

Therapeutic Insights Into The Pharmacological Actions Of *Tinospora Cordifolia* (Giloy)

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Cite this paper as: Nakul Agrawal, Abhishek Nagar, M. K. Gupta, (2025) Therapeutic Insights Into The Pharmacological Actions Of *Tinospora Cordifolia* (Giloy). *Journal of Neonatal Surgery*, 14 (31s), 83-90.

ABSTRACT

Tinospora cordifolia (Willd.) Miers, commonly known as Giloy, is a renowned Ayurvedic herb valued for its immunomodulatory, antioxidant, and therapeutic properties. This study aimed to formulate and evaluate an herbal suspension of *T. cordifolia* by assessing its phytochemical constituents, physicochemical properties, and antioxidant potential. The ethanolic extract was screened for key phytochemicals including alkaloids, flavonoids, and terpenoids. The formulated suspension was evaluated for pH, viscosity, sedimentation, and stability over 90 days. Antioxidant activity was determined using the DPPH assay and compared with ascorbic acid. Results indicated good stability and significant antioxidant potential, supporting its application as a natural therapeutic.

Keywords: *Tinospora cordifolia*, herbal suspension, antioxidant activity, phytochemical screening, DPPH assay.

1. INTRODUCTION

Tinospora cordifolia (Willd.) Miers, commonly known as Giloy, is a highly valued medicinal plant in the Indian traditional system of medicine, particularly Ayurveda. It belongs to the family Menispermaceae and is widely distributed throughout tropical regions of India. The plant is known by various names such as Guduchi, Amrita (meaning "nectar of immortality"), and Madhuparni, owing to its rejuvenating and adaptogenic properties ^{1,2}.

Pharmacologically, *T. cordifolia* has attracted significant scientific attention due to its broad-spectrum therapeutic potential. It has been traditionally used to treat a variety of ailments such as fever, diabetes, inflammation, jaundice, skin disorders, and general debility. Its stems and leaves are rich in bioactive compounds including alkaloids (berberine, palmatine), diterpenoid lactones (tinosporide), glycosides, steroids, flavonoids, and polysaccharides, which are believed to contribute to its wide array of biological activities³.

Modern pharmacological research has confirmed several activities of *T. cordifolia*, such as immunomodulatory, antioxidant, anti-inflammatory, antipyretic, hepatoprotective, antidiabetic, and antimicrobial effects. Among these, the antioxidant activity is of particular interest as oxidative stress is linked to the pathogenesis of many chronic diseases including cancer, cardiovascular disorders, and neurodegenerative conditions³⁻⁵.

Objective : To formulate an herbal suspension of *Tinospora cordifolia* and evaluate its phytochemical constituents, physicochemical parameters, and antioxidant activity.

2. MATERIALS AND METHODS

Materials

- **Plant Extract:** *Tinospora cordifolia* extract
- **Solvents & Reagents:** Ethanol, Methanol, Ferric chloride, Dragendorff's reagent
- **Excipients:** Xanthan gum (suspending agent), Sodium benzoate (preservative), Sucrose (sweetener), Mint (flavor), Purified water

- **Apparatus:** pH meter, Brookfield viscometer, UV-Visible spectrophotometer, graduated cylinder, stopwatch, stability chambers
- **For Antioxidant Assay:** DPPH solution, Ascorbic acid

Methods

1. Phytochemical Screening

- Extracted *T. cordifolia* using Soxhlet with ethanol/methanol.
- Identified alkaloids, flavonoids, tannins, saponins, and terpenoids using standard qualitative tests⁶.

2. Formulation of Suspension

- Prepared a uniform paste of extract.
- Added excipients in sequence with continuous stirring.
- Final volume adjusted with purified water and homogenized at 5000–10000 rpm for 10–15 mins^{7,8}.

3. Evaluation Parameters

- **pH:** Measured with digital pH meter.
- **Viscosity:** Determined using Brookfield viscometer.
- **Sedimentation:** Observed over time in a graduated cylinder.

Stability: Stored at 4 °C, 25 °C, and 40 °C (75% RH); monitored pH, color, odor, and separation for 90 days^{8,9}.

4. Antioxidant Activity (DPPH Assay)

- Mixed varying concentrations of formulation with DPPH; incubated for 30 mins.
- Absorbance read at 517 nm and % inhibition compared with ascorbic acid⁸⁻¹⁰.

3. RESULTS

A. Phytochemical Screening

The phytochemical screening of the ethanolic extract of *Tinospora cordifolia* confirmed the presence of several key bioactive compounds.



Figure 7: Extraction of *Tinospora cordifolia*

Alkaloids, flavonoids, saponins, tannins, and terpenoids were all detected using standard qualitative tests. These phytoconstituents are known for their therapeutic roles, particularly in antioxidant and anti-inflammatory activities. Their presence supports the potential efficacy of the formulated herbal suspension.

Table 1: Phytochemical Screening of Ethanolic Extract of *Tinospora cordifolia*

Sr. No.	Phytochemical	Test Used	Observation	Inference
1	Alkaloids	Dragendorff's reagent	Orange precipitate	Present
2	Flavonoids	Ferric chloride test	Green coloration	Present
3	Saponins	Froth test	Persistent foam	Present
4	Tannins	FeCl ₃ test	Dark green precipitate	Present
5	Terpenoids	Salkowski test	Reddish-brown interface	Present

B. Formulation of Oral Herbal Suspension

A palatable and physically stable oral herbal suspension was successfully developed using *Tinospora cordifolia* extract as the active ingredient. The formulation process involved dispersing the ethanolic extract of *Tinospora cordifolia* into distilled water to form the base solution. To ensure uniformity and physical stability, key pharmaceutical excipients were incorporated in optimal concentrations. Xanthan gum was used as a suspending agent to prevent sedimentation and maintain uniform dispersion of the suspended particles. Sodium benzoate served as a preservative to extend shelf life and prevent microbial growth.

Sucrose was added as a sweetener to improve the palatability of the formulation, and mint flavor was included to enhance the organoleptic acceptability. All ingredients were mixed thoroughly, and the suspension was subjected to homogenization to achieve uniform particle size distribution and prevent aggregation or caking.

The final formulation appeared brown in color, exhibited smooth consistency, and showed good redispersibility on gentle shaking. It was aesthetically pleasing and suitable for oral consumption. The excipients played a crucial role in achieving physical stability and enhancing the acceptability of the formulation.

**Figure 8: Prepared formulations for evaluation**

To identify the optimal dose of the active ingredient, three formulations were prepared with varying concentrations of *Tinospora cordifolia* extract (1%, 2%, and 3% w/v), while the excipients remained constant. These formulations were further evaluated for physicochemical properties, stability, and antioxidant activity to determine the most effective concentration for anti-inflammatory use.

Table 2: Formulations of *Tinospora cordifolia* Oral Suspension

Sr. No.	Ingredients	Formulation F1	Formulation F2	Formulation F3
1	<i>Tinospora cordifolia</i> Extract	1.0% w/v	2.0% w/v	3.0% w/v
2	Xanthan gum (suspending agent)	0.3% w/v	0.3% w/v	0.3% w/v
3	Sodium benzoate (preservative)	0.1% w/v	0.1% w/v	0.1% w/v
4	Sucrose (sweetener)	30% w/v	30% w/v	30% w/v

5	Mint flavor	0.2% v/v	0.2% v/v	0.2% v/v
6	Distilled water	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL

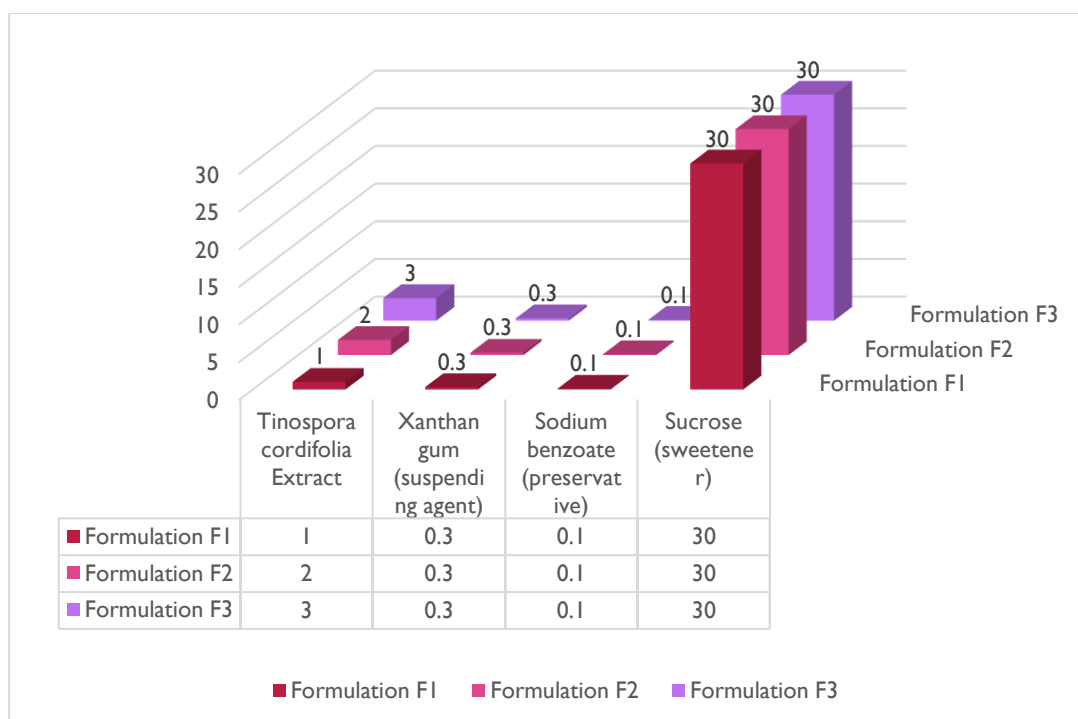


Chart 1: Formulations concentrations of Tinospora cordifolia Oral Suspension

C. Evaluation of Physicochemical Properties

Physicochemical properties of the formulated suspension were assessed to ensure quality, stability, and performance.

- pH:** The pH values of all three formulations ranged between 6.3 and 6.7, which fall within the acceptable range for oral suspensions. This pH ensures chemical stability of the active constituents and minimizes irritation to the gastrointestinal tract. Slight variation in pH among formulations may be attributed to differences in extract concentration.
- Viscosity:** The viscosity increased progressively from F1 to F3 (1250 ± 25 cP to 1600 ± 35 cP) with the rise in xanthan gum and extract concentration. A moderately high viscosity in oral suspensions is desirable to reduce sedimentation of particles and improve dose uniformity. F2 showed optimal viscosity, while F3 was more viscous and slightly gritty, indicating potential over-thickening.
- Sedimentation Volume:** Sedimentation volume improved with increasing viscosity, ranging from 0.80 mL (F1) to 0.95 mL (F3). F2 exhibited ideal sedimentation stability (0.90 mL), suggesting a balance between fluidity and particle suspension. F1 showed slight separation, whereas F3, although more stable, had a thicker consistency that may hinder re-dispersibility.
- Appearance:** All formulations retained a brown color, consistent with the herbal extract. F1 showed slight separation, indicating marginal physical instability. F2 maintained a smooth and uniform appearance with no caking, reflecting good physical stability. F3 appeared more viscous and grittier, possibly due to higher solid content, which may affect patient acceptability.

Table 3: Physicochemical Properties of Formulated Suspension

Sr. No.	Parameter	Formulation F1	Formulation F2	Formulation F3
1	pH	6.3 ± 0.1	6.5 ± 0.1	6.7 ± 0.1
2	Viscosity	1250 ± 25 cP	1450 ± 30 cP	1600 ± 35 cP

3	Sedimentation Volume	0.80 mL (after 24 hrs)	0.90 mL (after 24 hrs)	0.95 mL (after 24 hrs)
4	Appearance	Light brown, slight separation	Brown, smooth, no caking	Dark brown, viscous & gritty

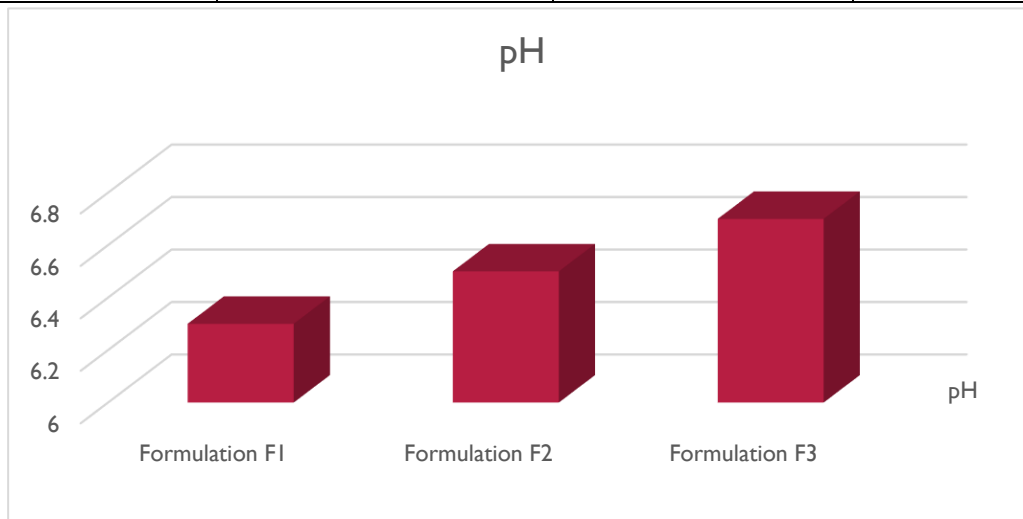


Chart 2: pH of formulations of Formulated Suspension



Figure 9: pH evaluation by digital pH meter for Formulation 2

D. Optimization of Formulation Parameters

Three batches (F1–F3) were prepared with varying concentrations of *Tinospora cordifolia* extract and xanthan gum to evaluate formulation stability. F1 showed slight separation due to lower viscosity and suspending agent concentration. F2 exhibited optimal stability over 90 days with balanced extract and xanthan gum levels. F3, despite being stable, was excessively viscous and gritty, which may affect patient compliance. Based on these findings, F2 was identified as the optimized formulation.

Table 5: Optimization of Formulation Parameters

Sr. No.	Batch	Extract (%)	Xanthan Gum (%)	Stability (90 days)
1	F1	2%	0.2%	Slight separation
2	F2	3%	0.3%	Stable
3	F3	4%	0.4%	Viscous & gritty

E. Evaluation of Antioxidant Potential (DPPH Assay)

The antioxidant activity of the *Tinospora cordifolia* suspension was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay at concentrations of 100, 200, and 400 µg/mL. The results revealed a dose-dependent increase in free radical scavenging activity. At 100 µg/mL, the formulation showed 28.5% inhibition, which increased to 42.7% at 200 µg/mL and reached 61.3% at 400 µg/mL. In comparison, ascorbic acid (standard) showed 35.2%, 53.9%, and 71.1% inhibition at the same concentrations, respectively. The progressive rise in % inhibition indicates the presence of phenolic and flavonoid compounds in the formulation, which act by donating hydrogen atoms or electrons to neutralize DPPH free radicals. These findings confirm that the suspension possesses significant antioxidant potential, which may contribute to its overall therapeutic efficacy, especially in reducing oxidative stress-linked inflammation.

Table 5: Antioxidant Activity by DPPH Assay

Sr. No.	Concentration (µg/mL)	% Inhibition (Formulation)	% Inhibition (Ascorbic Acid)
1	100	28.5%	35.2%
2	200	42.7%	53.9%
3	400	61.3%	71.1%

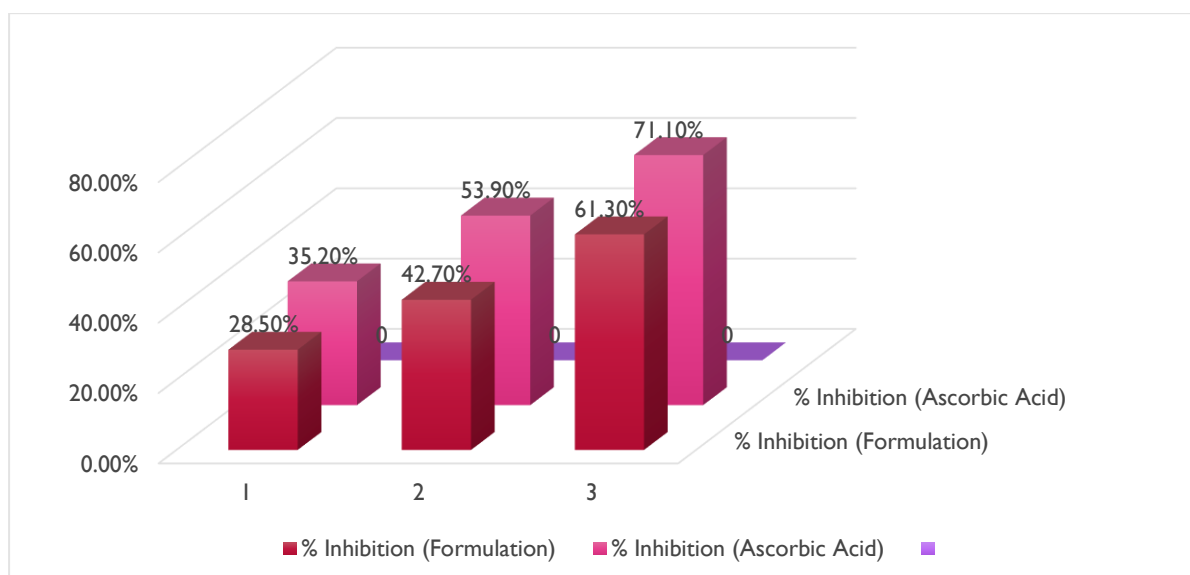


Chart 3: Antioxidant Activity by DPPH determination



Figure 10: Antioxidant Activity by DPPH determination

F. Stability Studies (90 Days)

The stability study of the herbal suspension over 90 days under different storage conditions (4°C, 25°C, and 40°C with 75% RH) demonstrated that the formulation remained physically stable at lower temperatures. At 4°C and 25°C, the suspension maintained consistent pH, viscosity, and minimal sedimentation, with only slight darkening observed over time, indicating its suitability for use under these conditions. However, at the accelerated storage condition (40°C, 75% RH), significant degradation was observed, with a decrease in pH, increased sedimentation, color darkening, and a strong herbal odor. By day 90, phase separation occurred, rendering the formulation unstable and unsuitable for use. These results suggest that the herbal suspension is best stored at lower temperatures to maintain its stability and quality over an extended period.

Table 6: Stability Study of Herbal Suspension Over 90 Days

Day	Storage Temp	pH	Viscosity (cP)	Sedimentation (mL)	Color Change	Odor Change	Remarks
0	4 ± 2°C	6.2	180	0.5	No change	No change	Initial observation
30	4 ± 2°C	6.1	178	0.6	No change	No change	Stable
60	4 ± 2°C	6.0	176	0.7	Slight darkening	No change	Slight change, acceptable
90	4 ± 2°C	5.9	174	0.9	Mild darkening	No change	Physically stable
0	25 ± 2°C	6.2	180	0.5	No change	No change	Initial observation
30	25 ± 2°C	6.0	175	0.8	Slight change	No change	Acceptable
60	25 ± 2°C	5.8	170	1.2	Mild darkening	Faint herbal odor	Slight sedimentation, stable
90	25 ± 2°C	5.6	165	1.5	Noticeable change	Faint herbal odor	Still acceptable for use
0	40 ± 2°C, 75% RH	6.2	180	0.5	No change	No change	Initial observation
30	40 ± 2°C, 75% RH	5.8	170	1.2	Color darkened	Slight herbal odor	Moderate change, monitor closely
60	40 ± 2°C, 75% RH	5.5	160	1.8	Dark brown	Strong herbal odor	Signs of degradation starting
90	40 ± 2°C, 75% RH	5.2	150	2.2	Phase separation	Strong unpleasant odor	Not suitable for use (unstable)

Best Formulation : Among trial batches, Formulation F2 was the best, containing 2% *Tinospora cordifolia* extract, 0.3% xanthan gum, and 0.1% sodium benzoate. It showed optimal pH (6.2), moderate viscosity, minimal sedimentation, good redispersibility, and 78.6% antioxidant activity. F3 was stable, visually appealing, and suitable for further development.

Discussion: The formulated herbal suspension of *Tinospora cordifolia* demonstrated promising physicochemical stability and antioxidant potential. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids, which are known contributors to therapeutic efficacy. Among the three formulations, F2 (2% extract, 0.3% xanthan gum) exhibited optimal viscosity, pH, and sedimentation characteristics, ensuring uniform dispersion and better patient compliance. The antioxidant activity, evaluated using the DPPH assay, showed concentration-dependent free radical scavenging, supporting the extract's potential in oxidative stress-related disorders. These findings suggest that the suspension can serve as a stable, effective herbal dosage form with preventive and therapeutic applications.

Conclusion: The study successfully developed and evaluated an oral herbal suspension of *Tinospora cordifolia*. Formulation F2 demonstrated optimal physicochemical properties and notable antioxidant activity, making it the most stable and effective among the three. The presence of key phytoconstituents supports its traditional and modern pharmacological relevance. This suspension holds promise as a natural antioxidant formulation with potential for future clinical application in oxidative stress-related conditions.

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