

# “Evaluation of Anti-inflammatory Activity of *Bryophyllum pinnatum* Leaf Extract in Experimental Animal Models”

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## Abstract

Inflammation is a protective physiological response; however, prolonged inflammation can lead to various chronic diseases. The present study was undertaken to evaluate the anti-inflammatory activity of *Bryophyllum pinnatum* leaf extract using experimental animal models. Preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, tannins, glycosides, and triterpenoids. The anti-inflammatory activity was assessed using carrageenan-induced paw edema, formaldehyde-induced paw edema, and cotton pellet-induced granuloma models. The extract exhibited significant and dose-dependent inhibition of inflammation in both acute and chronic models, as evidenced by reduced paw edema and decreased granuloma formation. Histopathological studies further confirmed the anti-inflammatory effects by showing reduced inflammatory cell infiltration and improved tissue architecture. The observed activity may be attributed to the presence of bioactive phytoconstituents with known anti-inflammatory and antioxidant properties. The findings suggest that *Bryophyllum pinnatum* leaf extract possesses promising anti-inflammatory potential and may serve as a natural source for the development of safer therapeutic agents for inflammatory disorders.

**Keywords:** *Bryophyllum pinnatum*, Anti-inflammatory activity, Carrageenan-induced paw edema, Cotton pellet granuloma, Phytochemical screening, Flavonoids, Phenolic compounds, Herbal medicine, Inflammation, Experimental animal models

## INTRODUCTION

### Inflammation and Its Pathophysiology

Inflammation is a complex protective response of the body to injury, infection, or harmful stimuli, aimed at eliminating the cause of injury, removing damaged tissue, and initiating the healing process. It involves a highly coordinated interaction between immune cells, vascular components, and chemical mediators.

Following tissue injury, immune cells such as macrophages, neutrophils, and mast cells are activated and release a variety of inflammatory mediators, including histamine, prostaglandins, leukotrienes, bradykinin, and cytokines like TNF- $\alpha$ , IL-1 $\beta$ , and IL-6. These mediators induce vasodilation, increased vascular permeability, and migration of leukocytes to the affected site, which manifest as the classic signs of inflammation: redness, swelling, heat, pain, and loss of function. (Oladejo et al., 2024; Lopes Andrade et al., 2020).

Enzymatic pathways, particularly cyclooxygenase (COX) and lipoxygenase (LOX), play a central role by producing prostaglandins and leukotrienes that amplify the inflammatory response. In addition, nitric oxide

(NO) and reactive oxygen species (ROS) are generated, which can further damage tissue if produced in excess. Persistent or uncontrolled release of these mediators may lead to chronic inflammation, causing prolonged tissue injury and delayed healing.

Understanding these mechanisms is crucial for evaluating anti-inflammatory agents, such as *Bryophyllum pinnatum* leaf extract, which may exert therapeutic effects by modulating inflammatory mediators, reducing oxidative stress, and stabilizing vascular and immune responses. (Sharma et al., 2024)

### Limitations of Conventional Anti-Inflammatory Drugs (NSAIDs and Steroids)

Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are commonly used to manage inflammation and pain, but their long-term use is associated with significant adverse effects.

NSAIDs inhibit cyclooxygenase (COX) enzymes to reduce prostaglandin synthesis, but this can lead to:

#### Gastrointestinal irritation, ulcers, and bleeding

Renal toxicity due to reduced renal blood flow

Cardiovascular risks, including hypertension and increased risk of heart attack or stroke  
 Corticosteroids are highly effective anti-inflammatory agents but prolonged therapy may cause:

- **Immune suppression**, increasing susceptibility to infections
- **Metabolic effects**, such as hyperglycemia, weight gain, and osteoporosis
- **Hormonal disturbances**, including adrenal suppression

#### Drug Profile

Name of the drug: *Bryophyllum pinnatum* Leaf Extract (Miracle Leaf)



Fig: *Bryophyllum pinnatum* Plant

**Biological Source:** Dried leaves of *Bryophyllum pinnatum*. It belongs to the family  
**Phytochemical Constituents:** -The leaf extract of *Bryophyllum pinnatum* contains various important phytochemicals responsible for its medicinal properties such as anti-inflammatory, antioxidant,

antimicrobial, wound healing, and analgesic activities.

- Flavonoids
- Tannins
- Saponins
- Terpenoids
- Steroids / Phytosterols
- Phenolic compounds

- These phytochemicals mainly contribute to the anti-inflammatory activity of the leaf extract. (Ojha et al., 2019; Sharma et al., 2024)

*Bryophyllum pinnatum* is a perennial succulent medicinal plant widely distributed in tropical and subtropical regions. It is commonly known as “Patharchatta”, “Life plant”, or “Miracle leaf” due to its ability to reproduce vegetatively from leaf margins and its wide range of therapeutic applications in traditional medicine systems such as Ayurveda, African folk medicine, and Chinese traditional medicine. (Ukamaka et al., 2023; Pawar et al., 2022).

The plant is characterized by thick, fleshy leaves that store water and contain a rich variety of secondary metabolites. Phytochemical investigations have revealed the presence of flavonoids (kaempferol, quercetin derivatives), phenolic acids, tannins, alkaloids, triterpenoids, glycosides, steroids, bufadienolides, and organic acids, which contribute to its diverse pharmacological activities. Among these, flavonoids and phenolic compounds are considered major contributors to its anti-inflammatory and antioxidant effects.

Traditionally, *Bryophyllum pinnatum* has been used for the treatment of inflammation, wounds, burns, abscesses, ulcers, kidney stones, hypertension, diarrhea, asthma, and infections. The leaf juice is often applied topically for wound healing, while oral preparations are used for systemic disorders such as fever, pain, and inflammatory conditions.

From a pharmacological perspective, the anti-inflammatory activity of *Bryophyllum pinnatum* is mainly attributed to the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to reduced synthesis of prostaglandins and leukotrienes. In addition, the plant extract has been shown to suppress the release of pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6, which play a key role in the progression of inflammation.

The antioxidant potential of the plant further enhances its anti-inflammatory effect by scavenging reactive oxygen species (ROS) and reducing oxidative stress-induced tissue damage. This dual action—anti-inflammatory and antioxidant—supports its effectiveness in both acute and chronic inflammatory conditions. (Johnson et al, 2022)

Preclinical studies have demonstrated that *Bryophyllum pinnatum* exhibits significant analgesic, antipyretic, antimicrobial, antiulcer, antidiabetic, nephroprotective, hepatoprotective, and immunomodulatory activities, indicating its broad therapeutic potential.

In experimental models, the leaf extract has shown dose-dependent reduction in carrageenan-induced paw edema and inhibition of granuloma formation in cotton pellet models, supporting its efficacy in both early and late phases of inflammation. Its safety profile is generally favorable in acute toxicity studies, although standardized dosing and clinical validation are still required. (Lopes Andrade et al., 2020)

*Bryophyllum pinnatum* is a pharmacologically important medicinal plant with significant potential as a natural anti-inflammatory agent, and it serves as a promising candidate for the development of plant-based therapeutic formulations with reduced side effects compared to synthetic drugs.

**Objectives:**

1. To collect, authenticate, and prepare the ethanolic leaf extract of *Bryophyllum pinnatum*.
2. To perform preliminary phytochemical screening of the leaf extract to identify the major bioactive constituents responsible for anti-inflammatory activity.
3. To evaluate the acute and sub-acute anti-inflammatory activity of *Bryophyllum pinnatum* leaf extract using the carrageenan-induced paw edema model and Formaldehyde-Induced Paw Edema Model in experimental animals.
4. To evaluate the chronic anti-inflammatory activity of *Bryophyllum pinnatum* leaf extract using the cotton pellet-induced granuloma model in experimental animals.
5. To compare the anti-inflammatory effects of the extract with a standard anti-inflammatory drug and scientifically validate the traditional use of *Bryophyllum pinnatum* in the treatment of inflammatory conditions.

**List of Materials**

Sr. No.	Material	Specification / Purpose
1	Fresh leaves of <i>Bryophyllum pinnatum</i>	Plant material for extraction (Collected locally and authenticated by recognized Botanist)
2	Carrageenan	Induction of acute inflammation (paw edema model)
3	Formaldehyde	Induction of sub-acute inflammation
4	Indomethacin	Standard anti-inflammatory drug
5	Ethanol	Extraction solvent
9	Distilled water	Solvent and preparation medium
10	Normal saline	Control solution / vehicle
11	Magnesium turnings	Detection of Flavonoids
12	Concentrated Hydrochloric Acid	Detection of Flavonoids
13	Ferric Chloride	Detection of Phenolic compounds and Tannins
14	Acetic Anhydride	Detection of Steroids and Triterpenoids
15	Concentrated Sulfuric Acid (H <sub>2</sub> SO <sub>4</sub> )	Detection of Steroids and Triterpenoids
16	Wistar albino rats	Experimental animals
17	Cotton, gloves, disinfectants	General laboratory and safety use
18	Labeling materials	Sample identification and documentation

Sr. No.	Equipment	Purpose
1	Plethysmometer	Measurement of paw volume in inflammation models
2	Analytical balance	Weighing of drugs, extracts, and materials
3	Hot air oven	Drying of plant material
4	Soxhlet apparatus	Extraction of plant material
5	Rotary evaporator	Concentration of extracts
6	Oral gavage needle	Oral administration of test samples
7	Syringes and needles	Drug administration and injections
8	Animal cages with bedding	Housing of experimental animals
9	Feed and water bottles	Maintenance of animals during study

## Methods:

### Extraction Process of *Bryophyllum pinnatum* Leaf Extract (Simple Method)

- Collection of Leaves: Collect fresh and healthy leaves of *Bryophyllum pinnatum*. After Wash properly with water to remove dust.
- Drying: Dry the leaves in shade for 5–7 days. Avoid direct sunlight to protect active constituents.
- Powder Preparation: Grind the dried leaves into coarse powder using a grinder. After grinding process store the powder in an airtight container.
- Extraction: Solvent repeatedly passes through powder and extracts phytochemicals.

The dried and powdered leaves of *Bryophyllum pinnatum* were placed in a thimble and loaded into a Soxhlet apparatus. Ethanol was added as the extraction solvent in a round-bottom flask. The apparatus was heated continuously for 6–8 hours, allowing the ethanol to repeatedly pass through the leaf powder and extract the phytochemical constituents. After completion of the extraction process, the solvent was evaporated to obtain the concentrated ethanolic leaf extract.

- Filtration: Filter the extract to remove plant particles.
- Concentration: Evaporate solvent using water bath. Thick semisolid extract is obtained.
- Storage: Store extract in airtight container at cool temperature for further animal studies.

### Preliminary Phytochemical screening:

Test	Observation	Result
Shinoda Test	Red colour observed	Flavonoids are Present.
Ferric Chloride Test	Blue-black colour observed	Tannins are Present
Foam Test	Persistent froth or foam formation observed	Saponins are Present

Liebermann–Burchard Test	Green or bluish-green color observed	Terpenoids are Present
Ferric Chloride Test	Blue, green, or violet coloration observed	Presence of phenolic compounds

## 2. Carrageenan-Induced Paw Edema Model (Acute Inflammation)

Anti-inflammatory activity was evaluated by the method of Winter et al. using a plethysmograph to measure paw edema in Wistar rats (150–200 g). Animals were divided into Five groups (n=6). The anti-inflammatory activity of the ethanolic leaf extract of *Bryophyllum pinnatum* was evaluated using the carrageenan-induced paw edema model in Wistar rats. The animals were divided into five groups, each consisting of six rats. Group I served as the normal control and received distilled water (1 mL/kg, p.o.). Group II served as the disease control and received distilled water (1 mL/kg, p.o.). Group III received indomethacin (10 mg/kg, p.o.) as the standard drug. Groups IV and V received the ethanolic extract of *Bryophyllum pinnatum* at doses of 100 mg/kg and 200 mg/kg, respectively, by oral administration.

Before treatment, the initial paw volume of the right hind paw of each rat was measured using a digital plethysmometer and recorded as the baseline value (0 hour). The respective treatments were then administered orally. One hour after drug administration, acute inflammation was induced by injecting 0.1 mL of 1% carrageenan solution into the subplantar region of the right hind paw of each rat.

The paw volume was measured using the plethysmometer at 1, 2, 3, 4 and 6 hours after carrageenan injection. The increase in paw volume was considered an index of edema formation. The anti-inflammatory activity of the test extract was assessed by comparing the paw edema volume of the treated groups with that of the disease control group.

The percentage inhibition of paw edema was calculated using the following formula:

$$\text{Percentage of inhibition} = 100 (1 - V_t / V_c)$$

Where,

$V_t$  is increase in paw volume in treated groups  $V_c$  is increase in paw volume in control groups.

A significant reduction in paw edema volume and a higher percentage inhibition in the extract-treated groups compared to the disease control group was considered indicative of anti-inflammatory activity of the *Bryophyllum pinnatum* leaf extract.

### Grouping of Animals

-Fasted overnight with free access to water

-Animals divided into 5 groups (n = 6)

Group	Treatment	No. of animals	Observation parameter
Group-I	Normal control (1ml Distilled water)	06	Carrageenan induced paw edema in rats
Group-II	Carrageenan control (Carrageenan 0.1ml)	06	

Group-III	Standard drug (indomethacin 10 mg/kg)	06	Change in paw volume.
Group-IV	Extract low dose (100 mg/kg)	06	
Group-V	Extract high dose (200 mg/kg)	06	

## 2. Formaldehyde-Induced Paw Edema Model Using Bryophyllum pinnatum Leaf Extract

Formaldehyde injection into the rat hind paw produces inflammation and edema due to the release of inflammatory mediators like histamine, serotonin, prostaglandins, and bradykinin. Anti-inflammatory drugs or plant extracts reduce paw swelling.

The initial paw volume of the left hind paw of each rat was measured using a digital plethysmometer and recorded as the baseline value (0 hour). The animals then received their respective treatments orally. The control group received distilled water (1 ml/kg), the standard group received indomethacin (10 mg/kg), and the test groups received the ethanolic leaf extract of *Bryophyllum pinnatum* at doses of 100 mg/kg and 200 mg/kg.

One hour after treatment administration, acute inflammation was induced by injecting 0.1 ml of 2% formaldehyde solution into the sub-plantar region of the left hind paw of each rat.

The paw volume was measured using a plethysmometer immediately before formaldehyde injection (0 hour) and subsequently at 1, 3, 5 and 7 Days after formaldehyde administration. The increase in paw volume was considered as an indicator of edema formation.

The anti-inflammatory activity of the extract was evaluated by comparing the paw edema volume of the treated groups with that of the formaldehyde control group. The percentage inhibition of edema was calculated, and a significant reduction in paw swelling was considered indicative of anti-inflammatory activity.

### Grouping of Animals

- Fasted overnight with free access to water
- Animals divided into 5 groups (n = 6)

Group	Treatment	No. of animals	Observation parameter
Group-I	Normal control (1ml Distilled water)	06	Formaldehyde induced paw edema in rats  Change in paw volume.
Group-II	Formaldehyde control (Formaldehyde 0.1ml)	06	
Group-III	Standard drug (indomethacin 10 mg/kg)	06	
Group-IV	Extract low dose (100	06	

	mg/kg)		
Group-V	Extract high dose (200 mg/kg)	06	

### 3. Cotton Pellet Granuloma Model

To evaluate the chronic anti-inflammatory activity of *Bryophyllum pinnatum* leaf extract using the Cotton Pellet Granuloma Model in rats.

Implantation of sterile cotton pellets under the skin induces a chronic inflammatory response, resulting in granuloma tissue formation around the pellet.

On Day 0, sterile cotton pellets weighing  $10 \pm 1$  mg were prepared. The rats were anesthetized, and the dorsal (back) region was shaved and disinfected with alcohol. A small incision of approximately 1 cm was made in the dorsal region, and one sterile cotton pellet was implanted subcutaneously on each side of the back. The incision was then closed using sutures, and the animals were allowed to recover.

-Treatment was started immediately after pellet implantation. The animals received the vehicle, standard drug, or *Bryophyllum pinnatum* leaf extract orally once daily for seven consecutive days.

-On Day 8, the animals were humanely sacrificed, and the cotton pellets along with the surrounding granuloma tissue were carefully removed. The pellets were dried in a hot air oven at 60°C for 24 hours and then weighed. The increase in the dry weight of the pellets represented

granuloma formation, which was used to assess the chronic anti-inflammatory activity of the test extract.

#### Experimental Grouping and Dose Design

The animals will be randomly divided into the following groups:

Group	Treatment	No. of animals	Observation parameter
Group-I	Normal control (1ml Distilled water)	06	
Group-II	Disease/Negative Control		
	(Cotton pellet implantation +)	06	
	Distilled Water (1 ml/kg, p.o.)		Body weight, dry
Group-III	Standard drug (indomethacin) 10 mg/kg.p.o)	06	granuloma weight (mg), % inhibition of
Group-IV	<i>Bryophyllum pinnatum</i> Extract low dose (100 mg/kg,p.o)	06	granuloma formation

Group-V	<i>Bryophyllum pinnatum</i> Extract high dose (200 mg/kg,p.o)	06	
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## RESULTS AND DISCUSSION

### Preliminary Phytochemical Screening:

Components	Test	Result
Flavonoids	Shinoda Test	Present
Tannins	Ferric Chloride Test	Present
Saponins	Foam Test	Present
Terpenoids	Liebermann–Burchard Test	Present
phenolic compounds	Ferric Chloride Test	Present

### Percentage Yield:

Plant Material	Solvent	Weight of Powder(gm)	Weight Of Extract(gm)	Percentage Yield
<i>Bryophyllum pinnatum</i> Leaves	Ethanol	100	16.5	16.5 %

**Table: Effect of *Bryophyllum pinnatum* Extract on Carrageenan-Induced Paw Edema**

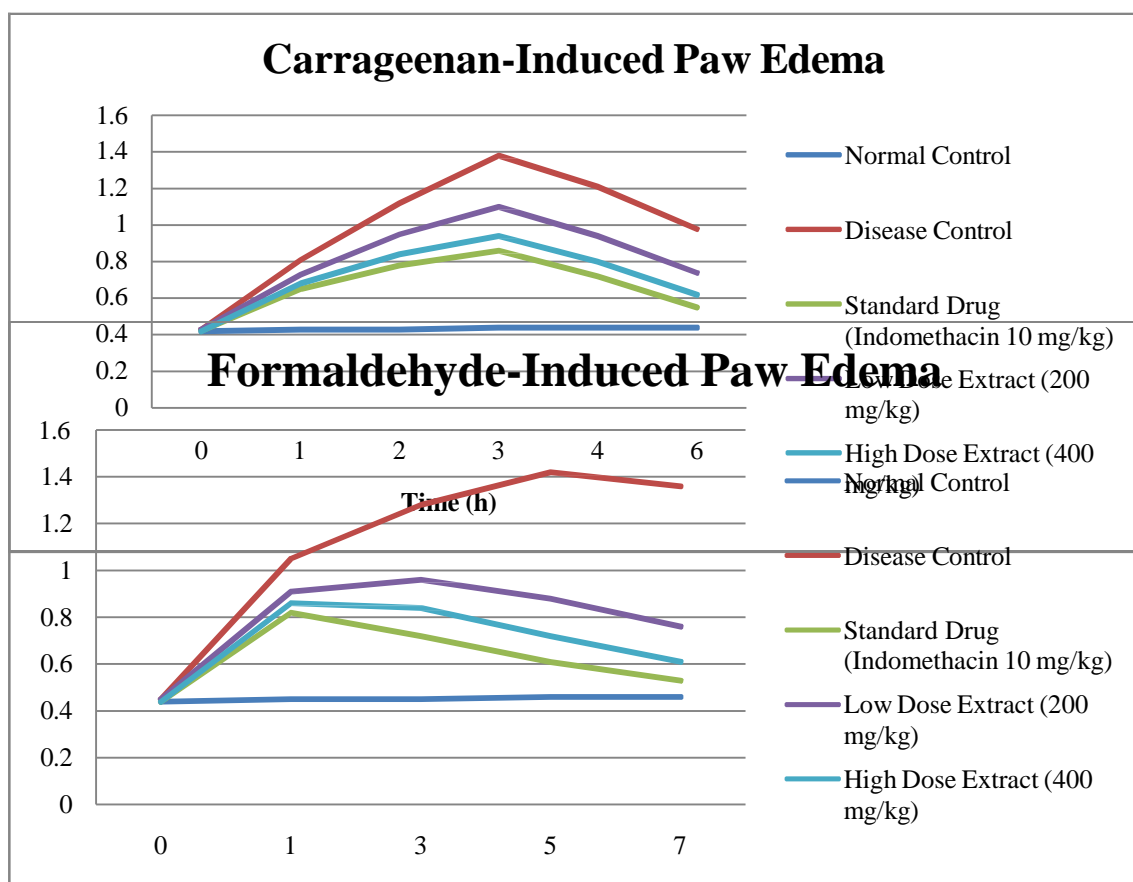
Group	0 h	1 h	2 h	3 h	4 h	6 h
Normal Control	0.42 ± 0.01	0.43 ± 0.02	0.43 ± 0.01	0.44 ± 0.01	0.44 ± 0.02	0.44 ± 0.01
Disease Control	0.43 ± 0.02	0.81 ± 0.03	1.12 ± 0.04	1.38 ± 0.05	1.21 ± 0.04	0.98 ± 0.03
Standard Drug (indomethacin 10 mg/kg)	0.42 ± 0.01	0.65 ± 0.02**	0.78 ± 0.03***	0.86 ± 0.03***	0.72 ± 0.02***	0.55 ± 0.02***
Low Dose Extract (200 mg/kg)	0.43 ± 0.01	0.73 ± 0.03*	0.95 ± 0.04*	1.10 ± 0.04*	0.94 ± 0.03**	0.74 ± 0.03**
High Dose Extract (400 mg/kg)	0.42 ± 0.02	0.68 ± 0.02**	0.84 ± 0.03**	0.94 ± 0.03***	0.80 ± 0.03***	0.62 ± 0.02***

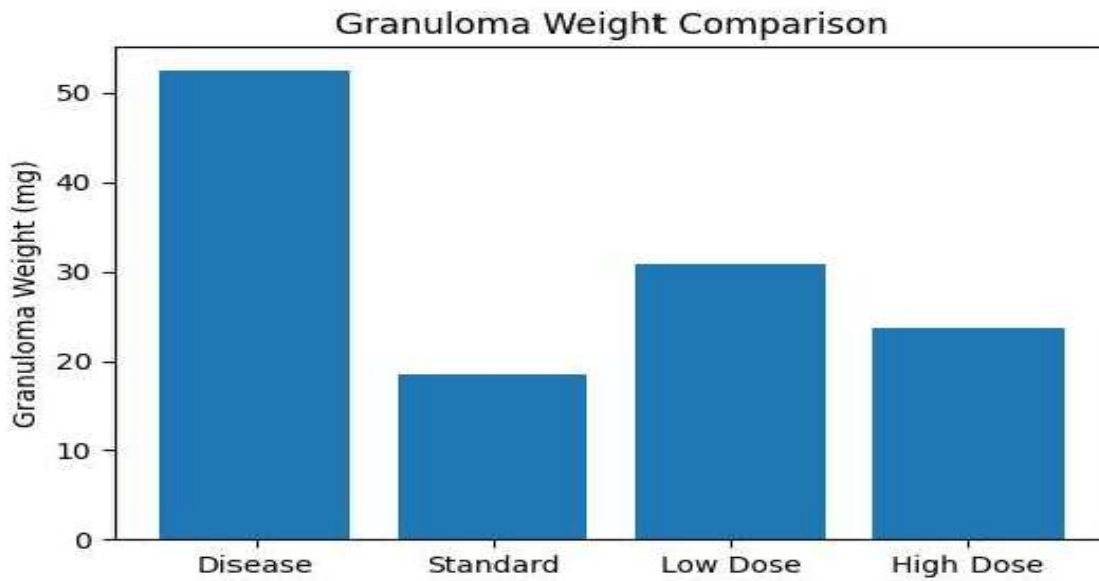
All values are presented as mean  $\pm$  SEM, n=6. One way ANOVA, followed by Dunnett's test was performed as the test of significance \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

**Table: Percentage Inhibition of Paw Edema**

Group	Initial Pellet Weight (mg)	Final Pellet Weight (mg)	Granuloma Weight (mg)	Percentage Inhibition (%)
Disease Control	20	72.5 $\pm$ 2.8	52.5 $\pm$ 2.8	0.00
Standard Drug (indomethacin 10 mg/kg)	20	38.4 $\pm$ 1.9***	18.4 $\pm$ 1.9***	64.95
Low Dose Extract (200 mg/kg)	20	50.8 $\pm$ 2.3*	30.8 $\pm$ 2.3**	41.33
High Dose Extract (400 mg/kg)	20	43.6 $\pm$ 2.1**	23.6 $\pm$ 2.1***	55.05

All values are presented as mean  $\pm$  SEM, n=6. One way ANOVA, followed by Dunnett's multiple comparison test was performed as the test of significance \*p<0.05, \*\*p<0.01, \*\*\*p<0.001





## SUMMARY AND CONCLUSION

The present study was designed to evaluate the anti-inflammatory activity of *Bryophyllum pinnatum* leaf extract using well-established experimental animal models. The leaves of the plant were collected, authenticated, and subjected to extraction using suitable solvents. The obtained extract was then evaluated for preliminary phytochemical constituents, which indicated the presence of bioactive compounds such as flavonoids, phenols, tannins, glycosides, and triterpenoids, which are known to possess anti-inflammatory and antioxidant properties.

The anti-inflammatory potential of the extract was assessed using carrageenan-induced paw edema (acute inflammation), formaldehyde-induced paw edema (sub-acute inflammation), and cotton pellet-induced granuloma (chronic inflammation) models. Paw volume, percentage inhibition of edema, onset and duration of inflammation, and granuloma weight were used as evaluation parameters. The results were compared with a standard anti-inflammatory drug such as indomethacin.

*Bryophyllum pinnatum* leaf extract significantly reduced carrageenan-induced paw edema in rats. The effect was dose-dependent and comparable to indomethacin, indicating that the extract possesses significant acute anti-inflammatory activity.

*Bryophyllum pinnatum* leaf extract significantly reduced granuloma formation in a dose-dependent manner, indicating potent chronic anti-inflammatory activity comparable to the standard drug. This suggests that the extract may be useful in the management of chronic inflammatory conditions.

Histopathological examination further supported the biochemical findings by showing reduced inflammatory cell infiltration, decreased edema, and improved tissue architecture in treated groups. Statistical analysis confirmed the significance of the observed effects.

The extract demonstrated dose-dependent anti-inflammatory activity across acute, sub-acute, and chronic models, suggesting its broad-spectrum efficacy.

The findings of the present study indicate that *Bryophyllum pinnatum* leaf extract possesses significant anti-inflammatory activity in experimental animal models. The extract effectively reduced inflammation in

both acute and chronic models, as evidenced by decreased paw edema and inhibition of granuloma formation.

The observed pharmacological activity may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, and triterpenoids, which are known to inhibit inflammatory mediators and oxidative stress pathways. The results were comparable, though standard anti-inflammatory drugs, indicating its potential therapeutic relevance.

Thus, *Bryophyllum pinnatum* can be considered a promising natural source for the development of safer anti-inflammatory agents. However, further studies are recommended to isolate the active compounds, elucidate detailed molecular mechanisms, and evaluate clinical efficacy and safety in humans.

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