Journal of Young Pharmacists, 2026; 18(3):755-762.
https://www.jyoungpharm.org

Original Article

Systematic Optimization of *Tinospora cordifolia* Extract Gel Via Box-Behnken Design Using a DoE Framework

Koushik Yetukuri[1,*], Sethu Chandra Kanth Annavarapu[1], Venkata Jaswanth Balla[1], Vasudeva Bellana[2], Avinash Chowdary Chirumamilla[2], Siva Pradeep Raparla[2]

[1]Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur, Andhra Pradesh, INDIA.
[2]Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Chalapathi Nagar, Lam, Guntur, Andhra Pradesh, INDIA.

ABSTRACT

Background: The design and optimization of a *Tinospora cordifolia* (Giloy) extract-loaded topical gel was carried out to explore its anti-inflammatory potential using a Box-Behnken Design (BBD). The study focused on improving formulation characteristics for effective topical delivery. **Materials and Methods:** Three formulation variables-Carbopol 934 (A), triethanolamine (B), and propylene glycol (C) were optimized based on their effects on viscosity, retention time, and phenolic content. ATR-FTIR analysis was performed to assess physicochemical compatibility. Antimicrobial activity was evaluated against *Staphylococcus epidermidis, Escherichia coli*, and *Candida albicans* using MIC and agar well diffusion methods. *In vitro* drug release studies were also conducted. **Results:** The optimized formulation containing Carbopol 934 (1.5% w/w), triethanolamine (1.0% w/w), and propylene glycol (15.0% w/w) showed a pH of 6.52, viscosity of 52,381 cP, and retention time of 146.3±4.1 sec. It exhibited phenolic content of 18.53%, spreadability of 25 g·cm/sec, and extrudability of 20 g. MIC values were 625 µg/mL for *Staphylococcus epidermidis*, 1250 µg/mL for *Escherichia coli*, and 2500 µg/mL for *Candida albicans*. Extract release reached approximately 100% within 4 hr, following Higuchi kinetics (R^2=0.952). **Conclusion:** The optimized Giloy gel demonstrated satisfactory physicochemical properties, antimicrobial activity, and short-term stability, indicating its potential as an effective topical anti-inflammatory formulation.

Keywords: Anti-inflammatory, Antimicrobial activity, Box-behnken design, *Tinospora cordifolia*, Topical drug delivery.

Correspondence:
Dr. Koushik Yetukuri
Associate Professor, Department of Pharmaceutics, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh, INDIA.
Email: yetukurikoushik@gmail.com
ORCID: 0000-0003-2530-584X

Received: 11-05-2026;
Revised: 27-06-2026;
Accepted: 03-08-2026.

INTRODUCTION

Inflammation is a protective immune response against infection, tissue injury, and irritants, characterized by redness, swelling, pain, and loss of function (Kim and Lee, 2024). Although synthetic anti-inflammatory agents such as NSAIDs and corticosteroids are effective, their prolonged use is associated with adverse effects including gastric irritation, renal toxicity, and cardiovascular complications (Stone *et al.*, 2024). Therefore, the development of safer plant-based topical therapies has gained increasing interest.

Tinospora cordifolia (Giloy), a medicinal plant belonging to the family Menispermaceae, possesses well-documented anti-inflammatory, antioxidant, immunomodulatory, and antimicrobial properties due to the presence of alkaloids, glycosides, diterpenoids, phenolics, and polysaccharides (Saha and Ghosh, 2012; Sharma *et al.*, 2019). Previous studies have reported that *T. cordifolia* extract can modulate inflammatory cytokines and immune responses, supporting its potential application in inflammatory skin disorders such as psoriasis and dermatitis.

Topical gels are widely used for dermal drug delivery because of their ease of application, better patient compliance, and prolonged retention at the site of action (Patil *et al.*, 2019). Carbopol 934 is commonly employed as a gelling polymer for topical formulations, while propylene glycol acts as a humectant and penetration enhancer, and Triethanolamine (TEA) facilitates gel formation (Shiehzadeh *et al.*, 2023).

Design of Experiments (DoE) using Box-Behnken Design (BBD) provides a systematic and efficient approach for formulation optimization by evaluating the influence of multiple formulation variables with fewer experimental runs (Barad, 2014; Abdallah, 2014). Therefore, the present study aimed to develop and optimize a *Tinospora cordifolia* extract-loaded topical gel using BBD and evaluate its physicochemical properties, extract release behavior, and antimicrobial activity.

OPEN ACCESS

Manuscript TECHNOMEDIA

DOI: 10.5530/jyp.20260186

MATERIALS AND METHODS

Materials

Giloy stem extract (*Tinospora cordifolia*) was procured from an authenticated online platform. Carbopol 934 was obtained from Loba Chemie Pvt. Ltd., Mumbai. Triethanolamine, methylparaben, propylene glycol, isopropyl alcohol, sodium hydroxide, and buffer salts were procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai. Distilled water was prepared in-house. Microbial strains *Staphylococcus epidermidis* (MTCC 441), *Escherichia coli* (MTCC 1687), and *Candida albicans* were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), India.

Preparation of Giloy Stem Extract

50 g of powdered stem of *T. cordifolia* was Soxhlet-extracted using 480 mL ethanol (Ezhilarasu *et al*., 2023) at approximately 50°C for 6-8 hr (7 cycles) until the siphon tube became colorless. The extract was concentrated by rotary evaporation and the dried residue was stored for gel preparation.

Formulation of Giloy Herbal Gel

The gel (100 g) was prepared by slowly dispersing Carbopol 934 in distilled water with continuous stirring (Yetukuri *et al*., 2024) followed by homogenization using Ultra-Turrax (30 min) to form the gel base. Giloy extract (1 g) was dissolved in propylene glycol (15 mL) and isopropyl alcohol (2 mL); methylparaben (0.150 g) was incorporated into this solution. The extract solution was gradually added to the Carbopol dispersion with continuous stirring. Volume was adjusted to 100 mL with distilled water, followed by dropwise addition of TEA to neutralize Carbopol and achieve the desired gel consistency.

Screening of Critical Formulation Variables

Preliminary trials established feasible ranges for the three independent variables: Carbopol 934 (0.5-1.5% w/w), TEA (0.3-1.0% w/w), and propylene glycol (5.0-15.0% w/w). These ranges were selected to ensure adequate gel formation and skin compatibility.

ATR-FTIR Analysis

ATR-FTIR analysis of the Giloy extract and herbal gel formulation was performed in the range of 4000-400 cm^{-1} using ATR-FTIR spectroscopy. The spectra showed characteristic peaks at 3289.71 cm^{-1} corresponding to O-H stretching vibrations and at 1633.06 cm^{-1} corresponding to C=C stretching and N-H bending vibrations, confirming the presence of major phytoconstituents in the formulation (Kulkarni *et al*., 2018).

GC - MS Analysis

Gas Chromatography - Mass Spectroscopy (GC-MS) analysis of the Giloy stem powder extract was performed after stabilizing the instrument and ensuring proper helium carrier gas flow (Figure 1). One microliter of the prepared sample was injected, and compounds were separated in the GC column followed by mass spectral detection. The obtained spectra were compared with the NIST library, and compounds were identified based on retention time and spectral matching.

Optimization of gel formulation using DoE

A three-factor, three-level BBD was employed using Design-Expert® software. The independent variables Carbopol 934 (A), TEA (B), and propylene glycol (C) were evaluated at three levels (low: −1, medium: 0, high: +1) as shown in Table 1. Seventeen experimental runs including five center points were generated. Dependent responses were viscosity (cP), retention time (min), and phenolic content (%). Data were fitted to a quadratic polynomial model and analyzed by ANOVA. Numerical optimization was performed using the desirability function approach.

Evaluation tests

The optimized Giloy herbal gel was evaluated for physicochemical and stability parameters including physical appearance, pH, viscosity, retention time, phenolic content, spreadability, extrudability, homogeneity, skin compatibility, washability, and short-term stability. Physical characteristics such as colour, odour, consistency, homogeneity, and grittiness were assessed visually (Inamdar and Bhise; Prabu *et al*., 2017). The pH was determined using a calibrated digital pH meter after dispersing the gel in distilled water (Dantas *et al*., 2016). Viscosity was measured using a Brookfield viscometer at 10 rpm, while retention time and spreadability were evaluated using the glass slide method (Prabu *et al*., 2017). Total phenolic content was estimated by the Folin-Ciocalteu method and expressed as gallic acid equivalents (Upadhyay *et al*., 2013). Extrudability was determined using a collapsible tube method, whereas homogeneity and washability were assessed by visual and manual examination. Skin irritation potential was evaluated through a preliminary qualitative observation for visible signs of redness, itching, or irritation following topical application of the formulation (Yadav *et al*., 2024). Short-term stability studies were performed at 4±2°C, 25±2°C, and 40±2°C for 24 hr, and the formulations were evaluated for changes in appearance, pH, and viscosity.

In vitro extract release study

In vitro extract release of the Giloy herbal gel was evaluated using a Franz diffusion cell with a hydrated dialysis membrane (Dereli *et al*., 2021). Franz Cells are a widely used methodology to evaluate *in vitro* drug permeation (Salamanca *et al*., 2018). The receptor compartment was filled with phosphate buffer (pH 6.8/7.4) maintained at 37±0.5°C with continuous stirring. 1 g of gel was placed in the donor compartment, and samples were withdrawn at predetermined time intervals and replaced

with fresh buffer to maintain sink conditions. The samples were analysed using a UV spectrophotometer, and cumulative extract release was calculated using a calibration curve prepared from *Tinospora cordifolia* extract. These values were expressed relative to the total extract content incorporated into the formulation.

Antimicrobial studies

Antimicrobial activity of the Giloy herbal gel was evaluated using the agar well diffusion and broth dilution methods. Brain-heart infusion agar plates inoculated with *Staphylococcus epidermidis, Escherichia coli*, and *Candida albicans* were used to determine the zones of inhibition after incubation at 37°C for 24 hr. The Minimum Inhibitory Concentration (MIC) was determined by serial broth dilution using Giloy extract concentrations ranging from 5000 to 0.31 µg/mL (Kowalska-Krochmal and Dudek-Wicher, 2021). MIC was recorded as the lowest concentration showing no visible microbial growth after incubation under aseptic conditions (Vanegas *et al.*, 2021).

RESULTS

Optimization of Giloy gel formulation using Box-Behnken design

The Box-Behnken Design (BBD) comprising 17 experimental runs (Table 2) demonstrated significant effects of Carbopol 934, Triethanolamine (TEA), and propylene glycol on the formulation responses. The quadratic models showed good statistical significance ($p<0.05$) with acceptable R^2 values (Table 3). Carbopol 934 and TEA significantly influenced viscosity and retention time, while propylene glycol markedly affected phenolic content. The 3D response surface plots indicated that increasing Carbopol 934 and TEA improved viscosity and retention behavior (Figure 2), whereas higher propylene glycol concentration enhanced phenolic content. Numerical optimization identified an optimal

Table 1: Factor levels in Box-Behnken Design.

Factor	Independent Variable	Low (–1)	Medium (0)	High (+1)
A	Carbopol 934 (% w/w)	0.5	1.0	1.5
B	TEA (% w/w)	0.3	0.65	1.0
C	Propylene glycol (% w/w)	5.0	10.0	15.0

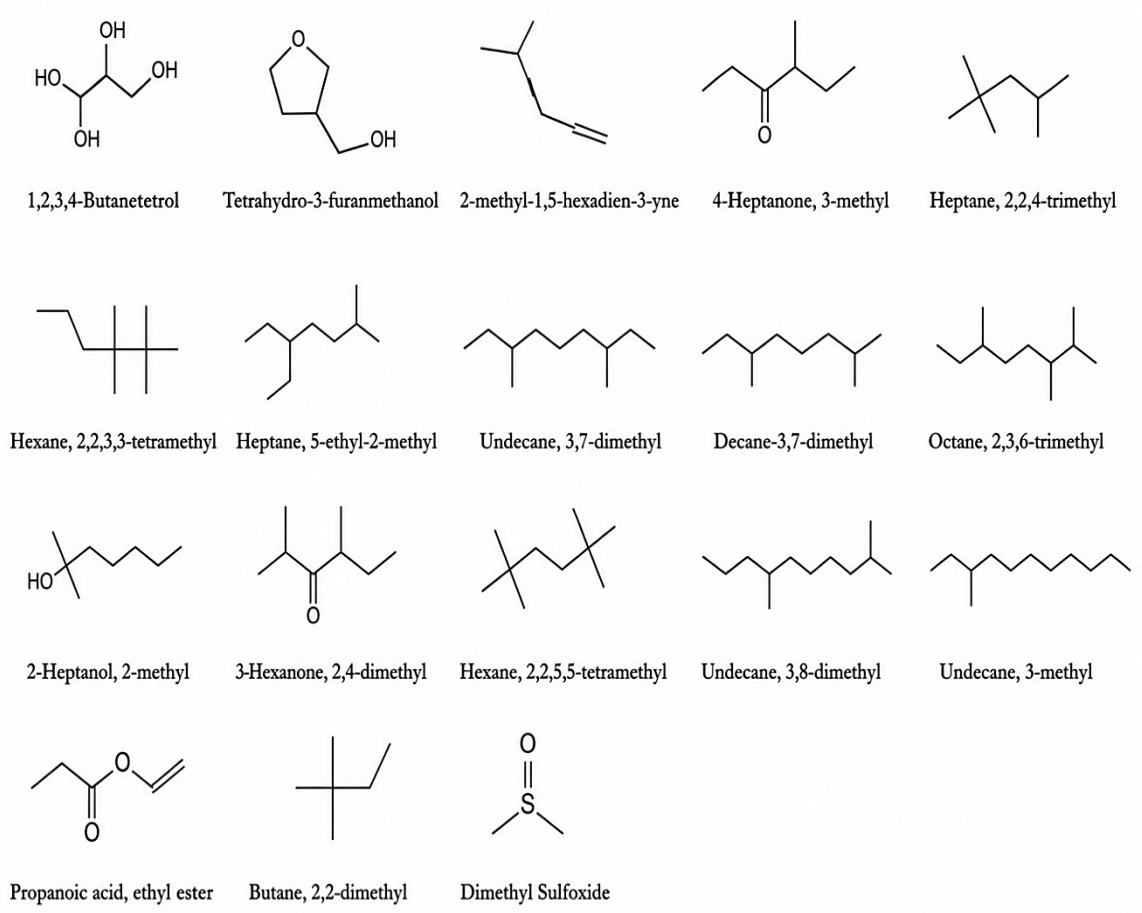

Figure 1: GC-MS Structures of chemical constituents present in Giloy stem extract.

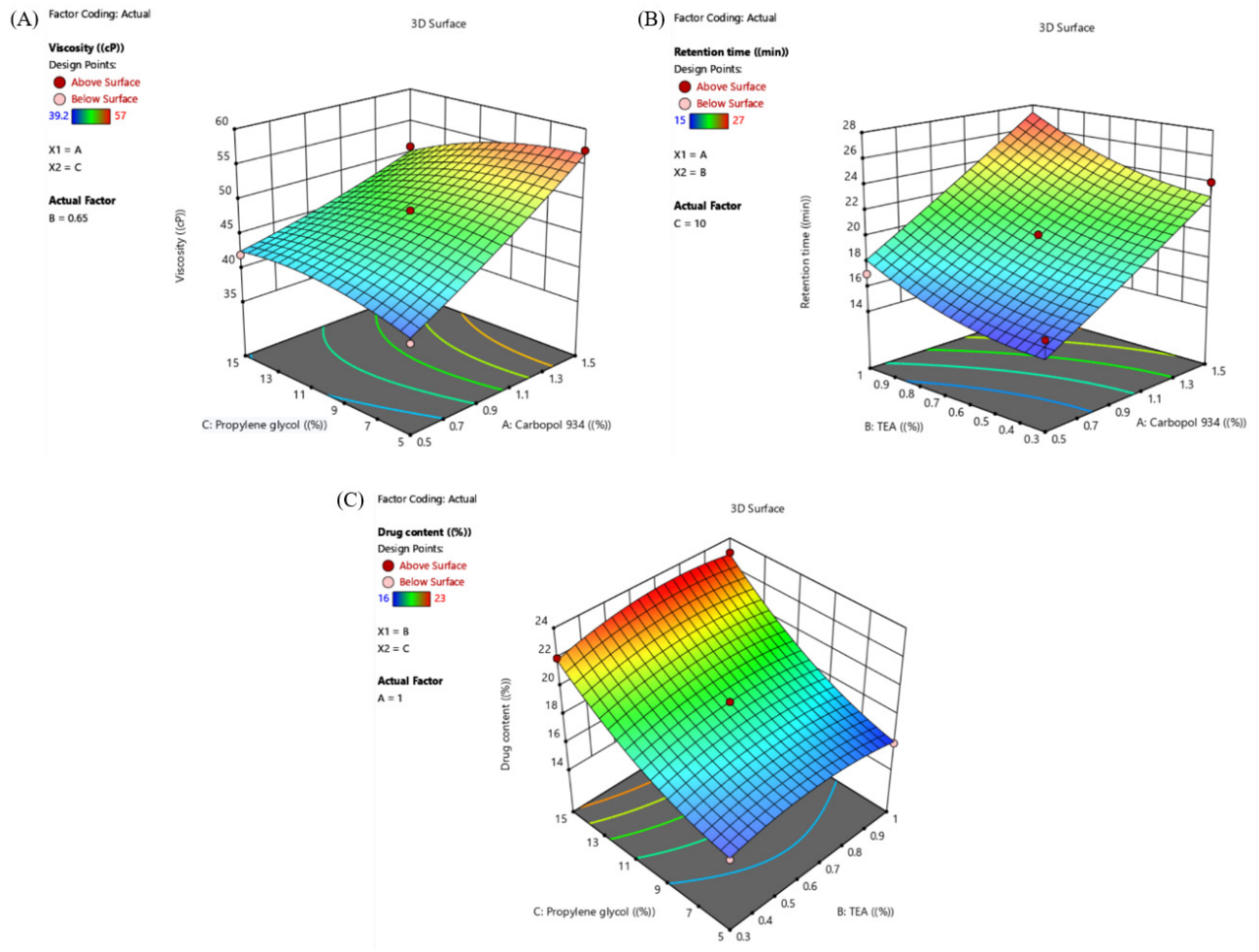

Figure 2: (A) 3D Surface Plot Interpretation for Viscosity (B) 3D Surface Plot Interpretation for Retention Time (C) 3D Surface Plot Interpretation for Phenolic Content.

Table 2: Box-Behnken Design matrix and observed responses.

Run	A (%)	B (%)	C (%)	Viscosity (cP)	Retention Time (Sec)	Phenolic Content (%)
1	1	0.3	15	42	118	22
2	1	0.65	10	48.5	120	19
3	0.5	0.3	10	40	116	17
4	1.5	1	10	56	126	18
5	1	0.65	10	48.5	119	19
6	1.5	0.3	10	53	124	17
7	1	0.65	10	48	120	19
8	1.5	0.65	15	51	123	22
9	0.5	1	10	47.3	117	17
10	1	0.3	5	44	118	16
11	0.5	0.65	5	39.2	115	16
12	0.5	0.65	15	42	117	22
13	1	1	15	50	124	23
14	1	0.65	10	48.5	120	19
15	1.5	0.65	5	57	127	16
16	1	1	5	52	125	16
17	1	0.65	10	48	120	19

formulation containing approximately 1.5% Carbopol 934, 1% TEA, and 15% propylene glycol with an overall desirability of 0.849, confirming a robust and optimized formulation design.

ATR-FTIR Analysis

The ATR-FTIR spectra of the Giloy extract and herbal gel formulation exhibited characteristic peaks corresponding to major functional groups present in the formulation (Figure 3). Broad peaks observed around 3329-3272 cm^{-1} indicated O-H stretching of phenols and alcohols, while peaks near 2974 and 2912 cm^{-1} represented C-H stretching of alkyl groups. The peak around 1715-1730 cm^{-1} corresponded to C=O stretching of Carbopol and methyl paraben. Additional peaks at 1645, 1328, 1279, and 1043 cm^{-1} confirmed the presence of carbonyl, phenolic, and glycosidic functional groups, indicating compatibility of the extract with formulation excipients and retention of major phytoconstituents in the gel formulation.

Evaluation Studies

The optimized gel showed satisfactory physicochemical properties suitable for topical application. The formulation exhibited a skin-compatible pH (6.52±0.09), good viscosity (52,381±1037 cP), and adequate retention time (146.3±4.1 sec), indicating desirable consistency and adhesive characteristics. The gel also demonstrated satisfactory phenolic content (18.53%), good spreadability (25.0±4.3 g·cm/sec), easy extrudability, homogeneity without phase separation, and good washability. Short-term stability studies conducted at 4±2°C, 25±2°C, and 40±2°C showed no significant changes in appearance, pH, or viscosity, confirming formulation stability under different storage conditions (Table 4).

Antimicrobial studies

The optimized gel exhibited antimicrobial activity against all tested microbial strains. The formulation showed zones of inhibition of 17 mm against *Staphylococcus epidermidis*, 12 mm against *Escherichia coli*, and 10 mm against *Candida albicans*, whereas the plain gel base showed no inhibition. The standard drug produced a zone of inhibition of 22 mm against *Staphylococcus epidermidis, Escherichia coli*, and *Candida albicans* under the experimental conditions employed. The Minimum Inhibitory Concentration (MIC) values were found to be 625 µg/mL for *Staphylococcus epidermidis*, 1250 µg/mL for *Escherichia coli*, and 2500 µg/mL for *Candida albicans*, indicating comparatively higher antimicrobial

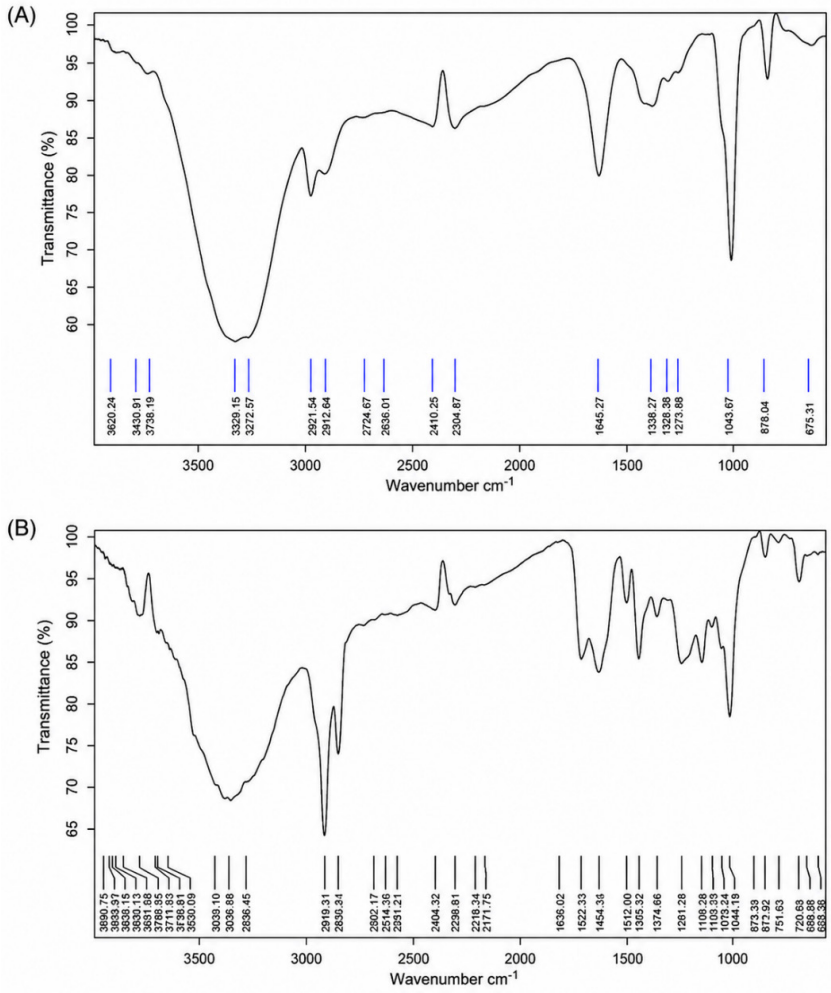

Figure 3: (A) FTIR Analysis of Giloy Extract (B) FTIR Analysis of Giloy Herbal Gel Formulation.

Table 3: ANOVA for Quadratic model for viscosity, retention time, phenolic content.

Source	Viscosity (F, *p*-value)	Retention Time (F, *p*-value)	Phenolic Content (F, *p*-value)
Model	27.65, <0.0001 (sig)	11.51, 0.0020 (sig)	278.86, <0.0001 (sig)
A (Carbopol 934)	86.06, <0.0001 (sig)	11.19, 0.0183 (sig)	3.50, 0.1036 (ns)
B (TEA)	78.56, <0.0001 (sig)	3.53, 0.0788 (ns)	14.00, 0.0072 (sig)
C (Propylene glycol)	5.99, 0.0455 (sig)	1.35, 0.4708 (ns)	2187.50, <0.0001 (sig)
AB	4.21, 0.0794 (ns)	0.13, 0.7299 (ns)	0.00, 1.0000 (ns)
AC	1.76, 0.2044 (ns)	4.05, 0.0690 (ns)	0.00, 1.0000 (ns)
BC	0.00, 1.0000 (ns)	0.13, 0.7299 (ns)	7.00, 0.0331 (sig)
A^2	1.11, 0.3277 (ns)	0.02, 0.8869 (ns)	117.89, <0.0001 (sig)
B^2	1.21, 0.3196 (ns)	0.09, 0.7689 (ns)	66.32, <0.0001 (sig)
C^2	96.01, 0.0001 (sig)	21.05, 0.0052 (sig)	117.89, <0.0001 (sig)
Lack of Fit	32.86, 0.0028 (sig)	21.25, 0.0064 (sig)	-

activity against the Gram-positive bacterium *Staphylococcus epidermidis*.

In vitro extract release study

The optimized formulation exhibited sustained extract release, with approximately 100% cumulative extract release achieved within 4 hr (Figure 4). Kinetic modeling demonstrated that the release profile showed the highest correlation with the zero-order model (R^2=0.975), followed by the Higuchi model (R^2=0.952), whereas the first-order model showed comparatively lower correlation (R^2=0.743). These findings suggest that the formulation exhibited near-constant extract release with diffusion-controlled release characteristics from the polymeric gel matrix.

DISCUSSION

The present study successfully developed and optimized a *Tinospora cordifolia* extract-loaded topical gel using Box-Behnken Design (BBD). Carbopol 934, triethanolamine (TEA), and propylene glycol significantly influenced the physicochemical characteristics of the formulation, particularly viscosity, retention time, and phenolic content. Carbopol 934 acted as the primary gelling polymer, while TEA facilitated gel network formation through neutralization. Propylene glycol contributed to improved spreadability and consistency due to its humectant and penetration-enhancing properties. Similar observations have been reported in previous Carbopol-based topical gel formulations (Prabu *et al.*, 2017).

The optimized formulation exhibited suitable physicochemical properties for topical application. The pH of the gel (6.52±0.09) was within the acceptable physiological skin range, indicating good skin compatibility and minimal irritation potential. The gel also demonstrated high viscosity, satisfactory retention time, good spreadability, and easy extrudability, which are important parameters for patient compliance and prolonged skin contact. Such properties are considered essential quality attributes for topical herbal formulations (Inamdar and Bhise).

In vitro extract release studies demonstrated sustained release behavior with approximately complete extract release within 4 hr. Release kinetics showed good fitting to both zero-order and Higuchi models, suggesting controlled diffusion-mediated release from the polymeric gel matrix. Sustained release of extract constituents from topical gels may prolong the availability of bioactive phytoconstituents at the site of application and enhance therapeutic efficacy. Similar diffusion-controlled release profiles have been reported for Carbopol-based hydrogels due to their crosslinked polymeric structure (Dantas *et al.*, 2016).

ATR-FTIR analysis confirmed compatibility between the Giloy extract and formulation excipients. Characteristic peaks corresponding to hydroxyl, carbonyl, alkyl, and glycosidic functional groups were retained without significant peak shifting or disappearance, indicating the absence of major chemical interaction. These findings confirm successful incorporation of the extract into the gel matrix and support formulation stability (Kulkarni *et al.*, 2018).

The optimized Giloy gel also demonstrated antimicrobial activity against *Staphylococcus epidermidis, Escherichia coli*, and *Candida albicans*, with comparatively higher activity against *S. epidermidis*. The antimicrobial activity may be attributed to phytoconstituents such as alkaloids, glycosides, diterpenoids, and phenolic compounds present in *T. cordifolia* (Upadhyay *et al.*, 2013). Short-term stability studies showed no significant changes in appearance, pH, or viscosity under different storage conditions, indicating acceptable formulation stability. Overall, the developed Giloy herbal gel demonstrated promising physicochemical, release, and antimicrobial properties for potential topical anti-inflammatory application.

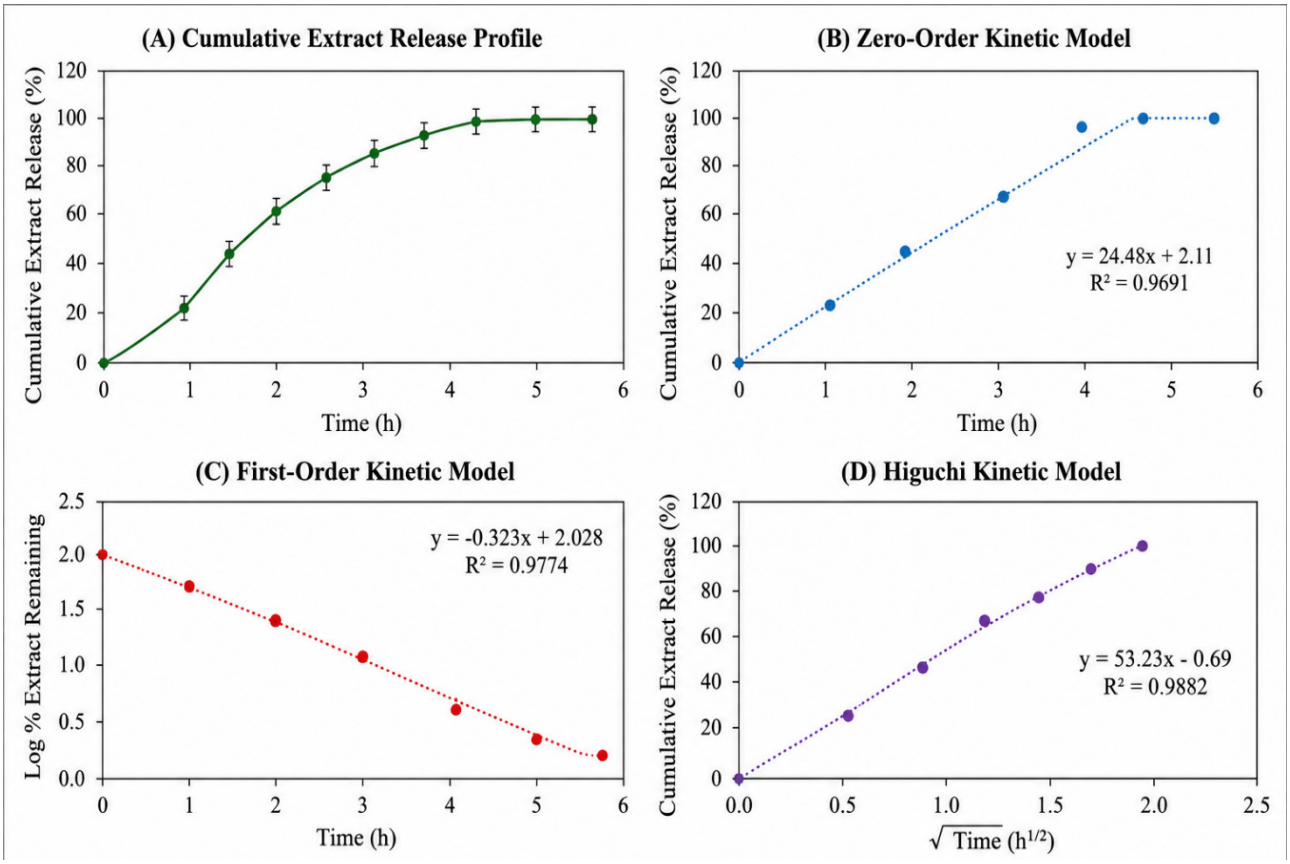

Figure 4: *In vitro* extract release profile and release kinetic models of the optimized *Tinospora cordifolia* gel formulation. (A) Cumulative extract release profile, (B) Zero-order kinetic model, (C) First-order kinetic model, and (D) Higuchi kinetic model.

Table 4: Evaluation results of optimized Giloy herbal gel.

Parameter	Observed Value (Mean±SD)	Inference
pH	6.52±0.09	Skin-compatible
Viscosity (cP)	52,381±1037	Good consistency for topical use
Retention time (sec)	146.3±4.1	Good adhesive properties
Phenolic content (%)	18.53%	Satisfactory phytoconstituent retention
Spreadability (g·cm/sec)	25.0±4.3	Adequate for topical application
Extrudability	20 g	Easy extrusion from tube
Homogeneity	Homogeneous	No lumps or phase separation
Skin irritation potential	No visible irritation observed	Preliminary qualitative assessment
Washability (sec)	23.3±5.1	Easily washable with water

CONCLUSION

A *Tinospora cordifolia* extract-loaded topical gel was successfully optimized using Box-Behnken Design. The optimized formulation exhibited suitable physicochemical properties, good skin compatibility, satisfactory phenolic content, and sustained extract release behavior. ATR-FTIR analysis confirmed compatibility between the extract and excipients. The formulation also demonstrated notable antimicrobial activity against *Staphylococcus epidermidis*, *Escherichia coli*, and *Candida albicans*. These findings suggest that the Giloy herbal gel is a promising candidate for topical anti-inflammatory and antimicrobial therapy, warranting further *in vivo* and long-term stability studies.

REFERENCES

Abdallah, M. H. (2014). Box-behnken design for development and optimization of acetazolamide microspheres.*International Journal of Pharmaceutical Sciences and Research*,*5*(4), 1228.

Agarwal, S., Ramamurthy, P. H., Fernandes, B., Rath, A., & Sidhu, P. (2019). Assessment of antimicrobial activity of different concentrations of *Tinospora cordifolia* against *Streptococcus mutans*: An: *In vitro*: Study.*Dental research journal*,*16*(1), 24-28.

Barad, M. (2014). Design of experiments (DOE)-A valuable multi-purpose methodology.*Applied Mathematics*,*5*(14), 2120.

Dantas, M. G. B., Reis, S. A. G. B., Damasceno, C. M. D., Rolim, L. A., Rolim-Neto, P. J., *et al.* (2016). Development and evaluation of stability of a gel formulation containing the monoterpene borneol.*The Scientific World Journal*,*2016*(1), 7394685.

Dereli, H. G., Şengün, E. K., Genç, C. O., Arslan, B. Ö, & Gökalp, M. U. (2021). Method development and validation of *in vitro* release test for fluorometholone ophthalmic suspension using franz diffusion cell system.*Global Journal of Pharmacy & Pharmaceutical Sciences [Internet]*.

Ezhilarasu, K., Kasiranjan, A., Priya, S., & Kamaraj, A. (2023). The antibacterial effect of *Tinospora cordifolia* (Guduchi) and its role in combating antimicrobial resistance.*Medeniyet Medical Journal*,*38*(3), 149-158.

Inamdar, A. S., & Bhise, K. S. (2023). Formulation and evaluation of topical nanosilver gel of *Tinospora cordifolia* (Guduchi). Indo American Journal of Pharmaceutical Research, 13(8), 1091–1101. https://doi.org/10.5281/zenodo.8307624

Kim, M. E., & Lee, J. S. (2024). Molecular foundations of inflammatory diseases: Insights into inflammation and inflammasomes. *Current Issues in Molecular Biology*, *46*(1), 469-484.

Kowalska-Krochmal, B., & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: methods, interpretation, clinical relevance.*Pathogens*,*10*(2), 165.

Kulkarni, P., Nale, A., Kharat, O., Sonwane, R., Lawand, D., & Khindkar, P. (2018). Phytochemical and FTIR analysis of *Tinospora cordifolia*. International Journal of Current Research, 10(11), 75331–75334. https://doi.org/10.24941/ijcr.33147.11.2018.

Patil, P. B., Datir, S. K., & Saudagar, R. B. (2019). A review on topical gels as drug delivery system. Journal of Drug Delivery and Therapeutics, 9(3–s), 989–994. https://doi.org/10.22270/jddt.v9i3-s.2930.

Prabu, S. L., Umamaheswari, A., Rajakumar, S., Bhuvaneswari, P., & Muthupetchi, S. (2017). Development and evaluation of gel incorporated with synthesized silver nanoparticle from *Ocimum gratissimum* for the treatment of acne vulgaris.*American Journal of Advanced Drug Delivery*,*5*(3), 107-117.

Saha, S., & Ghosh, S. (2012). *Tinospora cordifolia*: One plant, many roles.*Ancient science of life*,*31*(4), 151-159.

Salamanca, C. H., Barrera-Ocampo, A., Lasso, J. C., Camacho, N., & Yarce, C. J. (2018). Franz diffusion cell approach for pre-formulation characterization of ketoprofen semi-solid dosage forms.*Pharmaceutics*,*10*(3), 148.

Sharma, P., Dwivedee, B. P., Bisht, D., Dash, A. K., & Kumar, D. (2019). The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon, 5(9), Article e02437. https://doi.org/10.1016/j.heliyon.2019.e02437.

Shiehzadeh, F., Mohebi, D., Chavoshian, O., & Daneshmand, S. (2023). Formulation, characterization, and optimization of a topical gel containing tranexamic acid to prevent superficial bleeding: *In vivo* and *in vitro* evaluations.*Turkish Journal of Pharmaceutical Sciences*,*20*(4), 261.

Stone, W. L., Basit, H., Zubair, M., & Burns, B. (2024. Updated 2024, August 11). Pathology, inflammation. In StatPearls. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK534820/.

Upadhyay, N., Ganie, S. A., Agnihotri, R. K., & Sharma, R. (2013). Studies on antioxidant activity and total phenolic content of *Tinospora cordifolia* (Miers.) stem using *in vitro* models.*American Journal of Phytomedicine and Clinical Therapeutics*,*1*(8), 617-627.

Vanegas, D., Abril-Novillo, A., Khachatryan, A., Jerves-Andrade, L., Peñaherrera, E., *et al.* (2021). Validation of a method of broth microdilution for the determination of antibacterial activity of essential oils.*BMC Research Notes*,*14*(1), 439.

Yadav, R., Alagusundaram, M., Bhattacharya, V., Tiwari, P., Mishra, N., *et al.* (2024). Development and Characterization of *Tinospora cordifolia* extract Loaded Transethosomal Gel for Topical use.*Asian* Journal of Basic Science & *Research*,*6*(2), 100-112.

Yetukuri, K., Mohammad, B., Nookala, V., Pentyala, N., Kondaveeti, U., *et al.* (2024). Fabrication and Characterization of *Azadirachta indica* oil Induced Nanoemulgel Using 33 Central Composite Design (CCD): Assessment of Antibacterial Activity.*Journal of Young Pharmacists*,*16*(3), 535.

Cite this article: Yetukuri K, Annavarapu SCK, Balla VJ, Bellana V, Chirumamilla AC, *et al*. Systematic Optimization of *Tinospora cordifolia* Extract Gel Via Box-Behnken Design Using a DoE Framework. J Young Pharm. 2026;18(3):755-62.