INTERNATIONAL JOURNAL OF PHARMACY WITH MEDICAL SCIENCES
Peer Reviewed, Referred & Indexed Journal
E-ISSN:3068-2738
Vol.6, No. 4 (2026)
www.ijpams.com

EVALUATION OF COX AND LOX INHIBITORY ACTIVITY OF *COMBRETUM INDICUM* LEAF EXTRACT IN RAW 264.7 CELLS

Susan Alby*, Jenila Jose Jancy.V, Parthiban.S, Anand,S.K, Prabhu.G,Vijesh.V.

Abstract

Background

Inflammation is a protective biological response initiated by the body against harmful stimuli such as pathogens, tissue injury, and toxic substances. Cyclooxygenase (COX) and lipoxygenase (LOX) are key enzymes involved in the biosynthesis of inflammatory mediators, including prostaglandins and leukotrienes. Medicinal plants rich in bioactive phytochemicals are increasingly investigated as alternative sources of anti-inflammatory agents. Combretum indicum (syn. Quisqualis indica L.) is traditionally used for various medicinal purposes, including the treatment of inflammatory disorders.

Objective

To evaluate the in vitro anti-inflammatory activity of ethanolic leaf extract of Combretum indicum using COX and LOX inhibitory assays in RAW 264.7 murine macrophage cells.

Materials and Methods

Fresh leaves of Combretum indicum were shade-dried, powdered, and extracted with ethanol using the maceration method. Preliminary phytochemical screening was performed using standard qualitative methods. Anti-inflammatory activity was assessed in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells by determining the inhibitory effects on COX and LOX enzymes. Diclofenac sodium was used as the reference standard. Percentage inhibition and IC_{50} values were calculated.

Results

Phytochemical screening revealed the presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, tannins, phenolic compounds, and saponins. In the COX assay, the extract demonstrated concentration-dependent inhibition with a maximum inhibition of 51.19% at 100 µg/mL and an IC_{50} value of 93.41 µg/mL. In the LOX assay, the extract exhibited 50.70% inhibition at 100 µg/mL with an IC_{50} value of 96.10 µg/mL. Diclofenac showed superior inhibitory activity in both assays, with maximum inhibitions of 70.06% (COX) and 69.14% (LOX).

Conclusion

The ethanolic leaf extract of Combretum indicum exhibited significant in vitro anti-inflammatory activity through inhibition of both COX and LOX pathways. The observed activity may be attributed to the presence of flavonoids, phenolics, and other bioactive phytoconstituents. These findings support the traditional medicinal use of Combretum indicum and suggest its potential as a natural source of anti-inflammatory agents.

Keywords: Combretum indicum, Anti-inflammatory activity, RAW 264.7 cells, Cyclooxygenase, Lipoxygenase, Diclofenac, Phytochemicals.

Introduction

Inflammation is a complex physiological response of living tissues to harmful stimuli such as pathogens, damaged cells, and irritants. Although inflammation is essential for defense and tissue repair, persistent or chronic inflammation contributes to numerous pathological conditions including rheumatoid arthritis, cardiovascular diseases, diabetes mellitus, and cancer.

The inflammatory process is mediated through the release of various chemical mediators, including prostaglandins, leukotrienes, cytokines, histamine, and nitric oxide. Among the major pathways involved in inflammation, cyclooxygenase (COX) and lipoxygenase (LOX) play crucial roles in converting arachidonic acid into prostaglandins and leukotrienes, respectively. Therefore, inhibition of these enzymes is considered an effective strategy for controlling inflammation.

Medicinal plants represent an important source of bioactive compounds with therapeutic potential. Combretum indicum (L.) DeFilipps, commonly known as Rangoon Creeper, belongs to the family

Combretaceae and is widely distributed throughout tropical Asia. Various parts of the plant have been reported to possess antioxidant, antimicrobial, anthelmintic, wound-healing, and anti-inflammatory properties.

However, scientific evidence supporting the anti-inflammatory activity of Combretum indicum leaves remains limited. Therefore, the present study was undertaken to evaluate the anti-inflammatory potential of ethanolic leaf extract of Combretum indicum using RAW 264.7 macrophage cell line-mediated COX and LOX inhibition assays.

Materials and Methods

Plant Material Collection and Authentication

Fresh and healthy leaves of Combretum indicum were collected from Karungal, Kanniyakumari District, Tamil Nadu, India. The leaves were washed thoroughly, shade-dried for seven days, and pulverized into coarse powder using a mechanical grinder.

Preparation of Extract

The powdered leaf material was extracted with ethanol using the maceration technique. The powder was soaked in ethanol for seven days with occasional shaking. The extract was filtered and concentrated to obtain a crude ethanolic extract, which was stored for further analysis.

Preliminary Phytochemical Screening

The ethanolic extract was subjected to qualitative phytochemical analysis using standard protocols for:

Carbohydrates (Molisch's test)

Alkaloids (Dragendorff's test)

Flavonoids (Shinoda test)

Cardiac glycosides (Keller-Killiani test)

Tannins and phenolic compounds (Ferric chloride test)

Saponins (Froth test)

Cell Line Maintenance

RAW 264.7 murine macrophage cells were obtained and maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO_2.

Cyclooxygenase (COX) Inhibition Assay

RAW 264.7 cells were stimulated with lipopolysaccharide (LPS) and treated with varying concentrations of the extract. COX enzyme activity was assessed spectrophotometrically at 632 nm. Diclofenac sodium served as the standard anti-inflammatory drug.

Lipoxygenase (LOX) Inhibition Assay

LPS-stimulated RAW 264.7 cells were treated with different concentrations of the extract. LOX activity was measured by monitoring absorbance at 234 nm following sodium linoleate oxidation. Diclofenac sodium was used as the reference drug.

Statistical Analysis

Percentage inhibition was calculated using the standard formula:

$$\text{Percentage Inhibition} = \frac{\text{Control OD - Sample OD} \times 100}{\text{Control OD}}$$

IC_{50} values were determined from concentration-response curves.

Results

Phytochemical Screening

Phytochemical Constituent	Result
Carbohydrates	Present
Alkaloids	Present
Flavonoids	Present

Concentration (µg/mL)	% Inhibition
6.25	7.90
12.5	14.22
25	22.61
50	37.84
100	51.19

Cardiac Glycosides	Present
Tannins	Present
Phenolic Compounds	Present
Saponins	Present

COX Inhibition Activity
IC_{50} = 93.41 µg/mL
LOX Inhibition Activity

Concentration (µg/mL)	% Inhibition
6.25	7.36
12.5	14.54
25	22.49
50	35.28
100	50.70

IC_{50} = 96.10 µg/mL
Standard Diclofenac Activity
COX Inhibition

Concentration (µg/mL)	% Inhibition
6.25	11.84
12.5	21.50
25	35.86
50	52.22
100	70.06

IC_{50} = 52.19 µg/mL
LOX Inhibition

Concentration (µg/mL)	% Inhibition
6.25	6.84
12.5	14.34
25	30.03
50	47.96
100	69.14

IC_{50} = 60.22 µg/mL

Received: 02-10-2026 | Accepted: 06-11-2026 | Published: 13-11-2026

INTERNATIONAL JOURNAL OF PHARMACY WITH MEDICAL SCIENCES
Peer Reviewed, Referred & Indexed Journal
E-ISSN:3068-2738
Vol.6, No. 4 (2026)
www.ijpams.com

Discussion

COX Inhibitory Activity

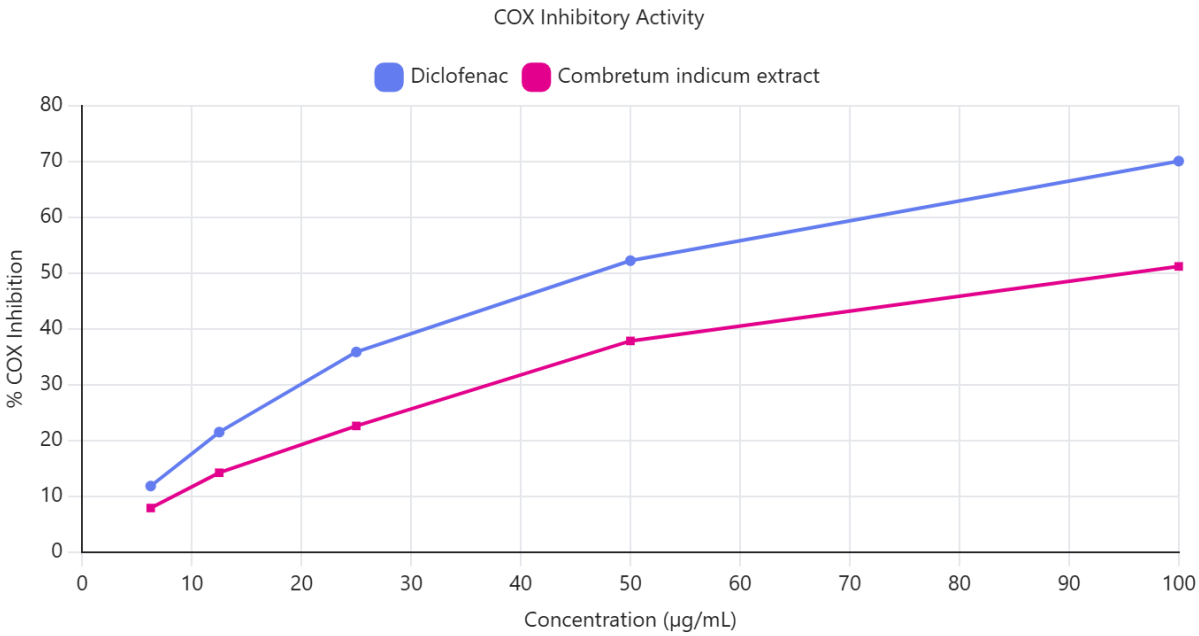


Fig.1

LOX Inhibitory Activity

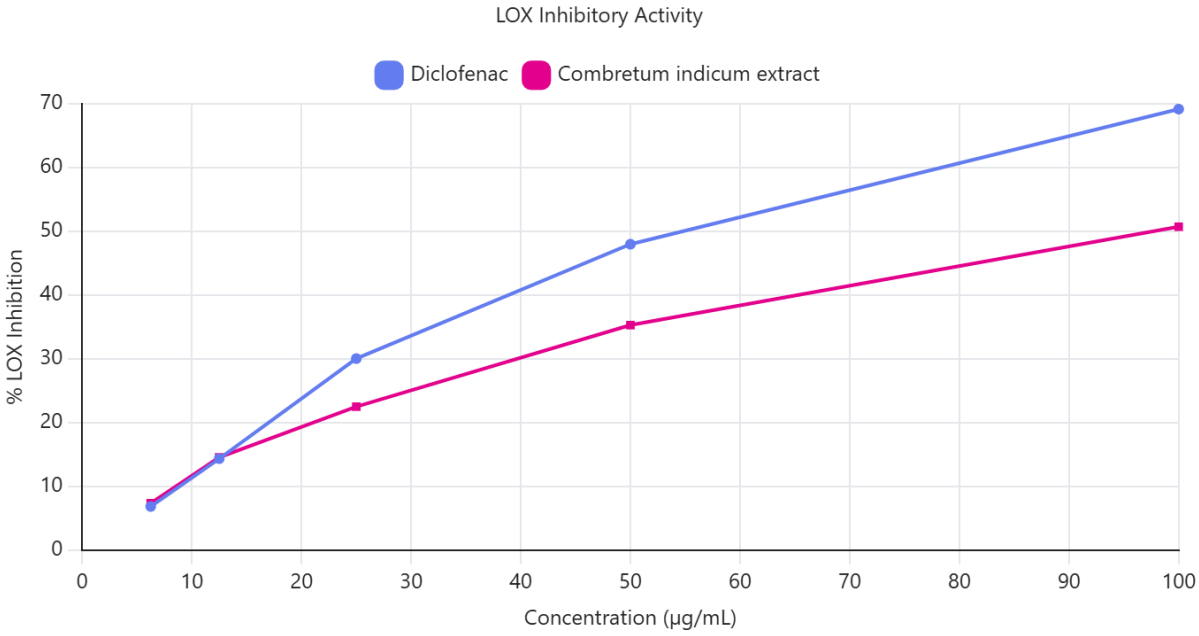


Fig.2

Figure 1. Concentration-dependent cyclooxygenase (COX) inhibitory activity of Combretum indicum leaf extract compared with Diclofenac. Values are expressed as percentage inhibition of COX enzyme activity at different concentrations (6.25-100 µg/mL).

Figure 2. Concentration-dependent lipoxygenase (LOX) inhibitory activity of Combretum indicum leaf extract compared with Diclofenac. Values are expressed as percentage inhibition of LOX enzyme activity at different concentrations (6.25-100 µg/mL).

Received: 02-10-2026 | Accepted: 06-11-2026 | Published: 13-11-2026

The present investigation demonstrated that the ethanolic leaf extract of Combretum indicum possesses notable anti-inflammatory activity through inhibition of both COX and LOX enzymes. The extract produced a concentration-dependent inhibitory effect, indicating the presence of biologically active constituents capable of modulating inflammatory pathways.

Phytochemical analysis revealed the presence of flavonoids, phenolic compounds, tannins, alkaloids, and saponins. Previous studies have reported that flavonoids and phenolic compounds exert anti-inflammatory effects by suppressing the synthesis of prostaglandins, leukotrienes, and pro-inflammatory cytokines.

The extract exhibited maximum COX inhibition of 51.19% and LOX inhibition of 50.70% at 100 µg/mL. Although the activity was lower than that of diclofenac sodium, the results indicate significant inhibition of inflammatory mediator pathways. The observed anti-inflammatory activity may be attributed to the synergistic action of multiple phytochemicals present in the leaf extract.

These findings are consistent with previous reports demonstrating the pharmacological potential of Combretum indicum and other medicinal plants rich in phenolic constituents.

Conclusion

The ethanolic leaf extract of Combretum indicum demonstrated significant in vitro anti-inflammatory activity against both cyclooxygenase and lipoxygenase enzymes in RAW 264.7 macrophage cells. The extract showed dose-dependent inhibition with maximum activities observed at 100 µg/mL. Preliminary phytochemical screening confirmed the presence of several bioactive constituents, including flavonoids, phenolics, alkaloids, and saponins, which may contribute to the observed biological activity. The study supports the traditional use of Combretum indicum as a medicinal plant and highlights its potential as a source of natural anti-inflammatory compounds. Further investigations involving bioactive compound isolation, molecular mechanism studies, and in vivo evaluations are recommended to validate its therapeutic applications.

Reference:

1. K.,Sidharth; Narayanasamy, K.;Mohan, Sruthy;Baskaran, K.;N., Nirmala Devi;Ragunathan, R. “Phytometabolite Profiling Of Combretum Indicum (L.) Defilipps And Its Characterization Studies” 2024
2. Ajay Verma, AKS Rawat “Phytochemical analysis, antioxidant potential, and cytotoxic activity of extracts of Quisqualis indica L.” Journal of Applied Pharmaceutical Research 13 (3), 247-258, 2025
3. Pallavi Pal, Ajeet Singh “In-vitro Antioxidant and Anti-inflammatory Activities of Quisqualis indica Linn. Leaves Extract” Journal of Advances in Biology & Biotechnology 21 (1), 1-13, 2019
4. Ritika Agrahari1*, Divya Singh2, Sandeep Yadav3, Rashmi Agrahari4 “Comparative Evaluation for the Anti-Inflammatory & Anti-Oxidant Properties of Hydro methanolic Extract of Leaves, Flowers and Stems of Combretum Indicum Linn in Albino Wistars Rats” 2023
5. Mayank Kulshreshtha, Rajaneesh Kumar Chaudhary, Supriya Roy, Karuna Shanker Shukla, Anita Singh, Manjul Pratap Singh “Pharmacological Investigation on Un raveling Mechanism of Action of Quisqualis indica Leaves for Predicted Treatment of Peptic Ulcer Disease” Current Functional Foods 1 (2), E200323214728, 2023
6. Subha Rastogi, Madan Mohan Pandey, Ajay Kumar Singh Rawat, Vikas Kushwaha, P Kalpana Murthy “In vitro antifilarial activity, antioxidant potential and phenolic constituents of Quisqualis indica L.” Indian Journal of Traditional Knowledge (IJTK) 18 (4), 648-654, 2019.
7. SJ Hur, S Young Choi, B Ou Lim “In vitro anti-inflammatory activity of

Russula virescens in the macrophage like cell line RAW 264.7 activated by lipopolysaccharide" Journal of Nutrition & Food Sciences 2 (4), 2012
8. Mohammad Shoaib, Diksha Singh, Rajeev Shukla "Anti-Rheumatoid Arthritic and Inflammatory property of Quisqualis indica Linn in wistar rat" Turkish Online Journal of Qualitative Inquiry 13 (1), 2022
9. Gierse Walker MC, Gierse JK. In vitro assays for cyclooxygenase activity and inhibitor characterization. Methods Mol Biol. 644, 2010, 131-44