J. Phytol. Res. **38**(2): 193-207, 2025 **ISSN 0970-576**

COMPARATIVE ANALYSIS OF PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF *TINOSPORA CORDIFOLIA* GROWING ON HOST PLANT *AZARDIRACHTA INDICA* (NEEM), *SARACA ASOCA* (ASHOKA), AND *CITRUS LIMON* (LEMON)

NIDHI YADAV[1], SUMER SINGH[1], KESHU YADAV[2] and ALOK DHAKA[3*]
[1]Department of Biochemistry, Singhania University, Badi Pacheri, Jhunjhunu-333515, Rajasthan (India)
[2]Department of Biotechnology, Central University of Rajasthan, Ajmer-305817, Rajasthan (India)
[3]Department of Sports Bio-sciences, Central University of Rajasthan, Ajmer-305817, Rajasthan (India)
***Corresponding Author's Email:** alokdhaka07@gmail.com

Tinospora cordifolia, commonly known as Giloy, is a heart-leaved moonseed, also known as Guduchi, and is considered a divine herb. Twenty-four species of an herbaceous vine are found in tropical areas of India, Myanmar, and Sri Lanka, among others. This study aims to comparatively evaluate the influence of host plants *Azadirachta indica (Neem), Saraca asoca (Ashoka), Citrus limon (Lemon), and normal giloy (independently growing) based on their phytochemical screening,* antioxidant activity, antidiabetic activity, and total flavonoid content. Phytochemical screening reveals the presence of carbohydrates, steroids, glycosides, quinones, phytosterols, flavonoids, proteins, and amino acids. Quantitative estimation revealed that normal *T. cordifolia* without a host exhibited the highest total flavonoid content. Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, the neem-hosted plant shows the highest antioxidant activity, and the neem-hosted giloy and regular giloy showed the highest inhibitory activity in the antidiabetics (α-amylase test). The studies suggest that the host plant affects the bioactive profile of *T. cordifolia.*

Keywords: *Antioxidant, Azardirachta indica, Citrus limon, Phytochemicals, Saraca asoca, Tinospora cordifolia.*

Introduction

According to the World Health Organisation (WHO), medicinal plants are plants that contain compounds used for therapeutic purposes and as precursors for the synthesis of effective drugs. It has been estimated that 70–80 per cent of the population in developing countries relies on traditional medicine. *Tinospora cordifolia*, commonly known as "Guduchi" in Sanskrit. *T. cordifolia* is a member of the family Manispermaceae. The plant is genetically diverse, featuring large, climbing shrubs that shed their leaves seasonally. It possesses greenish-yellow flowers, which are found at higher altitudes [1-3]. The male flowers occur in clusters, whereas the female flowers occur. The flowering time extends throughout the summers and winters, and several active constituents, including alkaloids, steroids, diterpenoid lactones, aliphatics, and lither root, are isolated from various parts of the plant, i.e., from the root, stem, and whole plant. Scientists worldwide are keenly interested in this plant due to its noted medicinal properties. They encompass anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, antioxidant,

Received: 20 December,2025 *Revised: 26 December,2025* *Accepted: 6 January,2026*

anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, and anti-neoplastic activities. The *Tinospora* genus comprises approximately 34 species, distributed across the tropical and subtropical regions of Asia, Australia, and Africa. *T. cordifolia (Wild)* Hook. F & Thomson, *Tinospora* Gulancha Indian *Tinospora*, and Giloy are its Latin names *T. cordifolia* is also known as *Tinospora sinensis* (Lour) Merr. And Guduchi/Amrita[4-6]. It belongs to the family Menispermaceae and is indigenous to China, Myanmar, and Sri Lanka. The plant is traditionally used in Ayurvedic medicines. It can be used to treat jaundice, rheumatism, urinary diseases, skin conditions, diabetes, anaemia, inflammation, allergic conditions, and other conditions, as well as exhibit anti-periodic qualities and radioprotective properties[7,8]. The root of the giloy plant (*T. cordifolia) is used as an* emetic and to relieve intestinal obstruction. The starch of this plant serves as a good home remedy for recurring fevers, removing stinging sensations, and improving energy and appetite[9,10]. Whereas numerous studies have endeavored to detect the medicinal and useful properties of *Tinospora cordifolia*, fewer studies have been aimed at food containing the giloy stem. The use of medicinal herbs not only provides health benefits but can also provide nutritional products and functional health benefits. Thus, this study aimed to conduct a phytochemical screening of giloy stem powder.

Material and Methods

Sample collection

The present study involved the collection of plant samples, specifically the stems of *Tinospora cordifolia* (Giloy) growing on different host plants, namely *Azadirachta indica* (Neem), *Saraca asoca* (Ashoka), and *Citrus limon* (Lemon), from Huda district, Narnaul, Haryana, India, during the month of March,2024. The study duration was 15 months, covering all phases from sample collection to final data analysis and interpretation. Collected plant materials were thoroughly washed with distilled water, shade-dried for 10–12 days and subsequently ground into a fine powder using an electric grinder.

Preparation of plant extracts

We used natural sources of Unprocessed *Tinospora cordifolia* stems. To remove soil and other contaminants, we gently clean the stems of *T. cordifolia* with regular water, then rinse them again with distilled water. Afterwards, we dried the stem for 48 hours at 45 °C in an oven. Crushed the dried stem of *T. cordifolia* into fine powder using a grinder. This powdered stem was then used in the extraction process.

Preparation of sample extraction: -

5 g of *T. cordifolia* powder was soaked in 50 ml of methanol at a ratio of 1:10 and shaken thoroughly to create the extract. The solution was then left to shake at room temperature for 48 hours before being filtered through filter paper. The filtered solution was set aside to allow the solvent to evaporate. Finally, the following formula was used to calculate the percentage yield of the dried extracts-

$$\%\, Yield = \frac{Weight\ of\ Extract}{Weight\ of\ powdered\ drug\ taken} \times 100$$

Phytochemical qualitative analysis

Freshly prepared plant extracts were subjected to standard phytochemical analysis to detect the phytochemical constituents, including terpenoids, alkaloids, quinines, saponins, flavonoids, etc. Typical screening techniques were applied[11-17].

Detection of Alkaloids

- Alkaloid test: To 1 ml of the extract, a few drops of iodine solution were added. The presence of blue colour, which disappears upon boiling and reappears upon cooling, indicates the presence of alkaloids.
- Wagner's test: 2-3 drops of acid were added to 1 mL of the extract, followed by 1 mL of Wagner's reagent. The appearance of a reddish-brown precipitate indicates the presence of alkaloids. (Wagner's reagent: 2g of iodine and 6g of potassium iodide in 100mL of distilled water.)

Detection of Glycosides

- Keller-Killani test: 1 mL of extract was combined with 1.5 mL glacial acetic acid,1 drop of 5% ferric chloride, and concentrated sulfuric acid H_2SO_4(along the sides of the test tube). Blue colour appearance is an indicator of the presence of a glycoside.

Detection of Saponins

- Froth Test: Dilute 1mLof extracts with 5 mL of distilled water. Shake the diluted extract in a graduated cylinder for approximately 15 minutes. The development of a 1-centimetre layer of foam is proof of the presence of saponins.
- Foam Test: In a test tube, around 1mL of extract was shaken with 2 mL of water. The appearance of foam that persists for approximately 10 minutes upon shaking shows the presence of saponins.

Detection of Phytosterols

- Hesse's test: 2.5 mL aqueous extracts were taken in a test tube, and 1mL chloroform and 1ml concentrated H_2SO_4 were added to it. The formation of a pink ring or Red colour in the lower chloroform layer confirms the existence of phytosterols.

Detection of tannins:

- Braymer's test: Add 3mL of distilled water and 3 drops of 10% ferric chloride to 1 mL of extract (boil 0.3 gm powder in 5mL of distilled water for 3-5 min.). A blue-green colour solution will be produced if tannins are present.

Detection of Proteins and Amino Acids

- Biuret test: Add 1 drop of 2%$CuSO_4$ solution, 1mL of 95% ethanol, and a pellet of KOH to 1mL of extract. A pink colour solution indicates the presence of protein and amino acids.
- Ninhydrin test: 1mL of ninhydrin was mixed with 1mL of extract. When protein and amino acids are present, a blue or purple colour is formed. (5mL of ethanol with 0.1gm of ninhydrin)

Detection of Phenolic Compounds

- Lead Acetate test: 1.5 mL of 10% lead Acetate solution was mixed with 1mL of plant extract. The presence of phenols is indicated by White precipitates.

Detection of Oils and Fat Test

- Add 1% of NaOH and 1% $CuSO_4$ to 1mL of plant extract. The occurrence of fat and oils is indicated by the blue-coloured solution.

Detection of Quinones

- Concentrated HCl test: 1mL of plant extract was added along with 1mL concentrated HCl. A green colour solution formed due to the presence of quinones.

Detection of Flavonoids

- Alkaline reagent test: 2mL of 2% NaOH and 1 mL of plant extract were added with a few drops of diluted HCl. Upon the addition of diluted HCl, the intense yellow colour should change to

colourless, indicating the presence of flavonoids.

Detection of Triterpenoids

- Salkowski's test: 1mL of plant extract and 1 mL of chloroform are added with a drop of concentrated H_2SO_4, shaken well, and left to settle. The golden-yellow layer at the bottom indicates the presence of triterpenoids.

Detection of Carbohydrate

- Resorcinol test: Few crystals of resorcinol and the same or an equal amount of concentrated HCL are added to 2mL of aqueous extract solution and heated. Rose-red colour is an indication of the presence of carbohydrates.
- Molish's test: 0.5mL concentrated H_2SO_4, and 1 drop of alcoholic alpha naphthol were mixed with 1 mL of extract. Violet rings indicate the presence of carbohydrates.

Detection of Steroids

- 1mL of concentrated H_2SO_4 and 1mL of chloroform were mixed with 0.5 mL of the extract. The presence of steroids is indicated by the formation of a reddish-brown ring.

Sugar Reduction Detection

- Benedict's test: Add 0.5mL Benedict reagent to 0.5 mL of extract, then boil for 2 minutes. Reducing sugar is shown by the red colour.

Antioxidant activity by DPPH assay

- 15 mg of DPPH was dissolved in 10mL of methanol. From this 75 µL solution, 75 µL was taken, and the final volume was made up to 3 mL with methanol. For control reading, Absorbance was taken immediately at 517nm. 75 µl of DPPH was added to methanol and 50 µl of the extract. Again, the final volume was made up to 3mL. A decrease in absorbance of DPPH was measured at 517 nm.

$$\%\ Inhibition = \frac{A_0 - A_1}{A_0} \times 100$$

Table 1. Reagent composition and assay conditions for the determination of total flavonoid content (TFC) in *Tinospora cordifolia* using the aluminum chloride colourimetric method.

S. No.	D.W(mL)	Quercetin	5%$NaNO_2$	10%$AlCl_3$	1M NaOH
Blank	3	0	0.3	0.3	2
T_1	2.8	0.2	0.3	0.3	2
T_2	2.6	0.4	03	0.3	2
T_3	2.4	0.6	0.3	0.3	2
T_4	2.2	0.8	0.3	0.3	2
T_5	2.0	1.0	0.3	0.3	2
Sample 1	2.5	0.5	0.3	0.3	2
Sample 2	2.5	0.5	0.3	0.3	2
Sample 3	2.5	0.5	0.3	0.3	2
Sample 4	2.5	0.5	0.3	0.3	2
			Incubate for 5 min. in dark	Incubate for 5 min. in dark	Absorbance at 510 nm

Total flavonoid content

- The total flavonoid content (TFC) of T. *cordifolia* growing on different host plants was determined using the aluminium chloride colourimetric method, with quercetin as the standard. The absorbance of the samples was measured at 415 nm, and the TFC is given as mg quercetin equivalent (QE) per gram of dry weight (mg QE/g DW)[11,12]. The procedure was followed as described in Table 1.

Antidiabetic alpha amylase test[15]

- Preparation of Reaction Mixture:
  - Add 500 µL of plant extract (or distilled water for blank) to labelled test tubes.
  - Add 500 µL of α-amylase solution (1mg/ml) to the test tubes.
  - Incubate the mixture at room temperature for 10 minutes.
  - After incubation, add 500 µL of 1% starch solution.
  - Incubate the tubes again for 10 minutes at room temperature.
- Stopping the Reaction:
  - To stop the reaction, add 1ml of the (DNS) 3,5-Dinitrosalicylic acid reagent to each other.
  - Place the tubes in a boiling water bath for 5 minutes to develop the colour.
  - After boiling, add 3 ml of distilled water.
  - Then the absorbance was measured at 540 nm using a UV-VIS spectrophotometer.
- Control and Blank Setup:
  - Blank: All reagents except enzyme (enzyme replaced with phosphate buffer).
  - Control: Contains enzymes and starch but no plant extract.
  - Samples 1–4: Contain different plant extracts being tested.
- Calculation

$$\%\ Inhibition = \{(Control - A\ sample)\ A\ Control\} \times 100$$

Where: **A** – absorbance of the control (no plant extract)

A sample means the absorbance of the reaction containing the plant extract.

Results and Discussion

Yield of extracts

According to the results of percentage yield, it is clear that methanol was a better solvent for the extraction of normal *T. cordifolia* than *T. cordifolia* growing on other host plants. *T. cordifolia* growing on Ashoka shows a lower % of yield than the *T. cordifolia* growing on neem and lemon, as shown in Table 2 and Figure 1.

Phytochemical screening of the methanolic extract of giloy stem powder.

Phytochemical analysis is the most valuable step in medicinal plant research. It may be used for drug discovery and development. In this study, the extract of *T. cordifolia*, grown on different supportive plants, was used. This study on plants was conducted on *T. cordifolia* growing on different plants, which affected the phytochemical components present in the extract. The results of the phytochemical screening of giloy stem powder are presented in Table 3. Presence of carbohydrates, steroids, reducing sugar, glycosides, triterpenoids, oils and fat, quinines, phytosterol, glycosides, saponins, was detected in the methanolic extract, whereas alkaloids, flavonoids, phenolic compounds, protein, and amino acid were absent.

Antioxidant activity by DPPH assay

The results of the antioxidant activity, as measured by the DPPH assay, clearly show that the % inhibition of *T. cordifolia* growing on the host *Azardirachta indica* has a higher % inhibition than the others, as shown in Table 4 and Figure 2.

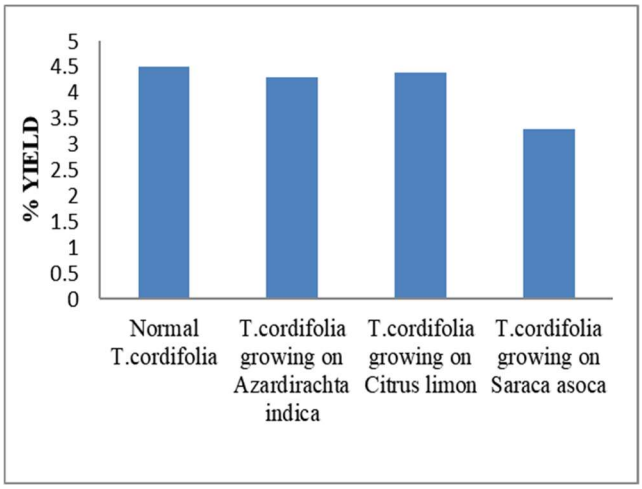

Figure 1. Comparative Graph of the percentage yield of sample extracts.

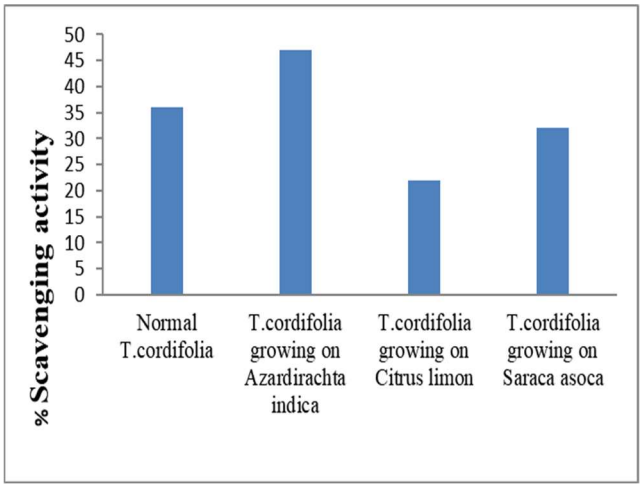

Figure 2. Percentage α-amylase inhibitory activity of *Tinospora cordifolia* stem extracts grown on different host plants.

Antidiabetic effect of plant extracts:

The α-amylase inhibitory activity of four different *Tinospora cordifolia* (Giloy) samples, grown on different host plants, was assessed using the DNS colourimetric assay. The control (without extract) showed an absorbance of 1.431. Among the tested samples, Giloy grown on lemon plant (Sample 3) exhibited the highest inhibitory effect, reducing the absorbance to 0.429, corresponding to 70.02% inhibition. This was followed by Giloy grown on a neem plant (Sample 2) and regular Giloy (Sample 4), which showed 63.80% and 65.19% inhibition, with absorbance values of 0.518 and 0.498, respectively, as shown in Table 5. Giloy grown on Ashoka plant (Sample 1) showed the lowest inhibition at 30.12%, with an absorbance of 1.000. These findings suggest that the host plant has a significant influence on the antidiabetic potential of Giloy, with the lemon-hosted variety demonstrating the most promising α-amylase inhibitory activity.

Total flavonoid content

The total flavonoid content (TFC) of *Tinospora cordifolia* growing on different host plants was estimated using the aluminium chloride colourimetric method, with quercetin as the reference standard. Quantification was performed based on the quercetin standard curve (Figure 3), which demonstrated a strong linear relationship between absorbance and concentration, as expressed by the regression equation $y = 3.0055x - 0.0351$, with a high coefficient of determination ($R^2 = 0.9933$), indicating the method's good accuracy and reliability.

The TFC values varied markedly depending on the host plant species (Table 6). The highest flavonoid content was recorded in *T. cordifolia* growing on Neem + Giloy mixed host (NG), with a value of 0.921 mg quercetin equivalent per gram of dry weight (mg QE/g DW). This was followed by samples growing on Neem (0.477 mg QE/g DW) and Lemon (0.376 mg QE/g DW) (Figure 4). The lowest TFC was observed in samples associated with Ashoka, which showed a value of 0.240 mg QE/g DW. The

flavonoid content varied significantly depending on the host plant on which *T. cordifolia* was growing.

The host-dependent variation in flavonoid accumulation is clearly illustrated in Figure 4, which demonstrates a pronounced enhancement of flavonoid content in plants growing on NG compared to other host species. These findings suggest that host plant association plays a significant role in modulating the biosynthesis of secondary metabolites in *T. cordifolia*. The higher flavonoid content observed in NG-associated plants may be attributed to differential nutrient availability, physiological interactions, or host-induced metabolic regulation, while the comparatively lower content in Ashoka-associated plants indicates a less favourable influence on flavonoid biosynthesis.

Table 2: Results of the percentage yield of different extracts (Methanol)

S. No.	Extracts	%Yield
1	Normal *T. cordifolia*	4.5
2	*T. cordifolia* growing on *Azardirachta indica*	4.3
3	*T. cordifolia* growing *on Citrus limon*	4.4
4	*T. cordifolia* growing on *Saraca asoca*	3.7

Table 2 and Figure 1 show that the percentage yield of methanolic extracts from *Tinospora cordifolia* exhibited noticeable variation among samples grown on different host plants, indicating that host-associated physiological and biochemical factors influence the extractability and overall phytochemical richness of the plant material. The highest yield was observed in the normal (un-hosted) *T. cordifolia*, which recorded 4.5%. This suggests that plants growing independently maintain a stable phytochemical profile and optimal biomass composition, allowing methanol to efficiently extract their metabolites. Methanol is a widely used solvent for extracting polar to moderately polar compounds such as glycosides, flavonoids, reducing sugars, phenolics, and alkaloids, which are abundantly present in Giloy under normal growth conditions[18]. The samples grown on *Citrus limon* (4.4%) and *Azadirachta indica* (4.3%) showed slightly lower but comparable yields. Citrus and Neem are known to influence the metabolic pathway of climbing or parasitic plants through nutrient, hormonal, or allelochemical interactions. The marginal reduction in yield in these samples may be due to host-induced biochemical adjustments that alter the distribution of extractable components. However, the yields remain relatively high, indicating that both hosts continue to support the synthesis of methanol-extractable metabolites, such as glycosides, sugars, and saponins.

The lowest yield was obtained from *T. cordifolia* growing on *Saraca asoca* (3.7%), suggesting that the Ashoka host plant may exert a suppressive effect on certain primary or secondary metabolite pathways. Ashoka is known for its tannin-rich and alkaloid-rich profile, which may interfere with or alter the synthesis of methanol-soluble compounds in Giloy. Lower biomass integrity or reduced accumulation of extractable phytochemicals such as flavonoids or triterpenoids may also contribute to the reduced yield. So, the data clearly demonstrate that the host plant significantly influences the extraction yield, likely due to differences in metabolite accumulation, tissue composition, and solvent–solute interactions[19]. The variation in extract yield corresponds with the phytochemical screening results, where normal and Neem-hosted samples showed a

Table 3: Phytochemical screening of methanolic extract of giloy stem powder.

Phytochemicals	Test	*T. cordifolia* growing on *A. indica*	*T. cordifolia* growing on *Citrus limon*	*T. cordifolia* growing on *Saraca asoca*	Normal *T. cordifolia*
Carbohydrates	Resorcinol test	+++	++	Less than 1	+
Alkaloid	Wagner's reagent	-	-	-	-
Steroid		++	+	++	Less than 1
Reducing sugar	Benedicts test	++	+++	+++	++
Glycosides	Keller-Killani test	++	++	+++	+
Flavonoids	Alkaline Reagent test	++	+	-	+++
Triterpenoids	Salkowski test	++	++	-	+++
Saponins	Foam test	++	++	++	++
Tannins	Braymer's test	-	-	-	-
Oils and Fat Test	Sodium hydroxide test	+	++	+++	+
Quinones	Conc. HCl test	+++	+++	+	+++
Phytosterols	Hesse's test	++	++	-	+++
Phenolics compound	Lead acetate test	-	-	-	-
Protein and Amino acid	Ninhydrin test	+	+++	++	+

-, absent; +, weakly present; ++, moderately present; +++, strongly present

stronger presence of multiple phytoconstituents, while Ashoka-hosted samples displayed reduced phytochemical diversity.

The qualitative phytochemical analysis of *Tinospora cordifolia* grown on different host plants revealed distinct variations in secondary metabolite composition, indicating that the host species plays a crucial role in modulating the phytochemical profile of Giloy. The presence or absence of key phytoconstituents, such as carbohydrates, glycosides, flavonoids, triterpenoids, saponins, and quinones, differed across samples, suggesting a significant biochemical interaction between the host and the climbing vine. In Table -3, we can see that across all samples,

carbohydrates, reducing sugars, glycosides, triterpenoids, saponins, oils and fats, and quinones were consistently present, although their intensities varied. These constituents are well-known for contributing to the therapeutic properties of *T. cordifolia*, including immune modulation, antioxidant potential, antidiabetic activity, and antimicrobial effects[20]. Among the host-associated samples, *T. cordifolia* growing on *Saraca asoca* exhibited a strong presence of glycosides, reducing sugars, oils, fats, and proteins, indicating a metabolic shift toward energy-rich and structurally complex compounds. However, flavonoids and triterpenoids were absent in Ashoka-hosted Giloy, which may explain the comparatively lower antioxidant and antidiabetic activities recorded in other assays. In contrast, *T. cordifolia* growing on *Azadirachta indica* (Neem) demonstrated a strong to moderate presence of carbohydrates, steroids, triterpenoids, quinones, and phytosterols. Neem is known to be rich in bioactive phytochemicals, and these may influence metabolite synthesis in Giloy through biochemical or allelopathic interactions[21]. The presence of flavonoids (++) in Neem-hosted samples corresponds with the higher antioxidant activity observed in the DPPH assay.

The *Citrus limon*–hosted samples showed intense reactions for reducing sugars (+++), oils and fats (+++), and quinones (+++), suggesting an enhanced production of energy-linked and stress-related metabolites. The acidic and limonoid-rich environment of citrus plants may induce metabolic stress, stimulate the accumulation of certain compounds, while suppressing others, such as flavonoids and triterpenoids. The normal (un-hosted) *T. cordifolia* displayed strong presence of flavonoids (+++), triterpenoids (+++), phytosterols (+++), and quinones (+++), demonstrating a balanced phytochemical profile. The high flavonoid content in the normal sample correlates with its moderate antioxidant activity, highlighting the role of host influence on metabolic pathways. Interestingly, alkaloids, tannins, and phenolic compounds were absent in all samples, regardless of the host plant[22]. This suggests that these constituents may require different extraction methods, solvents, or environmental stimuli for detectable expression.

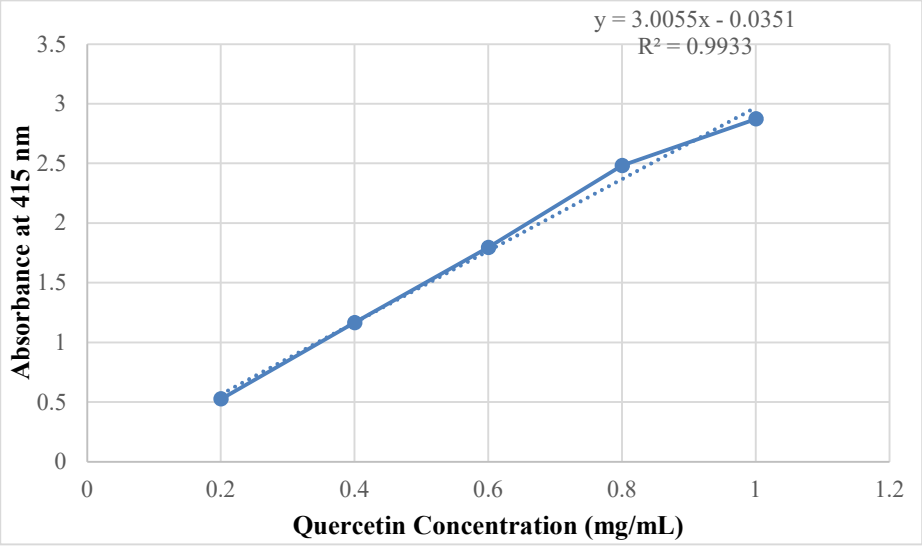

Fig. 3: Standard curve of total flavonoid content.

Overall, the findings clearly indicate that the phytochemical makeup of *T. cordifolia* is significantly altered by the host plant. Hosts rich in bioactive compounds (such as Neem) tend to enhance secondary metabolite production, whereas hosts with high tannin or alkaloid content (such as Ashoka) may suppress certain metabolic pathways. Variations in phytochemicals also correspond with the biological activities observed in antioxidant, antidiabetic, and antimicrobial assays, highlighting the importance of host selection in optimising the therapeutic potential of Giloy[23]. Table 4 and Figure 2 clearly show that the antioxidant potential of the *Tinospora cordifolia* extracts was evaluated using the DPPH free radical scavenging method, which measures the ability of plant phytochemicals to donate hydrogen or electrons to neutralise free radicals. The results demonstrated marked variability in antioxidant activity depending on the host plant supporting the growth of *T. cordifolia*, indicating a significant host-dependent modulation of secondary metabolite accumulation. Among all samples, *T. cordifolia* growing on *Azadirachta indica* exhibited the highest antioxidant activity, with 47% inhibition of the DPPH radical. Neem is well-known for its rich content of flavonoids, phenolic acids, and terpenoids, which may influence the phytochemical profile of the attached Giloy through metabolic or biochemical interactions. This enhanced activity may be attributed to increased levels of antioxidant compounds such as phenolics and flavonoids, which correlate strongly with free radical scavenging capability. Similar host-enhanced antioxidant responses in *T. cordifolia* or other medicinal climbers have been reported in earlier studies[24].

The normal (un-hosted) T. cordifolia showed moderate activity with 36% inhibition, suggesting that Giloy inherently possesses appreciable antioxidant capacity due to the presence of bioactive constituents like berberine, *Tinospora* side, and various phenolics. However, the improvement seen in Neem-hosted samples indicates that host plant characteristics can significantly affect metabolite accumulation. Extracts from *T. cordifolia* grown on *Saraca asoca* exhibited 32% inhibition, slightly lower than the normal sample. Ashoka is known for its high tannin and alkaloid content, which may alter

Table 4: % Scavenging activity of different extracts.

S.No.	Extracts	%Inhibition
1	Normal *T. cordifolia*	36
2	*T. cordifolia* growing on host *Azardirachta indica*	47
3	*T. cordifolia* growing on host *Citrus limon*	22
4	*T. cordifolia* growing on host *Saraca asoca*	32

Table 5. In vitro α-amylase inhibition assay.

Sample	Absorbance (450nm)	% Inhibition
Giloy host on Ashoka	1.0	30.12
Giloy host on Neem	0.518	63.80
Giloy host on Lemon	0.429	70.02
Regular Giloy	0.498	65.19
Control	1.431	-

or suppress certain metabolic pathways involved in the synthesis of antioxidant compounds. The reduced activity compared to Neem-hosted samples suggests that not all host plants enhance Giloy's bioactivity; some may exert a neutral or inhibitory influence. The lowest antioxidant activity was recorded in the *Citrus limon*-hosted sample, with 22% inhibition. Citrus species are rich in acidic compounds and limonoids, and the associated stress conditions may limit the biosynthesis of antioxidant phytochemicals in Giloy. Similar reductions in antioxidant activity due to host-related metabolic stress have been documented in studies of host-dependent medicinal plants[25].

The results clearly indicate that the antioxidant activity of *T. cordifolia* is strongly influenced by the type of host plant, with samples from Neem-hosted plants showing superior free radical scavenging potential. These findings correlate with phytochemical variations, as higher antioxidant activity is typically associated with increased levels of flavonoids and phenolics—compounds known for their strong electron-donating and radical-quenching capabilities. The α-amylase inhibition assay is a well-established in vitro method used to evaluate the antidiabetic potential of plant extracts, as inhibition of this enzyme delays carbohydrate breakdown and reduces postprandial hyperglycemia. In the present study (shown in Table 5), the α-amylase inhibitory activity of *Tinospora cordifolia* extracts varied substantially depending on the host plant on which the vine was growing, demonstrating clear host-dependent biochemical modulation. Among the tested samples, Giloy grown on Lemon exhibited the highest α-amylase inhibitory activity, with 70.02% inhibition. This strong activity may be attributed to increased levels of bioactive constituents—such as flavonoids, alkaloids, and phenolic compounds—known to contribute to antidiabetic effects. Citrus species are rich in limonoids and flavanones, which may induce stress-related metabolic enhancement in *T. cordifolia*, promoting the synthesis of enzyme-inhibitory phytochemicals. Similar synergistic effects between host plants and metabolic enhancement in parasitic/climbing plants have been documented in earlier studies[26].

Table 6: Total Flavonoid Content (TFC) of *Tinospora cordifolia* Growing on Different Host Plants.

Host Plant	TFC (mg QE/g DW)
Neem	0.477
Ashoka	0.24
Normal Giloy	0.921
Lemon	0.376

Regular Giloy and Neem-hosted Giloy also showed comparably high inhibitory activity (65.19% and 63.80%, respectively). Neem is particularly known for its potent antidiabetic phytoconstituents such as nimbidin and quercetin, which may influence metabolite transfer or elicit biochemical stimulation in the attached *T. cordifolia*, resulting in increased α-amylase inhibition. The findings align with previous research, which reports that host plants rich in bioactive compounds can positively impact the medicinal value of associated climbers.[27] In contrast, Giloy grown on Ashoka displayed the lowest inhibition (30.12%), indicating significantly reduced antidiabetic activity. The Ashoka plant is known for its higher levels of tannins and alkaloids, which are capable of modulating metabolic pathways and may interfere with the synthesis of α-amylase inhibitory compounds in *T. cordifolia*. A similar reduction in bioactivity due to host interference has been highlighted in studies evaluating host-dependent variations in medicinal climbers.[28] In this, results clearly

indicate that the host plant has a significant impact on the antidiabetic efficacy of *T. cordifolia*. The lemon-hosted sample showing the highest inhibitory activity suggests that certain hosts may enhance the therapeutic potential of Giloy by modulating the plant's secondary metabolite pathways. These variations correlate strongly with phytochemical findings such as total flavonoid content, providing biochemical justification for differences in biological activity.

The total flavonoid content (TFC) of *Tinospora cordifolia* varied markedly depending on the host plant on which it was growing, indicating a significant host-dependent influence on its secondary metabolite profile. In the present study, the TFC ranged from 0.240 to 0.921 mg QE/g DW, demonstrating almost a four-fold variation across host-associated samples. The highest flavonoid content was observed in Normal Giloy (NG), which recorded 0.921 mg QE/g DW (shown in Table 6 and Figure 4). This suggests that *T. cordifolia* growing independently (without host interference) may have a higher capacity for synthesising flavonoids, possibly due to unaltered physiological metabolism and optimal nutrient allocation. Similar findings have been reported in studies showing that metabolic pathways for phenolics and flavonoids are highly responsive to environmental and physiological conditions of the plant.[26] The next highest TFC was found in (shown in Figure 4) Neem-hosted Giloy (0.477 mg QE/g DW). Neem (*Azadirachta indica*) itself is rich in bioactive secondary metabolites, including phenolics and flavonoids, which may create a biochemical environment favourable for enhanced flavonoid synthesis in the attached *T. cordifolia*. Previous research suggests that host plants with strong antioxidant and phenolic profiles can influence parasitic or climbing plants through nutrient transfer or allelopathic interactions[27].

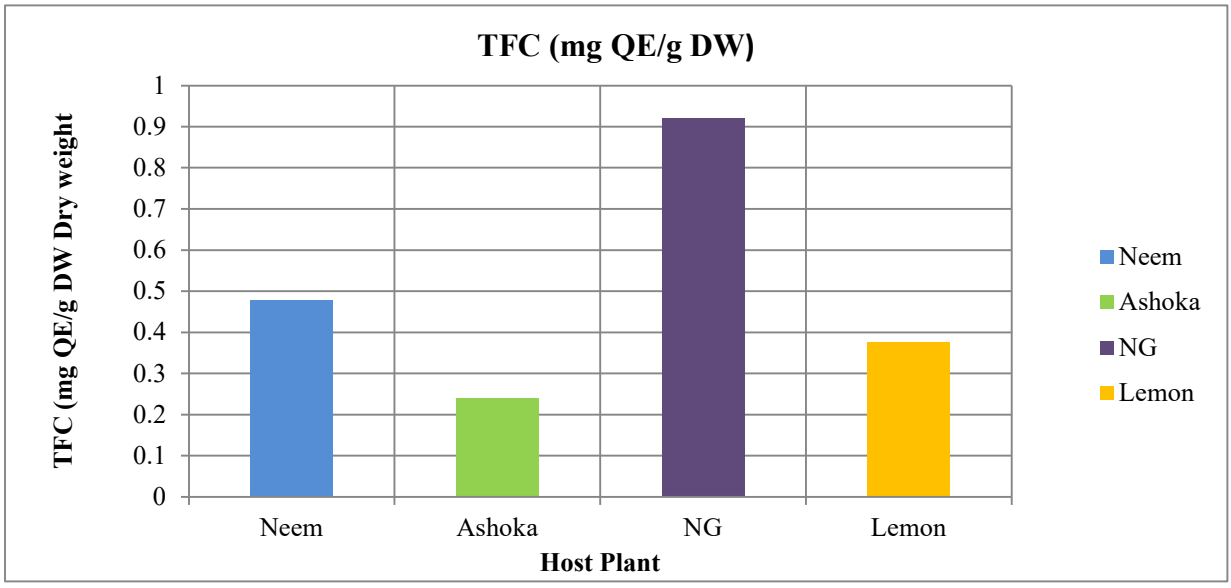

Figure 4. Total flavonoid content in *T. cordifolia* growing on different host plants. Values are expressed as mg quercetin equivalent per gram of dry weight (mg QE/g DW).

The sample growing on Lemon (0.376 mg QE/g DW) showed moderate flavonoid levels. Citrus species possess high acidic and flavonoid-rich tissues, which might modulate the stress physiology of *T. cordifolia*. However, the moderate values indicate that while lemons may offer certain metabolic advantages, they may also impose mild stress conditions that limit optimal flavonoid biosynthesis. The lowest TFC was recorded in Ashoka host plant samples (0.240 mg QE/g DW). *Saraca asoca* is known for its tannin-rich and alkaloid-rich phytochemistry, and such metabolites might competitively inhibit or alter the biosynthetic metabolic pathways of flavonoids in *T. cordifolia*. Stress or allelopathic factors may also contribute to the restriction of flavonoid accumulation. A similar suppression effect of host plants on secondary metabolites has been noted in previous studies involving host-dependent medicinal climbers.[28]

Overall, the results indicate a clear host-dependent modulation of flavonoid synthesis in *T. cordifolia*. Since flavonoids are crucial contributors to antioxidant, anti-inflammatory, and antimicrobial activity, the variation in TFC may correlate with the observed differences in biological activities across host-associated samples. Plants with higher TFC, such as those hosting NG and Neem, may exhibit stronger pharmacological potential.

Conclusion

India's rich flora provides a wealth of medicinal plants that can be incorporated into everyday dietary practices due to their numerous health and therapeutic benefits. The therapeutic and antioxidant effects of various phytochemicals present in these plants contribute to their medicinal properties. This study examines the influence of host plants, including *Azardirachta indica* (Neem plant), *Saraca asoca* (Ashoka plant), *Citrus limon* (Lemon), and normal giloy (independently growing), on the qualitative phytochemical screening of giloy stem. Phytochemical screening revealed the presence of carbohydrates, steroids, glycosides, quinones, phytosterols, flavonoids, proteins, and amino acids. Quantitative estimation revealed that normal *T. cordifolia* without a host exhibits the highest total flavonoid content. Using the DPPH radical scavenging method, Neem-hosted plant shows the highest antioxidant activity, and Neem-hosted giloy and regular giloy show the highest inhibitory activity in the antidiabetic assay (α-amylase test). The studies suggest that the host plant affects the bioactive profile of *T. cordifolia*. Therefore, further research is needed to determine the suitability of these phytochemical-rich plant resources for your health. It has been found that the stem of *T. cordifolia* growing on neem has more antioxidant activity. Therefore, it can be used as a natural antioxidant. For drug discovery and development, phytochemical screening is a valuable tool. This study revealed that the medicinal properties of *T. cordifolia* vary depending on the host plant on which it grows. So, we can use this plant as a reliable source for beneficial drugs.

References

1. Rana V, Thakur K, Sood R, Sharma V and Sharma TR 2012, Genetic diversity analysis of *Tinospora cordifolia* germplasm collected from northwestern Himalayan region of India. *Journal of Genetics* **91** 99–103.
2. Parthipan M, Aravindhan V and Rajendran A 2011, Medico-botanical study of Yercaud hills in the Eastern Ghats of Tamil Nadu, India. *Ancient Science of Life* **30** 104–109.
3. Department of Ayush 2001, *Ayurvedic Pharmacopoeia of* 2001, Part I, Vol. I.

Ministry of Health and Family Welfare, New Delhi, pp. 53–55.
4. Upadhyay AK, Kumar K, Kumar A and Mishra HS 2010, *Tinospora cordifolia* (Willd.) Hook. f. & Thoms. (Guduchi): validation of Ayurvedic pharmacology through experimental and clinical studies. *International Journal of Ayurveda Research* **1** 112–121.
5. Udayan PS, Pradeep AK and Balachandran I 2009, A new species of *Tinospora* (Menispermaceae) from South India. *Edinburgh Journal of Botany* **66** 77–80.
6. Saha S and Ghosh S 2012, *Tinospora cordifolia*: one plant, many roles. *Ancient Science of Life* **31** 151.
7. Meena AK, Singh A, Panda PPP, Mishra S and Rao MM 2010, *Tinospora cordifolia*: bioactivities and evaluation of physicochemical properties. *International Journal of Pharmacy and Pharmaceutical Research* **2** 50–55.
8. Sharma U, Bala M, Kumar N, Singh B, Munshi RK and Bhalerao S 2012, Immunomodulatory active compounds from *Tinospora cordifolia*. *Journal of Ethnopharmacology* **141** 918–926.
9. Goel HC, Prasad J, Singh S, Sagar RK, Agrawala PK, Bala M, Sinha AK and Dogra R 2004, Radioprotective potential of an herbal extract of *Tinospora cordifolia*. *Journal of Radiation Research* **45** 61–68.
10. Sonkamble VV and Kamble LH 2015, Antidiabetic potential and identification of phytochemicals from *Tinospora cordifolia*. *American Journal of Phytomedicine and Clinical Therapeutics* **3** 97–110.
11. Jayanthi P, Lalitha P and Sripathi SK 2011, Phytochemical investigation of the extracts of *Eichhornia crassipes* and its solvent fractionates. *Journal of Pharmaceutical Research* **4**.
12. Pavni K, Esha B, Neha J, Tushar A, Shrey K, Suchit A and Neeraj W 2011, Phytochemical screening of developing garlic and effect of its aqueous extracts on viability of cardiac cell line. *Journal of Pharmaceutical Research* **4**.
13. Abdulrazaq NB, Akram HB, Bero DN, Mohamad MYB, Malik IA and Rahman MT 2013, Addition of selenium to *Carica papaya* pulp extract enhances dermal wound healing activity. *Tropical Journal of Pharmaceutical Research* **12** 77–84.
14. Saranya MR 2013, Pharmacognostic profile and phytochemical investigation on the leaves of *Achyranthes aspera*. *International Journal of Pharmacy and Pharmaceutical Sciences* **5**.
15. Singh KL, Singh LR, Devi PG, Devi NR, Singh LS and Bag GC 2013, Comparative study of phytochemical constituents and total phenolic content in three species of *Hedychium*. *International Journal of PharmTech Research* **5** 601–606.
16. Sama K and Sivaraj R 2012, Pharmacognostical and phytochemical screening of fruits and leaves of *Cissus arnottiana*. *Asian Journal of Pharmaceutical and Clinical Research* **5** 64–66.
17. Sharma AK, Gangwar M, Tilak R, Nath G, Sinha ASK, Tripathi YB and Kumar D 2012, Comparative antimicrobial and phytochemical evaluation of *Jatropha curcas*. *Pharmacognosy Journal* **4** 34–40.
18. Garg P and Garg R 2018, Qualitative and quantitative analysis of *Tinospora cordifolia* in different solvent extracts. *Journal of Drug Delivery and Therapeutics* **8** 259–264.

19. Nurcholis W, Putri DNS, Husnawati H, Aisyah SI and Priosoeryanto BP 2021, Total flavonoid content and antioxidant activity of *Amomum compactum*. *Annals of Agricultural Sciences* **66** 58.
20. Marseti SW, Hermanto FE, Widyananda MH, Rosyadah N, Kamila FS, Annisa Y, Dwijayanti DR, Ulfa SM and Widodo N 2024, Pharmacological potential of *Clinacanthus nutans* against lung cancer. *Journal of Bioactive Products from Nature* **14** 343.
21. Hossain MA and Nagooru MR 2011, Biochemical profiling and flavonoid content of *Cordyline terminalis*. *Pharmacognosy Journal* **3** 25–30.
22. Ministry of Health & Family Welfare – GOI 2010, *Indian Pharmacopoeia, Vol. III*. Indian Pharmacopoeia Commission. Ghaziabad.
23. Suthindhiran KR, Jayasri MA and Kannabiran K 2009, α-Glucosidase and α-amylase inhibitory activity of *Micromonospora* sp. *International Journal of Integrative Biology* **6** 115.
24. Singh A 2018, Host-dependent phytochemical variation in medicinal climbers. *Journal of Medicinal Plant Research*.
25. Sharma R and Gupta V 2020, Influence of host species on antioxidant profiles of *Tinospora cordifolia*. *Pharmacognosy Reviews*.
26. Kumar M, Kumari P, Kumari A, and Akhtar M S 2024 Occupancy of Flavonoids in Plants, Pathogen, and Opportunistic Fungi Interactions. In: *Opportunistic Fungi, Nematode and Plant Interactions: Interplay and Mechanisms* (pp. 125-145). Singapore: Springer Nature Singapore.
27. Barlaskar WH, Sharma NR, Kumar G and Kataria S 2017, Effect of host trees on berberine content in stem of *Tinospora cordifolia* climber: A preliminary study. *Research Journal of Pharmacy and Technology* **10(3)** 818–822.
28. Patel DK, Prasad SK, Kumar R and Hemalatha S 2012, An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific Journal of Tropical Biomedicine* **2(4)** 320–330.