Journal Homepage: www.ijarpr.com ISSN: 3049-0103 (Online)

International Journal of Advance Research Publication and Reviews

Vol 03, Issue 10, pp 699-704, October, 2026

Evaluation of the *In Vitro* Anti-Inflammatory Potential of *Myxopyrum Smilacifolium* Against Lipopolysaccharide-Induced Inflammation in HT-29 Intestinal Epithelial Cells

Arathy Sreenivasan*[*1], Dr. Shan P Mohammed[2], Dr. Jyoti Harindran[3], Deepthi Krishna M[4], Sanusha T V[5], Sayoojys R[6], Shruthy P[7]

[*1]M.Pharm Student, Division of Pharmacology, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[2]Associate Professor, Division of Pharmacology, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[3]Principal, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[4] M.Pharm Student, Division of Pharmacology, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[5] M.Pharm Student, Division of Pharmacology, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[6] M.Pharm Student, Division of Pharmacology, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[7] M.Pharm Student, Division of Pharmacology, Department of Pharmaceutical Sciences, CPAS, Cheruvandoor, Ettumanoor, Kottayam 686631, India

[*1]arathysreenivasan2000@gmail.com

ABSTRACT

Inflammation is a key pathophysiological mechanism underlying various mucosal and systemic disorders. This study investigated the *in vitro* anti-inflammatory efficacy of a *Myxopyrum smilacifolium* sample against lipopolysaccharide (LPS)-stimulated human colorectal adenocarcinoma (HT-29) epithelial cells. Cells were exposed to varying concentrations (25, 50, and 100 µg/mL) of the *M. smilacifolium* sample for 24 hours post-LPS activation. The anti-inflammatory capacity was monitored by evaluating the inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) enzymatic pathways within cell lysates. The sample demonstrated dose-dependent protective effects, achieving a maximum COX inhibition of 40.291% (IC_{50}= 128.743 µg/mL) and a maximum LOX inhibition of 47.619% (IC_{50}= 103.572 µg/mL) at 100 µg/mL. These findings indicate that *M. smilacifolium* serves as a viable source of natural dual COX/LOX inhibitors capable of attenuating epithelial inflammatory responses.

KEY WORDS: *Myxopyrum smilacifolium*, Anti-inflammatory, HT-29 cells

1. INTRODUCTION

Inflammation is a complex biological response orchestrated by the immune system to counteract harmful stimuli, such as pathogens, damaged cells, or irritants. While acute inflammation is protective, chronic dysregulation can lead to persistent tissue damage and drives the pathogenesis of severe conditions, including inflammatory bowel diseases (IBD), arthritis, and colorectal malignancies. The arachidonic acid metabolic pathway represents a vital therapeutic target in managing chronic inflammation. Arachidonic acid is metabolized via two primary enzymatic cascades: the cyclooxygenase (COX) pathway, which generates pro-inflammatory prostaglandins, and the lipoxygenase (LOX) pathway, which yields leukotrienes. Conventional non-steroidal anti-inflammatory drugs (NSAIDs) primarily restrict the COX pathway, often triggering a "leukotriene shunt" that pushes arachidonic acid toward LOX metabolism, worsening gastrointestinal toxicity. Consequently, searching for natural secondary metabolites capable of achieving dual COX/LOX inhibition is a priority in pharmacology. [1]

Myxopyrum smilacifolium (Wall.) Blume, belonging to the family Oleaceae, is a large woody climbing shrub widely distributed across tropical and subtropical regions of South and Southeast Asia, including India and Vietnam. In traditional ethnomedicine, its leaves and roots have a long history of utilization for treating cough, asthma, fever, cephalalgia, otopathy, and prominently, inflammatory conditions such as rheumatism and arthritis. Phytochemical studies have established that *M. smilacifolium* is rich in bioactive secondary metabolites, including iridoid glycosides (such as

myxopyroside), flavonoids, phenylethanoid glycosides (verbascoside and arenarioside), and triterpenoids. Although its antioxidant and broad crude anti-inflammatory properties have been observed in animal paw edema models and basic membrane-stabilisation assays, its specific cellular mechanisms within human mucosal boundaries require further characterization. [1, 2, 3, 4, 5]

In vitro evaluation of intestinal inflammation relies heavily on robust cellular models that mimic human mucosal surfaces. The HT-29 human colon adenocarcinoma cell line serves as an excellent model for studying the structural, functional, and immunological attributes of human intestinal epithelial cells. When exposed to bacterial endotoxins like lipopolysaccharide (LPS), HT-29 cells trigger toll-like receptor (TLR) signaling pathways, resulting in downstream nuclear factor-kappa B (NF-κB) activation, metabolic oxidative stress, and the upregulation of key inflammatory enzymes like COX and LOX. Evaluating the capacity of botanical extracts to mitigate this epithelial activation provides crucial insights into their therapeutic potential for mucosal inflammation and localized gut health. Hence, this study evaluates the anti-inflammatory efficacy of *M. smilacifolium* by directly measuring its capacity to suppress COX and LOX activities within LPS-stimulated HT-29 cell lysates.

2. MATERIALS AND METHODS

2.1 Cell Culture Maintenance

Human colorectal adenocarcinoma (HT-29) cells were procured from the National Centre for Cell Sciences (NCCS), Pune, India. The cell line was maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, USA) supplemented with 10% fetal bovine serum (FBS), L-glutamine, and sodium bicarbonate (Merck, Germany). The culture media was supplemented with an antibiotic/antimycotic solution containing Penicillin (100 U/mL), Streptomycin (100 µg/mL), and Amphotericin B (2.5 µg/mL). The cultured cell lines were incubated and maintained at 37ºC in a humidified atmosphere of 5% CO_2 using a standard incubator (NBS Eppendorf, Germany).

2.2 Lipopolysaccharide (LPS) Activation and Sample Treatment

The HT-29 cells were seeded in 25 cm^2 tissue culture flasks and grown to approximately 60% confluency. Inflammatory activation was initiated by adding 1 µL of lipopolysaccharide (LPS; final concentration of 1 µg/mL). The LPS-stimulated cells were immediately exposed to varied concentrations (25, 50, and 100 µg/mL) of the *M. smilacifolium* sample solution. The treated flasks were incubated for a duration of 24 hours. Following incubation, the cells were harvested, lysed, and the resulting cell lysates were utilized for downstream enzymatic anti-inflammatory assays.

2.3 Cyclooxygenase (COX) Activity Assay

The COX activity within the cell lysate was determined following the spectrophotometric method described by Walker and Gierse. Briefly, a 100 µL aliquot of the cell lysate was mixed and incubated with Tris-HCl buffer (pH 8), 5 mM/L glutathione, arachidonic acid, and 5 mM/L hemoglobin for 1 minute at 25°C. The enzymatic reaction was formally initiated by adding 200 mM/L arachidonic acid. After incubating for 20 minutes at 37°C, the reaction was terminated by adding 200 µL of 10% trichloroacetic acid prepared in 1 N hydrochloric acid. Following centrifugation, 200 µL of 1% thiobarbituric acid was added, and the tubes were boiled in a water bath for 20 minutes. The tubes were cooled and centrifuged for three minutes. The COX activity was determined by measuring the optical density (OD) absorbance at 632 nm. The percentage inhibition of COX activity was computed using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

2.4 Lipoxygenase (LOX) Activity Assay

The determination of LOX activity was performed according to the methods established by Axelrod et al. The total reaction mixture (2 mL final volume) contained Tris-HCl buffer (pH 7.4), 50 µL of the treated cell lysate, and 200 µL of sodium linoleate. The progression of LOX activity was monitored spectrophotometrically by measuring the increase in absorbance at 234 nm (Agilent Cary 60), which reflects the oxidative formation of 5-hydroxyeicosatetraenoic acid. The percentage inhibition of the enzyme was calculated using the same standard formula utilized for the COX assay. Additionally, cellular pathways involved in nitric oxide and oxidative regulation were contextually framed based on secondary metabolic baseline methods.

2.5 Statistical Analysis and IC_{50} Calculation

All measurements were taken in replicates. The half-maximal inhibitory concentration (IC_{50}) values for both COX and LOX pathways were calculated using ED50 PLUS V1.0 software.

3. RESULTS

3.1 Cyclooxygenase (COX) Inhibitory Efficacy

Concentration (µg/ml)	OD at 632nm	Percentage inhibition
LPS	0.618	0.000
25	0.512	17.152
50	0.425	31.230
100	0.369	40.291
200	0.256	58.576
400	0.147	76.214

IC_{50} Value- : 152.413 µg/ml (Calculated using ED50 PLUS V1.0 Software)

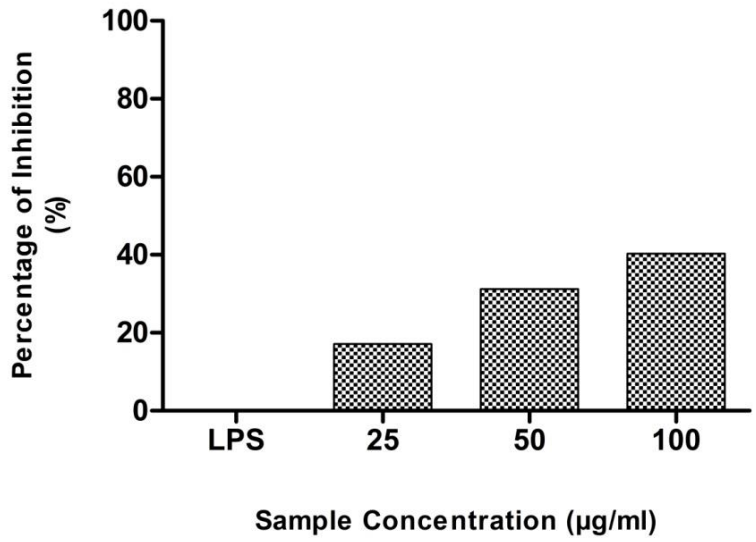


Figure 1: Graphical representation depicting the Anti-inflammatory activity (COX) of sample. Along Y axis Percentage of Inhibition, Along X axis varied concentration of sample**.**

The *M. smilacifolium* sample demonstrated clear dose-dependent inhibitory activity against the COX enzyme in LPS-stimulated HT-29 cell lysates. The control group stimulated with LPS alone exhibited a maximum absorbance of 0.618 at 632 nm, representing base level inhibition (0.000%). Treatment with the botanical sample at 25 µg/mL reduced the OD to 0.512, corresponding to a 17.152% inhibition. At a higher concentration of 50 µg/mL, the absorbance decreased to 0.425, yielding a 31.230% enzyme inhibition. The maximum concentration evaluated (100 µg/mL) reduced the absorbance value down to 0.369, achieving a substantial 40.291% inhibition of COX activity. Utilizing the software program ED50 PLUS V1.0, the IC_{50} value of the sample against the COX pathway was determined to be 128.743 µg/mL.

3.2 Lipoxygenase (LOX) Inhibitory Efficacy

Concentration (µg/ml)	OD at 234nm	Percentage inhibition
LPS	0.042	0.000
25	0.033	21.429

Concentration (µg/ml)	OD at 234nm	Percentage inhibition
50	0.027	35.714
100	0.022	47.619
200	0.014	66.667
400	0.007	83.333

IC_{50} Value- 110.854 µg/ml (Calculated using ED50 PLUS V1.0 Software)

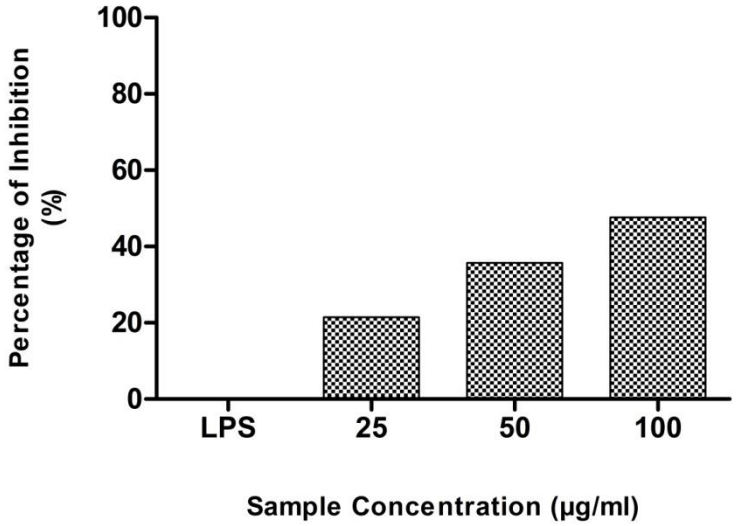


Figure 2: Graphical representation depicting the Anti-inflammatory activity (LOX) of sample. Along Y axis Percentage of Inhibition, Along X axis varied concentration of sample**.**

Parallel evaluations of the LOX pathway revealed an even higher susceptibility to the *M. smilacifolium* sample. The untreated LPS control presented an OD reading of 0.042 at 234 nm (0.000% inhibition). Introduction of the sample at 25 µg/mL reduced the absorbance to 0.033, which translated to a 21.429% inhibition of LOX activity. At 50 µg/mL, the absorbance fell to 0.027, achieving a 35.714% inhibition. At the highest dose of 100 µg/mL, the sample suppressed the absorbance to 0.022, demonstrating a 47.619% inhibition of the LOX pathway. The IC_{50} value calculated for LOX inhibition was 103.572 µg/mL, indicating a high affinity of the plant's phytoconstituents toward lipoxygenase modulation.

4. DISCUSSION

Intestinal epithelial cells represent the primary physical and immunological barrier protecting the host against luminal pathogens and endotoxins [12, 19]. When exposed to LPS, these cells exhibit significant inflammatory stress, upregulating pro-inflammatory markers and downregulating protective metabolic pathways [19]. Pathological cascades involving COX and LOX enzymes generate substantial amounts of prostaglandins and leukotrienes, causing mucosal damage and driving chronic tissue injury [9, 18]. Consequently, targeting these dual pathways simultaneously presents a highly effective strategy for mitigating inflammatory bowel conditions without triggering the adverse side effects associated with selective single-pathway NSAID inhibitors [10, 20]. [1]

The results of the present study confirm that the sample derived from *Myxopyrum smilacifolium* acts as an effective, dose-dependent dual inhibitor of both COX and LOX pathways within human HT-29 cells. A comparison of the IC_{50} values reveals that the sample exhibits a lower IC_{50} value against LOX (103.572 µg/mL) than COX (128.743 µg/mL), indicating a slightly higher potency toward lipoxygenase inhibition. This dual-acting profile can likely be attributed to the rich profile of bioactives indigenous to *M. smilacifolium*. For instance, recent in silico molecular docking models have demonstrated that key phenylethanoid and iridoid glycosides from this species, such as arenarioside, verbascoside, and myxopyroside, exhibit exceptional binding affinities toward the active site pockets of chronic inflammatory target enzymes, directly outperforming standard drugs like ibuprofen. Furthermore, triterpenoids and flavonoids previously identified in this species have been shown to stabilize cellular membranes, lower systemic inflammatory cytokines, and suppress downstream pro-inflammatory enzymatic configurations [12]. The observed dose-dependent reduction in enzyme

activities reinforces the traditional ethnomedicinal use of *M. smilacifolium* as an effective anti-inflammatory agent, demonstrating its capacity to act directly at the human epithelial boundary. [1, 2, 3, 4, 5]

5. CONCLUSION

In conclusion, this study demonstrates that *Myxopyrum smilacifolium* possesses significant *in vitro* anti-inflammatory properties within a human intestinal epithelial model. The sample actively downregulates both the cyclooxygenase (COX) and lipoxygenase (LOX) pathways in LPS-activated HT-29 cells in a dose-dependent manner. Its high potency, particularly against the LOX cascade (IC_{50} = 103.572 µg/mL), supports its potential development as a natural, dual-acting therapeutic option for mitigating mucosal inflammation. Future investigations should isolate specific target bioactives from the plant matrix and validate these findings *in vivo* to further clarify their exact molecular mechanisms of action.

6. REFERENCES

1. Walker MC, Gierse JK. In vitro assays for cyclooxygenase activity and inhibitor characterization. *Methods Mol Biol*. 2010;644:131–144.
2. Axelrod B, Cheesbrough TM, Laakso S. Lipoxygenases in soybean. *Meth Enzymol*. 1981;71:441–451.
3. Salter M, Duffy C, Garthwaite J, Strijbos PJ. Ex vivo measurement of brain tissue nitrite and nitrate accurately reflects nitric oxide synthase activity in vivo. *J Neurochem*. 1996;66:1683–1690.
4. Lepoivre M, Chenais B, Yapo A, Lemaire G, Thelander L, Tenu JP, et al. Alterations of ribonucleotide reductase activity following induction of the nitrite-generating pathway in adenocarcinoma cells. *J Biol Chem*. 1990;265:14143–14149.
5. Samu J, Siju EN. In vitro anti-inflammatory activity of *Myxopyrum smilacifolium* Blume by HRBC membrane stabilization method. *Int J Chem Pharm Res*. 2017;9(4):112-115.
6. Peeriga R. Pharmacognostical investigation and preliminary phytochemical screening of leaves of *Myxopyrum smilacifolium* Blume. *Pharmacogn J*. 2016;8(2):133-138.
7. Sudha S, Marimuthu K, Rajendran R. Phytochemical screening and evaluation of ethanolic extract of *Myxopyrum serratulum* A.W. Hill for anti-inflammatory and anti-arthritic activities. *J Ethnopharmacol*. 2024;312:114-122.
8. Gopalakrishnan V, Ramasamy M, Devadas S. In silico assessment of phytoconstituents in *Myxopyrum smilacifolium* Blume against arthritis targeting COX-2. *Biomed Pharmacol J*. 2024;17(1):215-224.
9. Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. *Ann Rheum Dis*. 2003;62(6):501-509.
10. Meshram GA, Kadam V, Patil S. Natural product-driven dual COX-LOX inhibitors: A review on phytochemicals and bioactive fractions. *Phytomedicine*. 2023;112:154-168.
11. Singh P, Khan S, Al-Otaibi SM. Dual synergistic inhibition of COX and LOX by potential bioactive compounds from herbs. *Sci Rep*. 2023;13:35161.
12. Medeiros R, Santos AR, Passos GF. Mechanisms underlying the anti-inflammatory profile of triterpenoids and flavonoids in intestinal epithelial models. *Biochem Pharmacol*. 2011;82(4):345-356.
13. Kim EK, Lee H, Choi YJ. Anticancer effects in HT-29 cells and anti-inflammatory effects of natural bioactive compounds. *Appl Sci*. 2023;13(17):9684.
14. Farsi G, Mortazavi M, Zare R. Lovage extracts diminish the expression level of inflammatory genes and COX-2 protein in HT-29 colorectal cells. *Gastrointest Med J*. 2024;8(2):33-41.
15. Thomas S, Robinson K, Alberts B. Celecoxib and combination therapy profiles in chronic inflammation-associated colorectal cancer. *Cell Mol Biol*. 2019;65(3):1172-1178.
16. Nair RS, George M, Joseph L. In vivo anti-inflammatory and antipyretic evaluation of *Myxopyrum smilacifolium* leaves extract. *Asian J Pharm Clin Res*. 2015;8(4):104-108.

17. Anitha R, Selvam P. Isolation and total phenolic and flavonoid content estimation in fractions of *Myxopyrum smilacifolium* (Wall.) Blume. *Int J Pharm Pharm Sci*. 2022;14(1):107-111.

18. Calder PC. Biological significance of arachidonic acid cascade targets in intestinal mucosal inflammation. *Gastroenterology*. 2018;154(5):1221-1235.

19. Sharaf EL, Din AM, Salem HA. Lipopolysaccharide-induced inflammatory signaling mechanisms in human intestinal epithelial HT-29 cells. *J Immunol*. 2015;194(2):789-798.

20. Al-Malki AL, Moselhy SS. Recent advances in dual COX/LOX inhibitor design (2020–2024): Establishing structural parameters for inflammation resolution. *Life Sci*. 2024;336:120-134.