RESEARCH PAPER

In Vitro Screening of Anti-Inflammatory Activity of Ficus racemosa L. Bark via Protein Denaturation Method

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Received: 2nd Mar, 2026 | **Revised:** 14th Mar, 2026 | **Accepted:** 4th Apr, 2026 | **Available Online:** 20th Apr, 2026

ABSTRACT

Background: Inflammation is a complex biological response associated with various chronic and acute disorders. Although conventional anti-inflammatory drugs are widely used, their long-term use is often associated with adverse effects. Hence, there is a growing interest in plant-based therapeutics. Ficus racemosa L., traditionally used in Ayurveda, possesses diverse pharmacological properties; however, its anti-inflammatory potential, particularly of the bark, remains underexplored.

Objective: The present study aimed to evaluate the in vitro anti-inflammatory activity of Ficus racemosa bark extracts using the protein denaturation method.

Methods: Hot and cold aqueous extracts of Ficus racemosa bark were prepared and tested at varying concentrations (0.01–1000 µg/mL). Anti-inflammatory activity was assessed using the egg albumin denaturation assay. Diclofenac and prednisolone were used as reference standards. Absorbance was measured at 680 nm, and percentage inhibition of protein denaturation was calculated. Statistical analysis was performed using SPSS software with significance set at $P < 0.05$.

Results: Both extracts exhibited significant inhibition of protein denaturation in a concentration-dependent manner. The hot water extract demonstrated comparatively higher inhibitory activity at lower concentrations (0.01 and 0.1 µg/mL). Notably, both extracts showed significantly greater inhibition than the reference drugs at higher concentrations ($P < 0.05$). Maximum inhibition was observed at 1000 µg/mL.

Conclusion: The findings indicate that Ficus racemosa bark possesses potent in vitro anti-inflammatory activity, surpassing standard drugs in the protein denaturation model. This supports its traditional use and highlights its potential as a natural anti-inflammatory agent. Further in vivo and mechanistic studies are warranted to validate these findings.

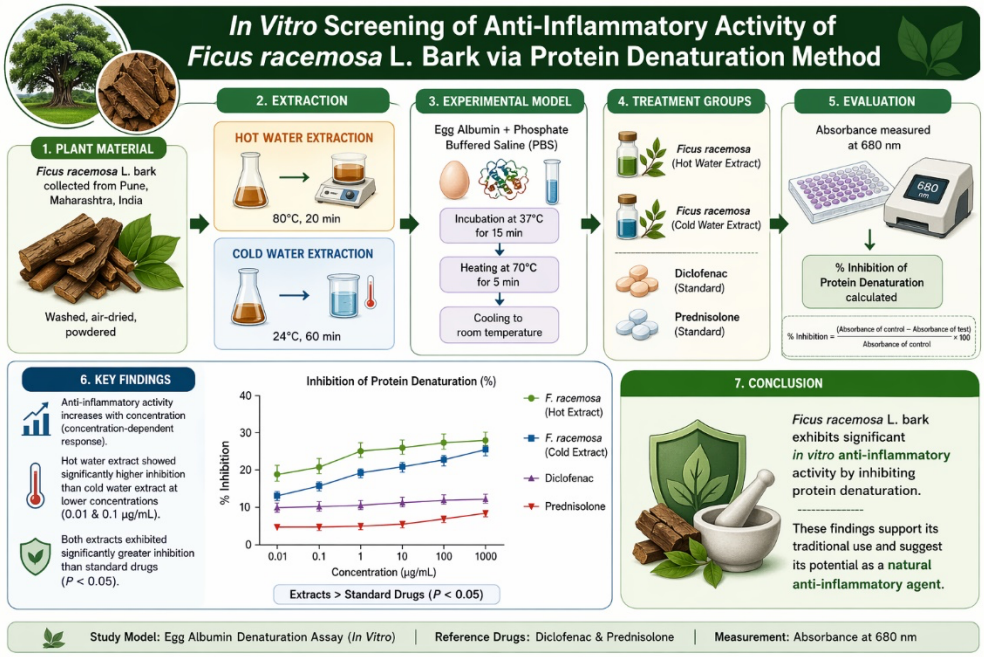
Keywords: Ficus racemosa, Anti-inflammatory activity, Protein denaturation, Egg albumin assay, Herbal medicine, Aqueous extract, Diclofenac, Prednisolone, Phytochemicals, In vitro study.

How to cite this article: Saste V, Mapari R, Bobade R, Mante A, Magar S, Lodhe N, Gite A, Mohrut S, Sawadadkar N, Kayande N. In Vitro Screening of Anti-Inflammatory Activity of Ficus racemosa L. Bark via Protein Denaturation Method. Int J Drug Deliv Technol. 2026;16(34s):217-223. DOI: 10.25258/ijddt.16.34s.27

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.

Graphical Abstract:



Introduction

Inflammation is a natural protective response to tissue injury, involving a complex cascade of events such as enzyme activation, mediator release, fluid exudation, cell migration, tissue degradation, and repair. It is a multifaceted process often associated with pain and characterized by increased vascular permeability, protein denaturation, and alterations in cellular membranes[1]. Harmful stimuli, including pathogens, irritants, or damaged cells, trigger the inflammatory response in vascular tissues. This response serves as a defense mechanism aimed at eliminating injurious agents and initiating tissue healing. However, if left uncontrolled or untreated, inflammation can contribute to the development of various disorders such as vasomotor rhinorrhea, rheumatoid arthritis, and atherosclerosis[2].

Understanding the inflammatory process requires recognizing the role of chemical mediators, which regulate and amplify the response. These mediators originate from plasma proteins or cells such as mast cells, platelets, neutrophils, and macrophages, and are activated by microbial products or host-derived signals. They exert their effects by binding to specific receptors, leading to increased vascular permeability, neutrophil chemotaxis, smooth muscle contraction, enzymatic activity, pain induction, and oxidative damage. Although most mediators are short-lived, they can produce significant biological effects. Key examples include vasoactive amines (histamine and serotonin), arachidonic acid metabolites (prostaglandins and leukotrienes), and cytokines such as tumor necrosis factor (TNF) and interleukin-1[3].Currently available anti-inflammatory drugs, including opioids and non-steroidal anti-inflammatory drugs (NSAIDs), are not universally effective and may cause adverse effects or exhibit limited potency in certain conditions. Therefore, the search for safer and more effective alternative therapies remains essential[4]. Inflammation continues to be a relevant and logical target in the search for novel anti-inflammatory agents. Medicinal plants represent a rich source of diverse bioactive compounds, many of which have contributed to the development of modern therapeutic agents. Over the past two centuries, extensive research on plant-derived compounds has led to the discovery of numerous drugs with significant biological activities[5-6]. Phytochemical investigations have revealed the presence of tetracyclic triterpenoid saponins such as bacosides A and B, hersaponin, alkaloids including herpestine and brahmine, and flavonoids[7-8].

The selected medicinal plants—leaves of Aloe vera, Bacopa monnieri, and Moringa oleifera, along with the rhizome of Zingiber officinale—were used to prepare a polyherbal formulation (HP-4). The present study aims to evaluate the in vitro anti-inflammatory activity of this formulation using red blood cell (RBC) membrane stabilization and protein denaturation assays[9-10].

In Ayurveda, numerous plant-derived compounds have been used for centuries to manage inflammatory conditions with minimal side effects. Ficus racemosa is an evergreen, deciduous tree widely distributed across Asia, Africa, America, and Australia, and is known for its diverse pharmacological properties, including antihyperglycemic, antioxidant, hepatoprotective, and antimicrobial activities. The bark of *Ficus racemosa* is traditionally utilized in the treatment of menorrhea,

hemoptysis, diabetes, dysentery, asthma, piles, burns, swelling, and leucorrhea, and is also applied externally as a wound wash[12].

Although several studies have reported the antibacterial and antioxidant potential of this plant, there is limited research evaluating its anti-inflammatory activity. Therefore, the present study was designed to investigate the anti-inflammatory potential of *Ficus racemosa* bark using the albumin denaturation method as an indirect indicator of anti-inflammatory activity[13].

Cyclooxygenase (COX) is a key rate-limiting enzyme involved in the conversion of arachidonic acid (AA) into prostaglandins (PGs), exhibiting both cyclooxygenase and peroxidase activities. Two major isoforms, COX-1 and COX-2, have been identified; these are encoded by separate genes and differ in their physiological roles. COX-1 is constitutively expressed in most tissues and is primarily responsible for maintaining normal cellular and physiological functions through basal prostaglandin production. In contrast, COX-2 is minimally expressed under normal conditions but is significantly upregulated during inflammation and in various tumor tissues.

Due to these functional differences, in vitro studies are essential to determine the selectivity of potential anti-inflammatory compounds. Currently, evaluation of anti-inflammatory activity, particularly for natural products and food-derived compounds, predominantly focuses on COX-2 inhibition.

The release of arachidonic acid is catalyzed by phospholipase A_2 (PLA_2) in response to physiological and pathological stimuli. Arachidonic acid is then metabolized by COX enzymes to form prostaglandin G_2 (PGG_2), which is subsequently reduced to prostaglandin H_2 (PGH_2). PGH_2 serves as a common precursor for various bioactive prostaglandins, including PGI_2, PGE_2, $PGF_2\alpha$, PGD_2, and thromboxane A_2 (TxA_2). These compounds are synthesized by specific enzymes such as prostacyclin synthase (PGIS), prostaglandin E synthase (PGES), prostaglandin F synthase (PGFS), prostaglandin D synthase (PGDS), and thromboxane synthase (TxS), respectively[14-16].

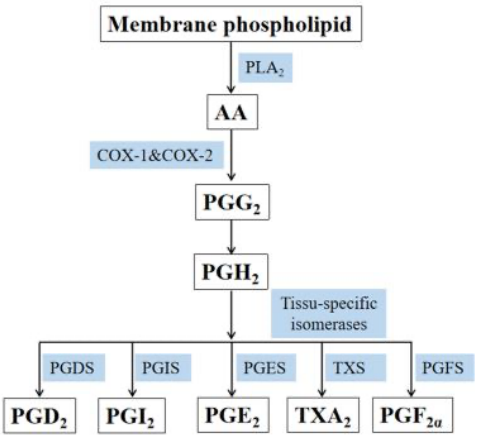


Figure No. 1. Cyclooxygenase (COX) involvement in the arachidonicacid pathway. AA, arachidonic acid; PGDS, PGD 2 synthetase; PGES,PGE2 synthetase; PGFS, PGF2α synthetase; PGG 2-prostaglandin;PGIS, PGI 2 synthetase; TXS, TxA 2 synthetase.

Collection of Plant Material and Extraction Procedure

The bark of *Ficus racemosa* was collected from the Sultanpur of Buldana, Maharashtra. The plant material was taxonomically authenticated by qualified Ayurveda practitioners. The collected bark was air-dried under ambient conditions, and a voucher specimen was preserved in the laboratory for future reference. The dried bark was then powdered using a traditional pestle and mortar and stored in an airtight container until further use.

Hot and Cold Water Extraction

The bark samples of *Ficus racemosa* were initially washed under running tap water, followed by rinsing with distilled water to remove surface impurities. The samples were air-dried at room temperature for one week until a constant weight was achieved, and subsequently ground into a fine powder. For hot water extraction, 0.2 g of the powdered bark was transferred into a conical flask containing 20 mL of distilled water. The mixture was first kept at room temperature for 15 minutes and then heated at 80°C for 20 minutes. The resulting extract was filtered through muslin cloth and stored at −4°C until further analysis.

For cold water extraction, 0.2 g of the bark powder was mixed with 20 mL of distilled water and maintained at 24°C for 60 minutes, followed by filtration using the same procedure[17].

Preparation of Reference Drugs (Positive Control)

Nonsteroidal anti-inflammatory drug (NSAID), Diclofenac, and a corticosteroid, prednisolone, were used as reference standards. Prednisolone tablets were finely powdered, and 0.2 g of the powder was accurately weighed using a digital analytical balance

(Adam PW 254). The weighed sample was dissolved in 20 mL of distilled water and mixed thoroughly using a vortex mixer. An identical procedure was followed for the preparation of the ibuprofen solution[16-17].

Inhibition of Protein Denaturation

The reaction mixtures were incubated in a water bath at 37°C ± 2°C for 15–20 minutes, followed by heating at 70°C for 5 minutes to induce protein denaturation. Subsequently, the mixtures were allowed to cool to room temperature for 15 minutes. The absorbance of each reaction mixture was measured before and after denaturation at 680 nm using a colorimeter across different concentrations (1000, 100, 10, 1, 0.1, and 0.01 µg/mL). Each experiment was performed in triplicate, and the mean absorbance values were recorded.

The percentage inhibition of protein denaturation was calculated relative to the control using the following formula:

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

All experimental data were statistically analyzed using IBM SPSS Statistics. Results were expressed as mean ± standard error of the mean (SEM). The differences in percentage inhibition among groups were evaluated using the independent samples *t*-test. A value of $P < 0.05$ was considered statistically significant[18s].

Results

The anti-inflammatory activity of Ficus racemosa bark was evaluated using the egg albumin denaturation assay. The highest percentage inhibition of protein denaturation was observed for both hot and cold water extracts at a concentration of 1000 µg/mL. Notably, the hot water extract exhibited significantly greater inhibitory activity than the cold water extract at lower concentrations (0.01 µg/mL and 0.1 µg/mL) (Table 1). When compared with the aqueous extracts, the reference drugs Prednisolone and Diclofenac demonstrated relatively lower inhibition rates. Furthermore, the inhibition of protein denaturation by both hot and cold water extracts, as well as prednisolone, showed a concentration-dependent increase. In contrast, ibuprofen exhibited a decreasing trend in inhibition with increasing concentration (Table 2).

At concentrations of 0.1 µg/mL and above, both hot and cold water extracts showed significantly higher inhibition rates compared to prednisolone and Diclofenac ($P < 0.05$).

Table 1: The percentage of inhibition rate of protein denaturation using cold and hot water

Concentration (µg/ml)	Rate of inhibition (%)		
	Cold water	Hot water	P
0.01	13.26±0.96	19.29±1.34	0.022
0.1	16.30±0.93	19.58±0.62	0.008
1	22.43±1.49	20.71±0.66	0.351
10	24.74±0.75	22.43±1.32	0.204
100	25.09±2.27	23.73±3.36	0.753
1000	27.71±0.72	27.65±0.73	0.954

Results are shown as mean±SEM. SEM: Standard error of the mean

Table 2: Percentage of inhibition rate of protein denaturation of reference drugs

Concentration (µg/ml)	Rate of inhibition (%)	
	Diclofenac	Prednisolone
0.01	15.13±3.56	5.43±0.14
0.1	12.09±0.44	3.95±1.05
1	11.71±0.51	5.03±1.04
10	11.29±4.27	5.21±1.04
100	12.16±1.96	6.47±1.51
1000	9.77±1.11	8.83±1.51

Results are shown as mean±SEM. SEM: Standard error of the mean

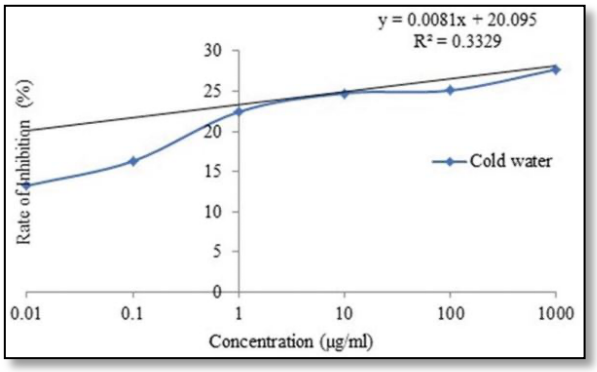


Figure 2: IC50 of Ficus racemosa cold water extract for protein denaturation

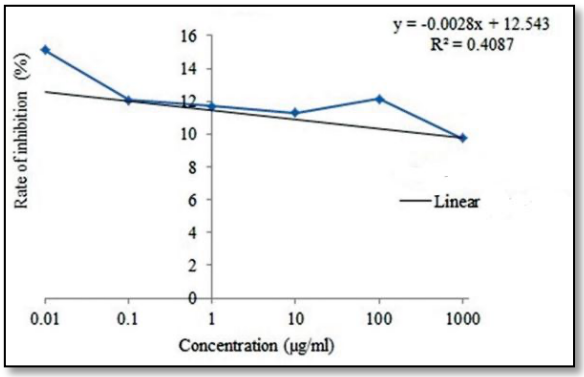


Figure 3: IC50 of protein denaturation in Diclofenac

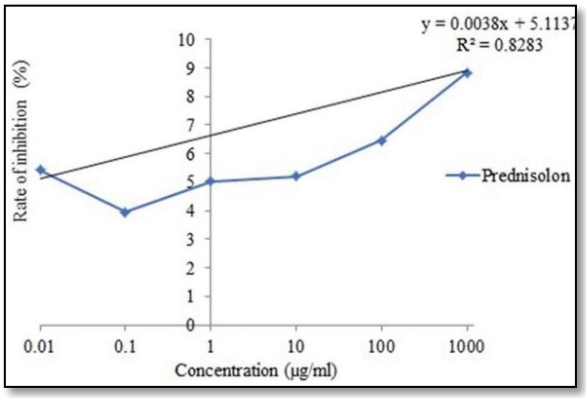


Figure 4: IC50 of protein denaturation in prednisolone

Discussion

Previous studies have reported the phytochemical composition and anti-inflammatory properties of the leaves and fruits of Ficus racemosa. However, the present investigation specifically evaluates the stem bark for its claimed therapeutic applications. The findings of this study support the traditional use of this plant in the management of pain and inflammatory conditions.

Phytochemical analyses of *Ficus racemosa* have revealed the presence of bioactive constituents such as flavonoids, tannins, phenolic compounds, and phytosterols. These compounds, either individually or synergistically, are likely responsible for the observed analgesic and anti-inflammatory effects. Further studies are currently underway to isolate and characterize the active principles from the stem bark of *Ficus racemosa*. The oral LD_{50} value obtained for the plant extract indicates a relatively wide safety margin, suggesting low acute toxicity and supporting its long-standing traditional use without significant reported adverse effects. Ibuprofen, a non-selective NSAID derived from propionic acid, is commonly used for its analgesic, anti-inflammatory, and antipyretic properties. Prednisolone, an oral corticosteroid, is widely prescribed for its potent anti-inflammatory effects, particularly in conditions such as asthma, allergies, and certain infections.

In the present study, the inhibition of protein denaturation by both cold and hot water extracts of *Ficus racemosa* bark, as well as prednisolone, increased in a concentration-dependent manner. In contrast, ibuprofen demonstrated a decreasing trend in inhibition with increasing concentration. The highest anti-inflammatory activity was observed at 1000 µg/mL of the bark extract. These findings are consistent with earlier reports, such as the study by Mandal et al. (2000), which demonstrated that leaf extracts of *Ficus racemosa* exhibited approximately 32% anti-inflammatory activity. Furthermore, at higher concentrations, the inhibitory effect of *Ficus racemosa* extracts on albumin denaturation was significantly greater than that of the reference drugs, indicating its promising potential as a natural anti-inflammatory agent.

The inhibitory effect of Ficus racemosa bark extracts was found to be greater than that of the reference drugs. Protein denaturation is known to generate autoantigens, which play a significant role in the pathogenesis of inflammatory conditions such as Rheumatoid arthritis, Cancer, and Diabetes mellitus. Therefore, inhibition of protein denaturation may contribute to the suppression of inflammatory responses. Future studies should focus on in vivo models, such as carrageenan-induced and egg albumin-induced rat paw edema assays, using varying concentrations of the extracts. Additional in vitro investigations, including bovine serum albumin denaturation and membrane stabilization assays, may further validate the anti-inflammatory potential of the plant. The egg albumin denaturation method offers a simple, cost-effective approach for preliminary screening of anti-inflammatory activity in herbal formulations; however, it requires further validation through comprehensive studies.

Overall, the findings demonstrate that *Ficus racemosa* bark exhibits significant in vitro anti-inflammatory activity, as evidenced by its ability to inhibit egg albumin denaturation. Both cold and hot water extracts showed notable effects, supporting its traditional therapeutic use.

Conclusion

The bark of *Ficus racemosa* possesses significant anti-inflammatory activity as demonstrated by the egg albumin denaturation assay, with greater efficacy than standard reference drugs such as Diclofenac and Prednisolone. Further detailed in vivo and in vitro studies are recommended to confirm and elucidate the

mechanisms underlying the anti-inflammatory effects of *Ficus racemosa* bark.

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