

# Ethnopharmacological Validation of *Barleria Terminalis* Nees: Comparative In Vitro Anti-Inflammatory Evaluation of Leaves, Stems and Flowers

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**Abstract**—Ethnopharmacological relevance: *Barleria terminalis* Nees (Acanthaceae) is traditionally used in Indian folk medicine for the treatment of inflammatory conditions, wounds, pain, and fever. However, experimental evidence supporting its anti-inflammatory potential, particularly across different plant parts, remains limited.

**Aim of the study:** The present study aimed to evaluate the in vitro anti-inflammatory activity of aqueous and ethanolic extracts of leaves, stems, and flowers of *Barleria terminalis*.

**Materials and methods:** Extracts of leaves, stems, and flowers were prepared using aqueous and ethanolic solvents and evaluated for in vitro anti-inflammatory activity using inhibition of protein denaturation, heat-induced haemolysis, human red blood cell (HRBC) membrane stabilization, and proteinase inhibitory assays. Diclofenac sodium was used as the reference anti-inflammatory drug. All experiments were performed in triplicate, and results were expressed as mean  $\pm$  SD.

**Results:** All extracts exhibited significant, concentration-dependent anti-inflammatory activity across the tested models. Ethanolic extracts showed consistently higher activity than aqueous extracts. Among the different plant parts, leaf extracts demonstrated the strongest anti-inflammatory effects, followed by flower and stem extracts. Ethanolic leaf extract showed pronounced inhibition of protein denaturation, effective stabilization of erythrocyte membranes, and notable proteinase inhibitory activity, with effects approaching those of diclofenac sodium at higher concentrations.

**Conclusion:** The findings provide experimental support for the traditional use of *Barleria terminalis* in inflammatory disorders. The observed in vitro anti-inflammatory activity, particularly of leaf extracts, suggests that the plant is a promising source of bioactive compounds warranting further phytochemical characterization and in vivo validation.

**Index Terms**—*Barleria terminalis*; Anti-inflammatory activity; Protein denaturation; HRBC membrane stabilization

## I. INTRODUCTION

Inflammation is a fundamental biological response to tissue injury, infection, or chemical insult and plays a crucial role in host defense. However, persistent or dysregulated inflammatory responses contribute to the development and progression of numerous chronic disorders, including rheumatoid arthritis, cardiovascular diseases, neurodegenerative conditions, and cancer. Although non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids remain the mainstay of inflammatory disease management, their long-term use is associated with adverse effects such as gastrointestinal irritation, renal toxicity, and cardiovascular complications. These limitations have stimulated growing interest in plant-derived anti-inflammatory agents with improved safety profiles. [1]

Ethnopharmacology provides a valuable framework for identifying medicinal plants with therapeutic potential based on traditional knowledge systems. Plants used in folk medicine often contain bioactive secondary metabolites capable of modulating inflammatory pathways through multiple mechanisms, including membrane stabilization, enzyme inhibition, and suppression of inflammatory mediators. Systematic scientific validation of such plants is essential to bridge traditional use and evidence-based pharmacology.[2]

The genus *Barleria* (family Acanthaceae) comprises several species widely used in traditional medicine across Asia and Africa for the treatment of

inflammatory disorders, pain, wounds, and infections. Previous pharmacological investigations on certain *Barleria* species have reported anti-inflammatory, analgesic, antioxidant, and antimicrobial activities, supporting their ethnomedicinal relevance. *Barleria terminalis* Nees is traditionally used in indigenous medical practices for managing inflammation, fever, pain, and wound-related conditions. Despite its frequent ethnobotanical mention, scientific studies validating its anti-inflammatory activity remain limited, particularly with respect to comparative evaluation of different plant parts.[3]

Plant secondary metabolites such as flavonoids, phenolic acids, triterpenoids, and tannins are well documented for their ability to stabilize biological membranes, inhibit protein denaturation, and suppress proteolytic enzymes involved in inflammatory processes. The distribution of these compounds often varies among plant parts, which can significantly influence pharmacological activity. Therefore, evaluating multiple plant parts is important for identifying the most therapeutically relevant material and promoting sustainable utilization of medicinal plants.

In vitro anti-inflammatory models provide a reliable and mechanistically informative approach for preliminary screening of plant extracts. Assays based on inhibition of protein denaturation, stabilization of human red blood cell (HRBC) membranes, prevention of heat-induced haemolysis, and inhibition of proteinase activity are widely employed to assess the anti-inflammatory potential of natural products and correlate well with in vivo inflammatory mechanisms.[4]

In this context, the present study aimed to scientifically validate the traditional use of *Barleria terminalis* by investigating the in vitro anti-inflammatory activity of its leaves, stems, and flowers using multiple experimental models. The comparative evaluation of different plant parts was undertaken to identify the most active fraction and to provide a rational basis for further phytochemical and pharmacological investigations.

## II. MATERIAL & METHODS

### 2.1. Plant material and extraction

Leaves, stems, and flowers of *Barleria terminalis* were collected, authenticated, shade-dried, and powdered.

The powdered material was extracted separately with distilled water and 95% ethanol using maceration. Extracts were filtered, concentrated under reduced pressure, and stored at 4 °C until use. Stock solutions were freshly prepared in appropriate solvents for in vitro assays.

### 2.2. Chemicals and reagents

Diclofenac sodium was used as the reference anti-inflammatory drug. Bovine serum albumin, trypsin, egg albumin, and all analytical-grade reagents were procured from standard commercial suppliers.

### 2.3. Human red blood cell (HRBC) membrane stabilization assay

The HRBC membrane stabilization assay was performed to evaluate protection against hypotonicity- and heat-induced haemolysis. Fresh human blood was collected from healthy volunteers with informed consent and ethical approval. Erythrocytes were separated, washed, and suspended in isotonic buffer. Extracts (aqueous and ethanolic; leaves, stems, flowers) were incubated with HRBC suspension and subjected to heat stress. Absorbance was measured at 560 nm. Percent membrane stabilization was calculated relative to control. Diclofenac sodium served as the standard. [5]

### 2.4. Heat-induced haemolysis assay

Extracts were evaluated for their ability to inhibit heat-induced haemolysis of erythrocytes. Reaction mixtures were incubated at elevated temperature and centrifuged, and absorbance of the supernatant was measured at 560 nm. Percentage inhibition of haemolysis was calculated. [6]

### 2.5. Inhibition of albumin denaturation

The anti-denaturation effect was assessed using bovine serum albumin. Reaction mixtures containing extract and albumin were incubated at elevated temperature, cooled, and absorbance was measured at 660 nm. Percent inhibition of protein denaturation was calculated using standard formulae.[7]

### 2.6. Proteinase inhibitory activity

Proteinase inhibition was evaluated using trypsin as the enzyme and casein as substrate. Extracts were incubated with trypsin, followed by substrate addition. The reaction was terminated, and absorbance was

measured at 210 nm. Percentage inhibition was calculated relative to control. [8]

2.7. Statistical analysis

All experiments were performed in triplicate. Results are expressed as mean ± SD (n = 3).

III. RESULTS

3.1. Effect of Barleria terminalis extracts on heat-induced haemolysis

Both aqueous and ethanolic extracts of Barleria terminalis leaves, stems, and flowers exhibited significant inhibition of heat-induced haemolysis in a concentration-dependent manner. Ethanolic extracts consistently showed greater protective effects than aqueous extracts across all tested plant parts. Among the different plant parts, leaf extracts demonstrated the highest percentage inhibition of haemolysis, followed by flower and stem extracts.

At the highest tested concentration, the ethanolic leaf extract produced inhibition values approaching those of the reference drug diclofenac sodium, whereas stem extracts exhibited comparatively lower activity. The results indicate that the extracts possess the ability to protect erythrocyte membranes against thermal stress, suggesting membrane-stabilizing properties (Table 1).

Table 1: Effect of Aqueous and Ethanolic Extracts of Barleria terminalis Leaves, Stems, and Flowers on Heat-Induced Haemolysis

S. No.	Treatment (500 µg/mL)	% Inhibition of haemolysis
1	Control (without extract)	0.00 ± 0.00
2	Aqueous leaf extract	58.42 ± 1.36*
3	Aqueous stem extract	41.28 ± 1.24*
4	Aqueous flower extract	49.76 ± 1.31*
5	Ethanolic leaf extract	72.84 ± 1.52**
6	Ethanolic stem extract	55.36 ± 1.41*
7	Ethanolic flower extract	63.48 ± 1.45**
8	Diclofenac sodium (standard, 500 µg/mL)	82.64 ± 1.18**

Values are expressed as Mean ± SD of triplicates (n = 3)

\*Significant compared with control (p < 0.05);

\*\*Highly significant compared with control (p < 0.001).

3.2. HRBC membrane stabilization activity

The HRBC membrane stabilization assay revealed marked protective effects of B. terminalis extracts against hypotonicity-induced haemolysis. Both aqueous and ethanolic extracts significantly stabilized the erythrocyte membrane in a dose-dependent manner. Ethanolic leaf extract showed the highest membrane stabilization among the test samples, followed by ethanolic flower and stem extracts.

Aqueous extracts exhibited moderate membrane stabilization compared to ethanolic extracts. The observed trend indicates that ethanol was more effective in extracting membrane-protective phytoconstituents. The degree of membrane stabilization produced by ethanolic leaf extract was comparable, though lower, than that of diclofenac sodium (Table 2; Fig. 2).

Table 2: Effect of Aqueous and Ethanolic Extracts of Barleria terminalis Leaves, Stems, and Flowers on HRBC Membrane Stabilization

S. No.	Treatment (500 µg/mL)	% Membrane stabilization
1	Control (without extract)	0.00 ± 0.00
2	Aqueous leaf extract	60.84 ± 1.42**
3	Aqueous stem extract	44.36 ± 1.28*
4	Aqueous flower extract	52.18 ± 1.35*
5	Ethanolic leaf extract	75.26 ± 1.58**
6	Ethanolic stem extract	57.42 ± 1.46*
7	Ethanolic flower extract	66.84 ± 1.49**
8	Diclofenac sodium (standard, 500 µg/mL)	85.48 ± 1.22**

Values are expressed as Mean ± SD of triplicates (n = 3)

\*Significant compared with control (p < 0.05);

\*\*Highly significant compared with control (p < 0.001).

### 3.3. Inhibition of albumin denaturation

All extracts significantly inhibited heat-induced denaturation of albumin in a concentration-dependent manner. Ethanolic leaf extract showed the highest inhibition of protein denaturation, followed by ethanolic flower extract, while stem extracts showed comparatively lower activity.

Aqueous extracts also demonstrated inhibitory effects, although their activity was consistently lower than ethanolic extracts. The reference drug diclofenac sodium exhibited the highest inhibition across all concentrations. The results suggest that *B. terminalis* extracts can effectively prevent protein denaturation under inflammatory conditions (Table 3).

Table 3: Effect of Aqueous and Ethanolic Extracts of *Barleria terminalis* Leaves, Stems, and Flowers on Albumin Denaturation

S. No.	Treatment (500 µg/mL)	% Inhibition of albumin denaturation
1	Control (without extract)	0.00 ± 0.00
2	Aqueous leaf extract	57.26 ± 1.34**
3	Aqueous stem extract	39.84 ± 1.21*
4	Aqueous flower extract	48.62 ± 1.29*
5	Ethanolic leaf extract	71.48 ± 1.46**
6	Ethanolic stem extract	53.72 ± 1.38*
7	Ethanolic flower extract	62.84 ± 1.42**
8	Diclofenac sodium (standard, 500 µg/mL)	83.26 ± 1.18**

Values are expressed as Mean ± SD of triplicates (n = 3)

\*Significant compared with control (p < 0.05);

\*\*Highly significant compared with control (p < 0.001).

### 3.4. Proteinase inhibitory activity

Extracts of *B. terminalis* showed significant inhibition of proteinase activity. Ethanolic leaf extract demonstrated the highest proteinase inhibitory effect, followed by ethanolic flower and stem extracts. Aqueous extracts showed moderate inhibition.

The inhibition increased with extract concentration, indicating a dose-dependent effect. Although the activity was lower than that of diclofenac sodium, the results confirm that *B. terminalis* contains constituents

capable of suppressing proteolytic enzymes involved in inflammatory tissue damage (Table 4).

Table 5.4: Effect of *Barleria terminalis* Leaves, Stems, and Flowers on Proteinase Inhibition

S. No.	Treatment (500 µg/mL)	% Proteinase inhibition
1	Control (without extract)	0.00 ± 0.00
2	Aqueous leaf extract	55.48 ± 1.32**
3	Aqueous stem extract	38.26 ± 1.18*
4	Aqueous flower extract	46.84 ± 1.24*
5	Ethanolic leaf extract	69.72 ± 1.44**
6	Ethanolic stem extract	51.36 ± 1.33*
7	Ethanolic flower extract	60.28 ± 1.39**
8	Diclofenac sodium (standard, 500 µg/mL)	81.64 ± 1.16**

Values are expressed as Mean ± SD of triplicates (n = 3)

\*Significant compared with control (p < 0.05);

\*\*Highly significant compared with control (p < 0.001).

## IV. DISCUSSION

The present investigation provides comprehensive in vitro evidence supporting the traditional use of *Barleria terminalis* in the management of inflammatory conditions. The anti-inflammatory activity observed across multiple experimental models reflects the ability of the plant extracts to modulate key processes involved in inflammation, including membrane destabilization, protein denaturation, and proteolytic enzyme activity.

Erythrocyte membrane stabilization is widely accepted as a reliable indicator of anti-inflammatory potential, as the erythrocyte membrane closely resembles lysosomal membranes. Stabilization of these membranes prevents the release of lysosomal enzymes that amplify inflammatory responses. The significant protection against heat- and hypotonicity-induced haemolysis observed in this study suggests that *B. terminalis* extracts possess membrane-stabilizing properties similar to those of non-steroidal anti-inflammatory drugs.

Protein denaturation is a well-recognized mechanism underlying inflammatory disorders such as rheumatoid arthritis. Denatured proteins can act as autoantigens, triggering immune responses and exacerbating inflammation. The strong inhibition of albumin

denaturation by *B. terminalis* extracts—particularly ethanolic leaf extract—indicates their potential to preserve protein structure under stress conditions and thereby attenuate inflammatory cascades.

Proteinases play a crucial role in inflammation by degrading extracellular matrix proteins and promoting tissue damage. The ability of *B. terminalis* extracts to inhibit proteinase activity further supports their anti-inflammatory potential. Although the inhibitory effect was lower than the standard drug, the activity observed is biologically relevant and consistent with plant-derived anti-inflammatory agents reported in the literature.

The consistently superior activity of ethanolic extracts compared to aqueous extracts highlights the influence of extraction solvent on pharmacological activity. Ethanol is known to extract a broader range of bioactive secondary metabolites, including flavonoids, phenolic compounds, and triterpenoids, which have been widely reported to exhibit anti-inflammatory effects through membrane stabilization, enzyme inhibition, and modulation of inflammatory mediators. Among the different plant parts, leaves exhibited the strongest anti-inflammatory activity, followed by flowers and stems. This plant-part specificity may reflect differences in the distribution and concentration of bioactive constituents. Such findings are important from an ethnopharmacological perspective, as they help identify the most therapeutically relevant plant part and promote sustainable utilization.

Overall, the multi-model approach employed in this study strengthens the validity of the findings and provides mechanistic insight into the anti-inflammatory properties of *Barleria terminalis*. While the present work is limited to in vitro evaluation, the results strongly justify further investigations, including phytochemical characterization, mechanism-based studies, and in vivo validation.

## V. CONCLUSION

This study provides in vitro evidence supporting the ethnopharmacological use of *Barleria terminalis* Nees in inflammatory conditions. Aqueous and ethanolic extracts of leaves, stems, and flowers exhibited significant anti-inflammatory activity through membrane stabilization, inhibition of protein denaturation, and suppression of proteinase activity. Ethanolic leaf extract consistently showed the highest

activity among the tested plant parts. Although less potent than diclofenac sodium, the findings validate the traditional relevance of *Barleria terminalis* and justify further phytochemical and in vivo investigations.

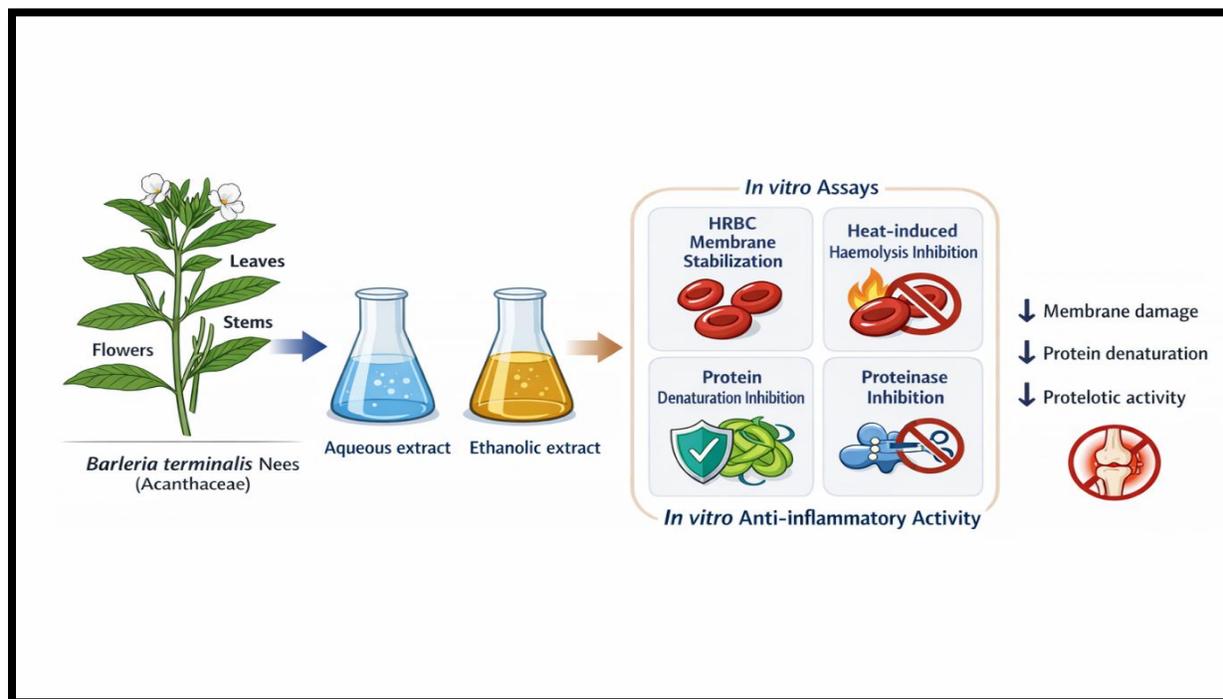
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GRAPHICAL ABSTRACT:



Schematic representation of the in vitro anti-inflammatory activity of aqueous and ethanolic extracts of *Barleria terminalis* Nees (Acanthaceae) leaves, stems, and flowers, evaluated through HRBC membrane stabilization, heat-induced haemolysis inhibition, inhibition of protein denaturation, and proteinase inhibition assays, demonstrating membrane-protective and anti-inflammatory effects.