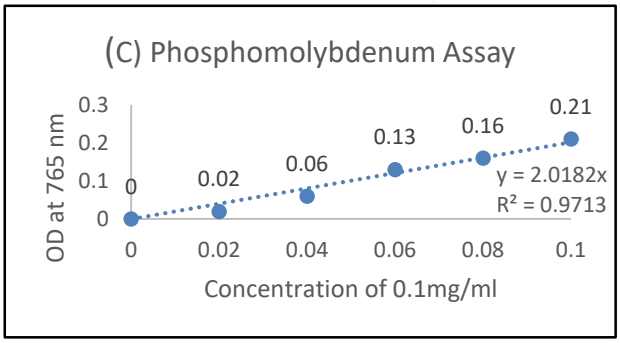
Sample	**Concentration (mg/ml)**	**Hydroxyl Radical Scavenging Assay**	**Phosphomolybdate assay**	**Ferric Reducing Antioxidant Power Assay**
Ascorbic acid standard	**0.02**	0.18	0.02	0.20
	0.04	0.20	0.06	0.40
	0.06	0.29	0.13	0.53

		0.08			0.35			0.17			0.58		
		1.0			0.59			0.28			0.72		
T. cordifolia		**M**	**E**	**D/W**	**D/W**	**M**	**E**	**D/W**	**M**	**D/W**	**E**	**D/W**	**M**
	0.1	0.55	0.013	0.55	0.55	0.23	0.16	0.20	0.23	0.16	0.16	0.20	0.23
	1.0	0.57	0.033	0.58	0.58	0.52	0.27	0.45	0.52	0.27	0.27	0.45	0.52

Table 3: antioxidant content of Hydroxyl Radical Scavenging Assay, Phosphomolybdate Assay and Ferric Reducing Antioxidant Power Assay (FRAP) of methanolic, aqueous and ethanolic extract of *T. cordifolia* stem.







Graph 2: Standard curve graph of **(A)** Hydroxyl Reducing Power Assay. **(B)** FRAP **(C)** phosphomolybdenum assay.

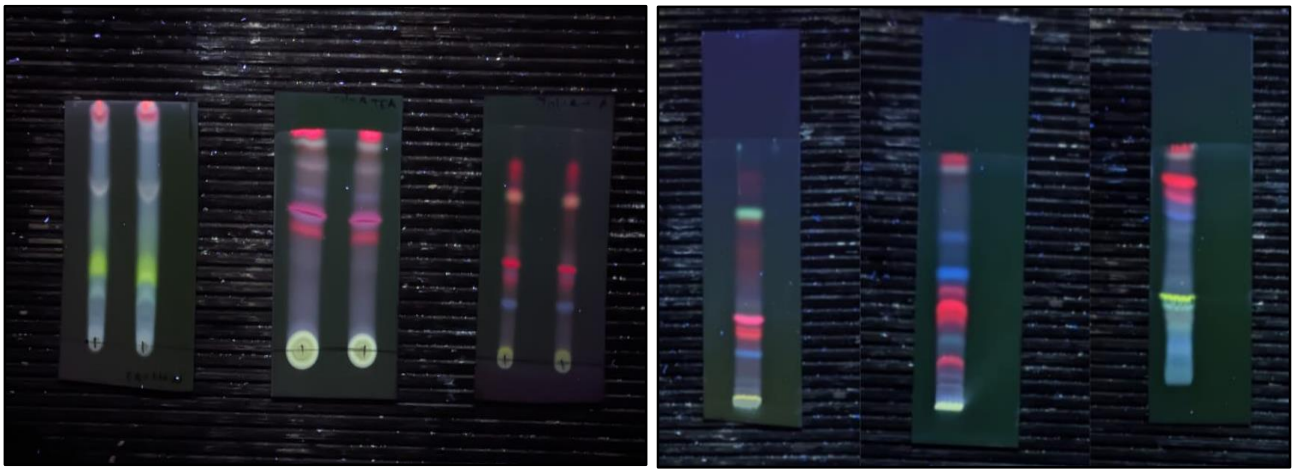
Discussion: Antioxidant activity in *Tinospora cordifolia* was assessed using various assays and solvent systems. Hydroxyl radical scavenging assay with ascorbic acid as the standard revealed methanol extract had **3.38%** antioxidant content, while distilled water extract showed **1.69%.** FRAP assay indicated methanolic extract had **27.77%** antioxidant content, and distilled water extract had **37.5%.** Phosphomolybdenum assay showed methanol extract had **17.85%** antioxidant content, and distilled water extract had **28.57%.** These results suggest significant antioxidant potential in *T. cordifolia*, particularly in extracts obtained with distilled water, indicating promising applications in nutraceuticals or pharmaceuticals. (Table 2, graph 2)

Thin Layer Chromatography (TLC) and High Pressure Thin Layer Chromatography (HPLTC fingerprinting):

No	Mobile phase	Extract	Colour	Retention factor, (R_f)
1	Toluene: acetone: formic acid (4: 1: 0.5)	Methanolic extract	White	0.58
			Green	0.36
			Pink	0.94
2	Toluene: acetone: ethyl acetate (2:1:4).	Methanolic extract	Violet	0.62
			White	0.86
			Pink	0.93
			Orange	0.53
3	Ethyl acetate: methanol: water (3.5: 2: 0.5).	Methanolic extract	Orange	0.85
			Blue	0.21
			Red	0.36
			Yellow	0.08
			Pink	0.89

Table 3: retention factors of the phytoconstituents separated during the TLC which was developed in 3 different solvents.

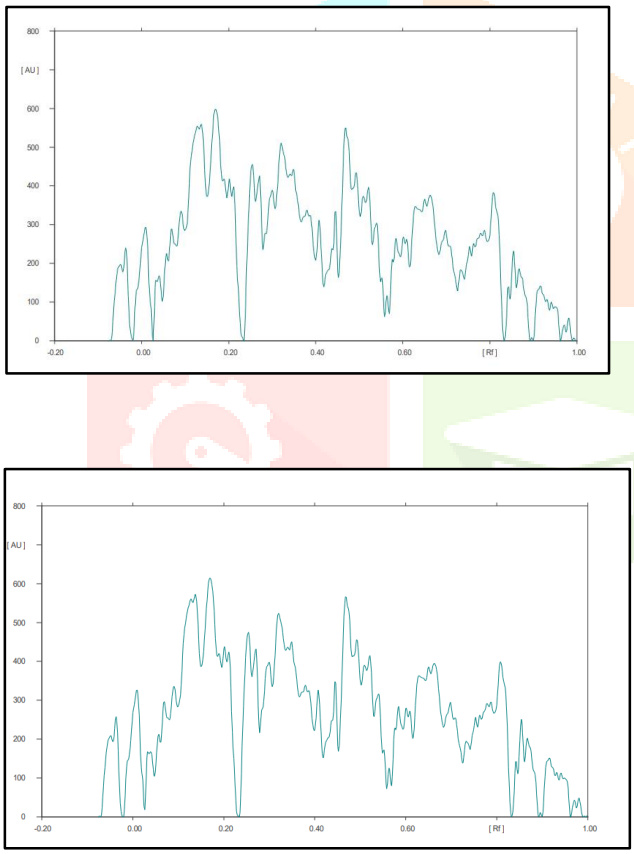
Discussion: The methanolic extract in toluene: acetone: formic acid (4:1:0.5) mobile phase exhibited white, green, and pink colours with retention factors of 0.58, 0.36, and 0.94, respectively. In toluene: acetone: ethyl acetate (2:1:4), the extract showed violet, white, pink, and orange colours with retention factors of 0.62, 0.86, 0.93, and 0.53, respectively. Lastly, in ethyl acetate: methanol: water (3.5:2:0.5), the extract displayed orange, blue, red, and yellow colours with retention factors of 0.85, 0.21, 0.36, and 0.08, respectively. The varying colours and



retention factors indicate different chemical compositions and polarities within the extracts across different mobile phases, suggesting potential variations in the presence of compounds based on their solubility and interaction with the stationary phase.

Figure 1: (1) Thin layer chromatography (HPTLC) plate showcasing separation of phytoconstituents using a solvent system comprising **(A)** Toluene: acetone: formic acid (4: 1: 0.5). **(B)** Toluene: acetone: ethyl acetate (2:1:4). **(C)** Ethyl acetate: methanol: water (3.5: 2: 0.5). **(2) (1)** High Performance Thin layer chromatography (TLC) plate showcasing separation of phytoconstituents using a solvent system comprising **(A)** Toluene: acetone: formic acid (4: 1: 0.5). **(B)** Toluene: acetone: ethyl acetate (2:1:4). **(C)** Ethyl acetate: methanol: water (3.5: 2: 0.5).

Discussion: The analysis of *Tinospora cordifolia* stem via High-Performance Thin-Layer Chromatography (HPTLC) across a spectrum of wavelengths (200-450 nm) using different solvent systems revealed varying numbers of phytoconstituents. At 200 nm, 25 constituents were detected, increasing to 31 at 400-450 nm. Notably, the solvent system Toluene: Acetone: Formic acid exhibited the highest diversity. This emphasizes the significance of wavelength variations in HPTLC fingerprinting, highlighting the optimal spectrum for lambda max (400-450 nm) to understand *T. cordifolia's* chemical profile for pharmacological insight.



Graph 3: HPTLC fingerprinting of maximum phytoconstituent present at 400nm

Anti- Diabetic Activity (DNSA method):

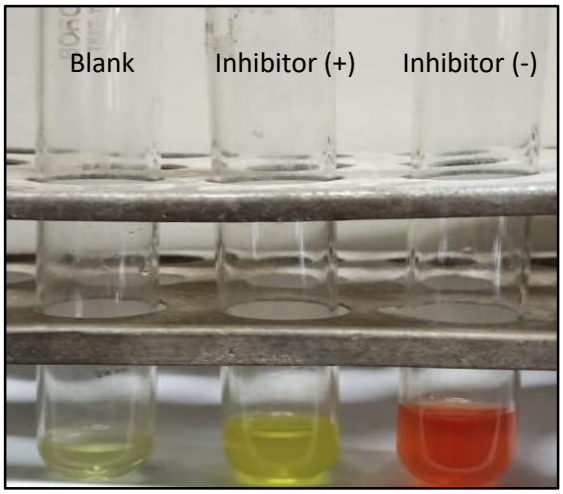


Figure 2: Image depicts three tubes: one blank, one with giloy sample as inhibitor, and one without inhibitor, illustrating inhibition study.

Discussion: The Anti-Diabetic analysis (Figure 2) reveals that *Tinospora cordifolia* inhibits Alpha-amylase enzyme activity, crucial for carbohydrate digestion and postprandial glucose levels. This inhibition, quantified using DNSA reagent, prevents the conversion to reduced sugar ANSA, indicative of enzyme inhibition. With *Tinospora cordifolia*, enzyme activity is 127.92 µmol/min compared to 209.07 µmol/min without, showing its efficacy as an inhibitor. This inhibition helps control postprandial hyperglycaemia, reducing diabetes risk and aiding in various disorders like obesity and dental issues.

Anti- inflammatory Activity:

	Proteinase Denaturation method	**Proteinase Inhibition Method**
% Inhibition	32.58%	57.14%

Table 4: Percentage inhibition of anti- inflammatory activity calculated by Proteinase Denaturation method and Proteinase Inhibition Method

Discussion: The results for anti-inflammatory activity were evaluated using two methods: inhibition of protein denaturation activity and proteinase inhibition activity. The percentage of inhibition of protein denaturation activity was found to be **32.85%,** while the percentage of proteinase inhibition activity was **57.14%.**The higher percentage of inhibition observed in the proteinase inhibition activity method suggests that it is more effective in preventing the activity of proteinases, which are enzymes that play a role in inflammatory processes. This indicates that the compound being tested may have a stronger anti-inflammatory effect through its action on proteinases. Therefore, focusing on compounds or treatments that exhibit higher proteinase inhibition activity may be more promising in terms of developing effective anti-inflammatory therapies.

Conclusion: In conclusion, the research conducted on *Tinospora cordifolia* has provided valuable insights into its phytochemical composition and pharmacological properties. The analysis revealed the presence of various phytoconstituents such as tannins, glycosides, terpenoids, flavonoids, alkaloids, phenols, proteins, carbohydrates, and amino acids in different solvent systems. Furthermore, the quantitative determination of phenolic content highlighted the significant presence of phenolic compounds in *T. cordifolia* extracts, indicating its potential medicinal applications. Moreover, the assessment of antioxidant activity demonstrated the considerable antioxidant potential of *T. cordifolia* extracts, with variations observed based on the solvent used for extraction. This underscores the importance of solvent selection in extracting bioactive compounds from herbal materials. Additionally, the analysis of the stem of *T. cordifolia* via High-Performance Thin-

Layer Chromatography (HPTLC) provided valuable insights into the chemical composition of the plant extract across different wavelengths, offering a comprehensive understanding of its phytoconstituent profile. Furthermore, the anti-diabetic and anti-inflammatory activities of *T. cordifolia* extracts were investigated, revealing promising results in inhibiting alpha-amylase enzyme activity and proteinase inhibition activity, respectively. These findings suggest the potential of *T. cordifolia* in managing diabetes and inflammation-related disorders. Overall, this research underscores the pharmacological potential of *Tinospora cordifolia* and provides a foundation for further exploration and development of herbal medicines derived from this plant species. Further studies are warranted to elucidate the mechanisms of action and therapeutic efficacy of *T. cordifolia* extracts for various health conditions.

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