Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

In Vitro Study of Anti-Inflammatory Activity of Ethanolic Extract of *Terminalia Cattappa* Red Leaves

Dasrao A Patil[1*], Dr. Disha Chouhan[2]

[1*]Research Scholar, Institute of Pharmaceutical Science and Research, Sardar Patel University, Balaghat, Madhya Pradesh, India 481001

[2]Professor, Institute of Pharmaceutical Science and Research, Sardar Patel University, Balaghat, Madhya Pradesh, India 481001

***Corresponding Author,**

Dasrao A Patil, Research Scholar, Institute of Pharmaceutical Science and Research, Sardar Patel University, Balaghat, Madhya Pradesh, India 481001

(Received: 16 February 2026 Revised: 25 March 2026 Accepted: 05 April 2026)

KEYWORDS:

Terminalia cattappa; Ethanolic extract; Anti-inflammatory activity; Antioxidant activity; DPPH assay; Nitric oxide scavenging; COX-2 inhibition; Protein denaturation; Phytoconstituents.

ABSTRACT:

The present study aimed to evaluate the in vitro anti-inflammatory and antioxidant activities of the ethanolic extract of Terminalia cattappa red leaves. The antioxidant potential was assessed using DPPH radical scavenging and nitric oxide scavenging assays, while anti-inflammatory activity was evaluated through cyclooxygenase-2 (COX-2) inhibition and protein denaturation methods.

The DPPH assay demonstrated a concentration-dependent increase in radical scavenging activity, with the extract showing 69.12 ± 0.86% inhibition at 100 µg/mL and an IC_{50} value of 74.49 ± 0.69 µg/mL. Similarly, nitric oxide scavenging activity increased with concentration, reaching 58.12 ± 0.86% inhibition at 100 µg/mL. In the COX-2 inhibition assay, the extract exhibited 56.70% inhibition at 100 µg/mL, indicating its potential to suppress prostaglandin-mediated inflammatory pathways. The protein denaturation assay further confirmed anti-inflammatory activity, showing 50.00% inhibition compared to 76.92% for the standard drug Ibuprofen.

The observed biological activities may be attributed to the presence of phytoconstituents such as flavonoids, tannins, and phenolic compounds, which possess strong antioxidant and anti-inflammatory properties. Overall, the results suggest that the ethanolic extract of Terminalia cattappa red leaves exhibits significant antioxidant and anti-inflammatory activities and may serve as a potential natural therapeutic agent for the management of inflammatory disorders.

INTRODUCTION:

Inflammation is a complex biological response of the body to harmful stimuli such as pathogens, damaged cells, or irritants. It plays a crucial role in the defense mechanism; however, prolonged or uncontrolled inflammation is associated with the development of various chronic disorders, including arthritis, cardiovascular diseases, diabetes, and neurodegenerative conditions.[1] The inflammatory process involves the release of mediators such as prostaglandins, cytokines, nitric oxide, and reactive oxygen species, which contribute to tissue damage and disease progression. Among these, the cyclooxygenase (COX) pathway, particularly COX-2, is a key regulator of prostaglandin synthesis and is widely targeted for anti-inflammatory therapy.[2]

Conventional anti-inflammatory drugs, especially non-steroidal anti-inflammatory drugs (NSAIDs) such as *Ibuprofen*, are commonly used for the management of inflammatory conditions. However, their long-term use is often associated with adverse effects, including gastrointestinal irritation, ulceration, and renal complications. These limitations have prompted the search for safer and more effective alternatives, particularly from natural sources. Medicinal plants have gained significant attention due to their rich content of bioactive compounds and their traditional use in the treatment of various ailments.[3-4]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

Terminalia cattappa, commonly known as Indian almond or tropical almond, is a medicinal plant widely distributed in tropical and subtropical regions. It has been traditionally used in various systems of medicine for its therapeutic properties, including anti-inflammatory, antioxidant, antimicrobial, and hepatoprotective effects. The plant is rich in phytoconstituents such as flavonoids, tannins, phenolic compounds, and triterpenoids, which are known to exhibit significant pharmacological activities.[5-8]

Oxidative stress is closely linked with inflammation, as excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can amplify inflammatory responses. Antioxidants play a vital role in neutralizing these free radicals and preventing cellular damage. Therefore, evaluating both antioxidant and anti-inflammatory activities provides a comprehensive understanding of the therapeutic potential of plant extracts. In vitro assays such as DPPH radical scavenging, nitric oxide scavenging, cyclooxygenase inhibition, and protein denaturation methods are widely employed to assess these activities.[9]

Despite the traditional use of *Terminalia cattappa*, there is a need for systematic scientific evaluation of its pharmacological properties, particularly using its red leaves, which are believed to possess higher concentrations of bioactive compounds. The present study was therefore designed to investigate the *in vitro* antioxidant and anti-inflammatory activities of the ethanolic extract of *Terminalia cattappa* red leaves using multiple experimental models, including DPPH radical scavenging assay, nitric oxide scavenging assay, cyclooxygenase-2 (COX-2) inhibition assay, and protein denaturation method.[10]

The findings of this study are expected to provide scientific evidence supporting the traditional use of *Terminalia cattappa* and may contribute to the development of novel plant-based therapeutic agents with improved safety and efficacy profiles for the management of inflammatory disorders.

MATERIALS AND METHODS:

Materials:

Fresh red leaves of *Terminalia cattappa* were collected and authenticated by a qualified botanist. The leaves were washed, shade-dried, powdered, and stored in airtight containers for further use. All chemicals and reagents used were of analytical grade. Methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium nitroprusside, and phosphate-buffered saline (PBS) were procured from Merck (India). Sulfanilamide, N-(1-naphthyl)ethylenediamine dihydrochloride, phosphoric acid, Tris-HCl buffer, hematin, arachidonic acid, and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) were obtained from Sigma-Aldrich (USA). Fresh hen's egg albumin was purchased from a local market. Standard compounds such as *Ascorbic acid* and *Ibuprofen* were obtained from HiMedia Laboratories. All solutions were prepared using double-distilled water. Absorbance measurements were carried out using a UV–Visible spectrophotometer (Shimadzu).

Methodology:

Pharmacological Screening: *In vitro* Antioxidant Activity

DPPH Radical Scavenging Assay[11-12]

The antioxidant activity of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, which is based on the ability of antioxidants to donate hydrogen atoms or electrons and neutralize free radicals. A freshly prepared 0.004% DPPH solution in methanol was used for the assay. The plant extract was dissolved in methanol to obtain different concentrations (20, 40, 60, 80, and 100 µg/mL). For the reaction, 1 mL of the extract solution at each concentration was mixed with 5 mL of DPPH solution. A control (negative control) was prepared by mixing DPPH solution with methanol, while the blank consisted of methanol and the respective sample solution. The reaction mixtures were incubated in the dark at 25°C for 30 minutes to allow complete interaction between DPPH radicals and the test samples. After incubation, the decrease in absorbance was measured at 517 nm using a UV–Visible spectrophotometer (Shimadzu model or equivalent). The discoloration from deep violet to yellow indicated the scavenging of DPPH radicals. A standard antioxidant, *Ascorbic acid*, was used for comparison under identical experimental conditions. All experiments were carried out in triplicate, and the mean values were calculated. The percentage of DPPH radical

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

scavenging activity was calculated using the following equation:

DPPH Scavenged (%) = ($A_{control}$ - A_{sample} / $A_{control}$) × 100

Where:

- $A_{control}$ = Absorbance of DPPH solution (control)
- A_{sample} = Absorbance of test sample

The IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined using linear regression analysis, and results were expressed as mean ± standard deviation.

Nitric Oxide (NO) Scavenging Assay[13-14]

The nitric oxide scavenging activity of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated based on its ability to inhibit nitric oxide generation from sodium nitroprusside under physiological conditions. Sodium nitroprusside in aqueous solution at physiological pH spontaneously releases nitric oxide, which subsequently reacts with oxygen to produce nitrite ions. These nitrite ions were quantified using the Griess reagent. In this assay, a reaction mixture containing sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.4) was prepared and mixed with different concentrations of the plant extract. The mixtures were incubated at room temperature for 150 minutes under light to facilitate nitric oxide generation. Following incubation, an equal volume of Griess reagent (comprising sulfanilamide, phosphoric acid, and N-(1-naphthyl)ethylenediamine dihydrochloride) was added to each sample. The resulting chromophore, formed due to the reaction between nitrite and Griess reagent, was measured spectrophotometrically at 546 nm. A control containing all reagents except the test extract was maintained for comparison. A standard antioxidant such as *Ascorbic acid* was used as a reference under identical experimental conditions. The percentage inhibition of nitric oxide was calculated using the following equation:

% Nitric Oxide Scavenging = ($A_{control}$ - A_{sample} / $A_{control}$) × 100

Where,

$A_{control}$ represents the absorbance of the control reaction and A_{sample} represents the absorbance in the presence of the extract. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation.

***In vitro* Anti-inflammatory Activity[15-29]**

Cyclooxygenase-2 (COX-2) Inhibition Assay[15-21]

The in vitro anti-inflammatory activity of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated by assessing its inhibitory effect on the cyclooxygenase-2 (COX-2) enzyme using a colorimetric assay. COX-2 is an inducible enzyme responsible for the synthesis of prostaglandins during inflammatory conditions, and its inhibition is widely considered a key mechanism for anti-inflammatory activity.

The assay was performed using a COX-2 inhibitor screening method with purified enzyme. The reaction mixture consisted of Tris-HCl buffer (100 mM, pH 8.0), hematin as a cofactor, and COX-2 enzyme solution. Different concentrations of the plant extract, prepared in a suitable solvent, were added to the reaction mixture and pre-incubated at 37°C for 10–15 minutes to allow interaction with the enzyme. The enzymatic reaction was initiated by adding arachidonic acid as the substrate. The mixture was further incubated at 37°C for 15 minutes. During this process, COX-2 catalyzes the conversion of arachidonic acid into prostaglandin intermediates. Following incubation, the reaction was terminated by the addition of a chromogenic reagent such as N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD), which produces a measurable color change proportional to enzyme activity. The absorbance was recorded at 590 nm using a UV–Visible spectrophotometer or microplate reader.

***In vitro* Anti-inflammatory Activity by Protein Denaturation Method[22-29]**

The anti-inflammatory potential of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated using the protein denaturation assay. The reaction mixture (total volume: 1 mL) was prepared by combining 0.1 mL of fresh hen's egg albumin, 0.5 mL of phosphate-

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

buffered saline (PBS, pH 6.4), and 0.4 mL of the test extract at the desired concentration. For the control group, an equal volume of double-distilled water was used instead of the extract.

All samples were incubated at 37 ± 2°C for 15 minutes, followed by heating at 70°C for 5 minutes to induce protein denaturation. After allowing the samples to cool to room temperature, the absorbance was measured at 660 nm using a spectrophotometer, with the vehicle serving as the blank. A standard drug, *Ibuprofen* (1 mg/mL), was used as a reference and treated under identical experimental conditions.

The percentage inhibition of protein denaturation was calculated using the following equation:

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100

Results and Discussion

DPPH Radical Scavenging Activity

The antioxidant potential of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated using the DPPH radical scavenging assay. The assay measures the ability of the extract to neutralize free radicals through hydrogen or electron donation, indicated by a decrease in absorbance at 517 nm.

Table 1: DPPH Radical Scavenging Activity

Sr. No.	Concentration (µg/ml)	Percentage inhibition	
		EETC	Ascorbic Acid (Standard)
1.	20	17.27 ± 0.75	29.42 ± 0.23
2.	40	28.76 ± 0.24	42.98 ±0.47
3.	60	43.57 ± 0.78	57.24 ±0.36
4.	80	56.83 ±0.53	71.87 ±0.89
5.	100	69.12 ±0.86	87.89 ±0.93
6.	IC_{50} (µg/ml)	74.49 ± 0.69	52.86 ± 1.48

The data is expressed as the mean ± standard error of the mean (SEM) for each set of triplicate tests.

The antioxidant activity of the ethanolic extract of *Terminalia cattappa* red leaves (CTLE) was evaluated using the DPPH radical scavenging assay and compared with the standard antioxidant, *Ascorbic acid*. The results demonstrated a clear concentration-dependent increase in radical scavenging activity for both the plant extract and the standard.

At lower concentrations (20 µg/mL), the extract exhibited 17.27 ± 0.75% inhibition, which gradually increased to 69.12 ± 0.86% at 100 µg/mL. Similarly, ascorbic acid showed higher scavenging activity across all concentrations, ranging from 29.42 ± 0.23% at 20 µg/mL to 87.89 ± 0.93% at 100 µg/mL. The consistent increase in percentage inhibition with rising concentration indicates the effective free radical neutralizing ability of the extract.

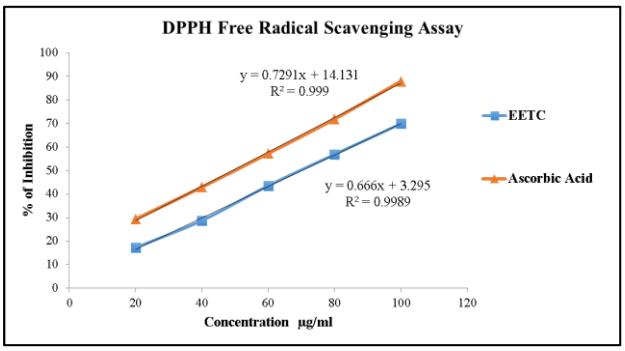

Figure 1: Percentage inhibition in DPPH assay of EETC at various concentrations

The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radicals, was found to be 74.49 ± 0.69 µg/mL for the plant extract, whereas the standard exhibited a lower IC_{50} value of 52.86 ± 1.48 µg/mL. This suggests that although the extract possesses considerable antioxidant activity, it is comparatively less potent than ascorbic acid.

The observed antioxidant effect of the extract can be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, and tannins, which are known to act as hydrogen donors and free radical scavengers. These compounds play an important role in reducing oxidative stress, which is closely linked to inflammatory processes.

Overall, the findings indicate that the ethanolic extract of *Terminalia cattappa* red leaves exhibits significant antioxidant potential in a dose-dependent manner. This

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

antioxidant activity may contribute to its therapeutic relevance by supporting its anti-inflammatory effects through the suppression of oxidative stress–mediated pathways.

Nitric Oxide (NO) Scavenging Assay

The nitric oxide scavenging activity of the ethanolic extract of *Terminalia cattappa* red leaves (EETC) was evaluated and compared with the standard antioxidant, *Ascorbic acid*. The results demonstrated that both the extract and the standard exhibited concentration-dependent nitric oxide scavenging activity.

Table 2: Nitric Oxide (NO) Scavenging Assay

Sr. No.	Concentration (µg/ml)	Percentage inhibition	
		EETC	Ascorbic Acid (Standard)
1.	20	11.79 ± 0.75	21.97 ± 0.23
2.	40	24.98 ± 0.24	43.78 ±0.47
3.	60	36.15 ± 0.78	56.53 ±0.36
4.	80	48.83 ±0.53	71.68 ±0.89
5.	100	58.12 ±0.86	8436 ±0.93
6.	**IC_{50} (µg/ml)**	58.12 ± 0.69	84.06 ± 1.48

The data is expressed as the mean ± standard error of the mean (SEM) for each set of triplicate tests.

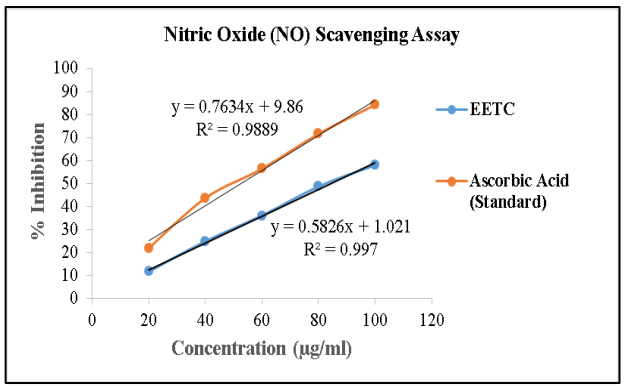

Figure 2: Percentage inhibition in Nitric oxide assay of EETC at various concentrations

At a concentration of 20 µg/mL, the extract showed 11.79 ± 0.75% inhibition, which progressively increased to 58.12 ± 0.86% at 100 µg/mL. Similarly, the standard ascorbic acid displayed higher inhibition values across all tested concentrations, ranging from 21.97 ± 0.23% at 20 µg/mL to approximately 84.36 ± 0.93% at 100 µg/mL. The steady increase in percentage inhibition with increasing concentration indicates the effective nitric oxide scavenging capacity of the plant extract.

The IC_{50} value of the extract was found to be 58.12 ± 0.69 µg/mL, whereas the standard showed an IC_{50} value of 84.06 ± 1.48 µg/mL. This suggests that the extract possesses notable nitric oxide scavenging potential and, based on the IC_{50} values, may exhibit comparatively efficient activity in this specific assay. However, the overall inhibition levels indicate that the standard compound still demonstrates stronger scavenging activity at higher concentrations.

Nitric oxide is an important inflammatory mediator produced during pathological conditions, and excessive NO generation contributes to inflammation and tissue damage. The ability of the extract to scavenge nitric oxide suggests its potential role in reducing inflammatory responses by limiting reactive nitrogen species. The observed activity of the extract may be attributed to the presence of phenolic compounds, flavonoids, and other bioactive constituents known for their free radical scavenging and anti-inflammatory properties. These compounds can interact with nitric oxide and its derivatives, thereby preventing oxidative damage and inflammation. Overall, the results indicate that the ethanolic extract of *Terminalia cattappa* red leaves exhibits significant nitric oxide scavenging activity in a dose-dependent manner, supporting its potential as a natural antioxidant and anti-inflammatory agent.

Cyclooxygenase-2 (COX-2) Inhibition Activity

The anti-inflammatory activity of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated using the COX-2 inhibition assay. The assay determines the ability of the extract to inhibit the activity of the COX-2 enzyme, which plays a crucial role in the synthesis of pro-inflammatory prostaglandins.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

Table 3: *In vitro* COX-2 Inhibition Activity

Sr. No.	Concentration (µg/mL)	Absorbance at 590 nm	Mean Absorbance	% Inhibition
Control	—	0.820 0.825 0.815	0.820	0.00
1	20	0.720 0.710 0.715	0.715	12.80
2	40	0.630 0.620 0.625	0.625	23.78
3	60	0.540 0.530 0.535	0.535	34.76
4	80	0.450 0.440 0.445	0.445	45.73
5	100	0.360 0.350 0.355	0.355	56.70
Ibuprofen (Standard)	100	0.140 0.135 0.138	0.138	83.17

The results of the present study demonstrate that the ethanolic extract of *Terminalia cattappa* red leaves exhibits significant inhibitory activity against the COX-2 enzyme in a concentration-dependent manner. As the concentration of the extract increased from 20 to 100 µg/mL, a progressive decrease in absorbance was observed, indicating effective inhibition of enzyme activity.

At the highest concentration (100 µg/mL), the extract showed 56.70% inhibition of COX-2, suggesting moderate to strong anti-inflammatory potential. In comparison, the standard drug ibuprofen exhibited a higher inhibition of 83.17%, confirming the sensitivity and reliability of the assay.

The observed inhibitory activity of the plant extract may be attributed to the presence of bioactive constituents such as flavonoids, polyphenols, and tannins, which are known to interfere with the cyclooxygenase pathway and reduce prostaglandin synthesis. These phytochemicals may act by binding to the active site of the COX-2 enzyme, thereby preventing the conversion of arachidonic acid into inflammatory mediators.

The concentration-dependent inhibition pattern indicates that the extract possesses dose-responsive pharmacological activity. Although the extract showed lower potency compared to ibuprofen, its significant inhibitory effect highlights its potential as a natural anti-inflammatory agent with possibly fewer side effects.

Overall, the findings suggest that the ethanolic extract of *Terminalia cattappa* red leaves can effectively inhibit COX-2 enzyme activity and may contribute to the management of inflammatory conditions.

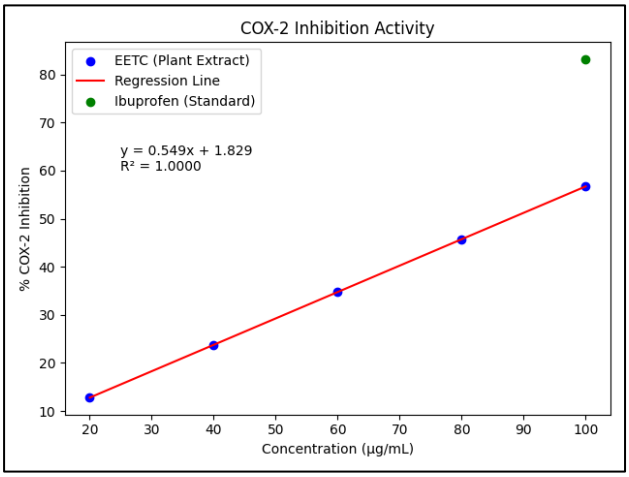

Figure 3: Cyclooxygenase-2 (COX-2) Inhibition Activity of EETC at various concentrations

***In-vitro* anti-inflammatory activity of the ethanolic extract of *Terminalia cattappa* red leaves**

The anti-inflammatory activity of the ethanolic extract of *Terminalia cattappa* red leaves was evaluated using the protein denaturation assay. The ability of the extract to inhibit heat-induced protein denaturation serves as an indicator of its anti-inflammatory potential.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

Table 4: *In vitro* Anti-inflammatory Activity by Protein Denaturation Method

Sample	Concentration	Absorbance (660 nm)	Mean Absorbance	% Inhibition
Blank	—	0.50 0.52 0.52	0.52	0.00
Ibuprofen	1 mg/mL	0.13 0.14 0.09	0.12	76.92
Plant Extract	1 mg/mL	0.20 0.31 0.29	0.26	50.00

The results of the present study demonstrate that the ethanolic extract of *Terminalia cattappa* red leaves exhibits significant anti-inflammatory activity, as evidenced by its ability to inhibit protein denaturation. Protein denaturation is a well-documented mechanism associated with inflammatory processes, and agents capable of preventing this denaturation are considered potential anti-inflammatory candidates.

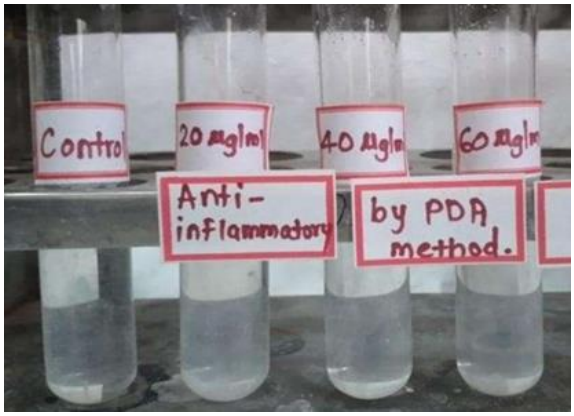

Figure 4: *In vitro* anti-inflammatory activity evaluation by protein denaturation method

The standard drug, ibuprofen (1 mg/mL), showed a high percentage inhibition of 76.92%, confirming the validity of the experimental model. In comparison, the plant extract at the same concentration exhibited 50.00% inhibition, indicating moderate yet significant anti-inflammatory activity.

The observed activity of the plant extract may be attributed to the presence of bioactive phytoconstituents such as flavonoids, tannins, and phenolic compounds, which are known to stabilize proteins and prevent denaturation under stress conditions. Although the extract demonstrated lower activity than the standard drug, its considerable inhibitory effect suggests its potential as a natural anti-inflammatory agent.

Overall, the findings support the traditional use of *Terminalia cattappa* and highlight its potential for further investigation, including isolation of active compounds and in vivo studies to establish its therapeutic efficacy.

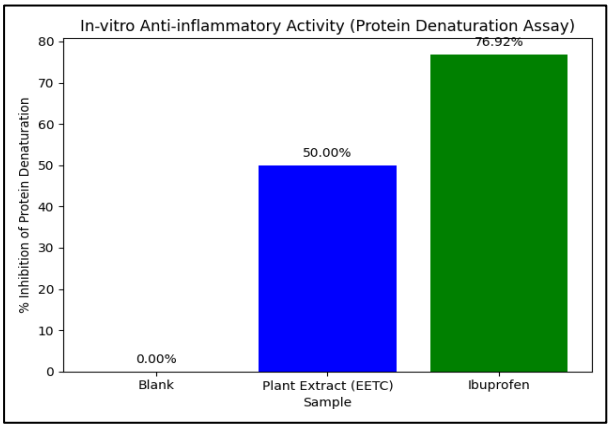

Figure 5: *In vitro* anti-inflammatory activity (protein denaturation method)

CONCLUSION:

The present investigation demonstrated that the ethanolic extract of *Terminalia cattappa* red leaves possesses significant in vitro antioxidant and anti-inflammatory activities. The extract exhibited dose-dependent free radical scavenging activity in both DPPH and nitric oxide assays, indicating its ability to neutralize reactive oxygen and nitrogen species. Furthermore, the extract showed appreciable inhibition of COX-2 enzyme activity and protein denaturation, confirming its potential to modulate key inflammatory pathways.

Although the activity of the extract was comparatively lower than standard compounds such as ibuprofen and ascorbic acid, its substantial biological effects highlight

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

its promise as a natural and safer alternative for anti-inflammatory therapy. The presence of bioactive phytochemicals such as flavonoids and phenolic compounds likely contributes to these pharmacological effects. In conclusion, the ethanolic extract of *Terminalia cattappa* red leaves can be considered a potential source of natural anti-inflammatory and antioxidant agents. However, further studies involving isolation of active constituents, mechanism-based investigations, and *in vivo* evaluations are necessary to validate its therapeutic applicability.

ACKNOWLEDGMENT:

The authors express their sincere gratitude to the Director, Dean, and faculty members of the Department of Pharmaceutical Sciences, *Institute of Pharmaceutical Science and Research*, Sardar Patel University, Balaghat, Madhya Pradesh, India, for providing the necessary facilities, infrastructure, and continuous support to successfully carry out this research work.

CONFLICT OF INTEREST:

The authors declare that there is no conflict of interest regarding the publication of this research work.

REFERENCES:

1. Kokate C. Practical Pharmacognosy, III-Edition 1991, Nirali Prakashan, Pune, 1996; 128.
2. Kosalge S, RA Fursule. Investigation of Anthelminetic Potential Some Plants. International Journal of Pharm Tech Research, 2009; 1: 68-72.
3. Coode MJ. Notes on Terminalia L. in Papuasia. Contribution from Hernarium Austra Hense, 1973; 2: 1-33.
4. Brand WW, Cuvelier H.E, Berset C: Use of a free radical method to evulate antioxidant activity. Food SciTechnol (1995); 82: 25-30.
5. Yagi, K. (1978). Lipid peroxides and human disease. Chem. Physiol. Lip. 45, 337-357.
6. Tripathi, 2008. Essential of Medical Pharmacology 7th Ed. New Delhi, Jaypee Brother's Medicinal Publishers Private Ltd
7. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. Molecules. 2022;27(4):1326. doi:10.3390/molecules27041326
8. Gulcin I. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2023;97:123–152. doi:10.1007/s00204-022-03395-x
9. Chaves N, Santiago A, Alías JC. Quantification of the antioxidant activity of plant extracts: analysis of sensitivity and mechanisms. Antioxidants (Basel). 2020;9(1):76. doi:10.3390/antiox9010076
10. Tabassum R, et al. DPPH and nitric oxide free radical scavenging potential of bioactive compounds. J Mol Struct. 2024;1290:136902. doi:10.1016/j.molstruc.2022.136902
11. Rasve VR, Chakraborty AK, Jain SK, Vengurlekar S., Study of phytochemical profiling and in vitro antioxidant activity of ethanolic extract of Clematis triloba. Eur Chem Bull. 2022;11(12):2658–2677. doi:10.53555/ecb/2022.11.12.2162022.
12. García Díaz J, et al. Inhibitory effect on nitric oxide release in LPS-stimulated macrophages. Antioxidants. 2022;11(10):1915. doi:10.3390/antiox11101915
13. Reeta KM, et al. Evaluation of free radical scavenging and nitric oxide inhibition activities of medicinal plants. Mater Sci Res India. 2023;20(Special Issue):1–10.
14. Dhanalakshmi S, Abinaya, Karthiga Devi K, Lakshmi. In vitro antioxidant study of herbal extract mixture by nitric oxide and DPPH method. Res J Pharm Technol. 2017;10(1):277–280. doi:10.5958/0974-360X.2017.00057.9
15. Lee JH, et al. Antioxidant, α-glucosidase inhibition and nitric oxide suppression activities of plant extracts. Saudi J Biol Sci. 2014;21(6):563–567. doi:10.1016/j.sjbs.2014.01.002
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199–1200. doi:10.1038/1811199a0
17. Sharma OP, Bhat TK. DPPH antioxidant assay revisited. Food Chem. 2009;113(4):1202–1205. doi:10.1016/j.foodchem.2008.08.008

Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 2738-2746 | ISSN:2251-6727

18. Vane JR, Botting RM. Mechanism of action of anti-inflammatory drugs. Am J Med. 1998;104(3A):2S–8S. doi:10.1016/S0002-9343(97)00203-1

19. Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. FASEB J. 2004;18(7):790–804. doi:10.1096/fj.03-0645rev

20. Sakat SS, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata. Int J Pharm Pharm Sci. 2010;2(1):146–155.

21. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins. J Pharm Pharmacol. 1968;20(3):169–173. doi:10.1111/j.2042-7158.1968.tb09718.x

22. Govindappa M, et al. Phytochemical screening, antimicrobial and antioxidant activity of plant extracts. J Pharm Res. 2011;4(3):686–688.

23. Rasve VR, Paithankar VV, Shirsat MK, Dhobale AV. Evaluation of antiulcer activity of Aconitum heterophyllum on experimental animals. World J Pharm Pharm Sci. 2018;7(2):819–839.

24. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti- inflammatory activity of methonal extract of oxalis corniculatelinn. International Journal of Pharma and Pharmacological Sciences (2010); 2(1): 146-155.

25. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol 1968;20(3):169-73.

26. Oyedepo OO, Femurewas AJ. Anti-protease and membrane stabilizing activities of extracts of Fagra santhoxiloides, Olax subscorpioides and *Tetrapluera tetraptera*. In. J Pharm 1995;33:65-9.

27. Sessa, G., Weisman, G. (1968). Effect of components of the polyene antibiotic. Fillipin on phospholipids spherules (liposome) and erythrocytes. J Biol Chem 243, 43644371.

28. Litman, G. W., Litman, R. T., Henry, C. J. (1976). Analysis of lipophilic carcinogen membrane interaction using human erythrocyte membrane system model. Cancer Res 243, 43644371.

29. Umapathy E. Ndebia EJ. Meeme A, Adam B, Menziwa P. NK Chchungag BN. An experimental evaluation of Albucasettosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. J Med Plant Res. (2010); 4: 789-795.