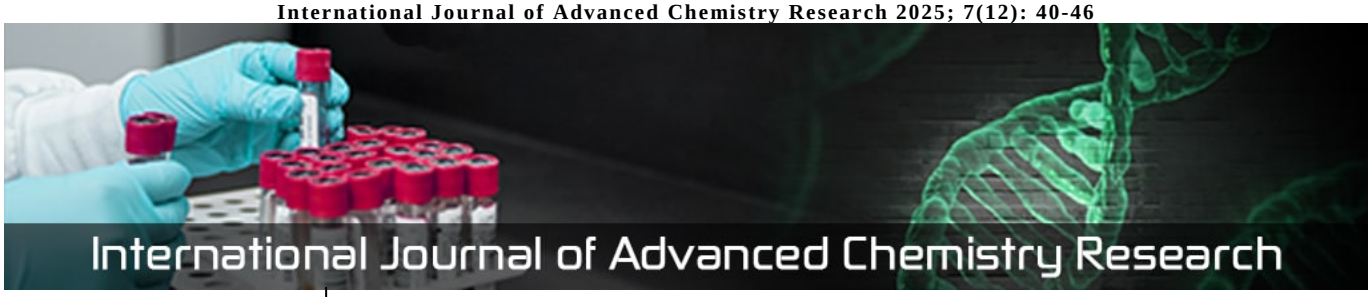

ISSN Print: 2664-6781
ISSN Online: 2664-679X
NAAS Rating (2025): 4.77
IJACR 2025; 7(12): 40-46
www.chemistryjournals.net
Received: 23-09-2025
Accepted: 25-10-2025

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Biochemical evaluation of xanthine oxidase inhibition and anti-inflammatory activity of *Drynaria quercifolia* (L.) J. Sm and *Curcuma aeruginosa* Roxb. Rhizomes

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DOI: https://www.doi.org/10.33545/26646781.2025.v7.i12a.345

Abstract
Gout is a form of metabolic inflammatory arthritis characterized by hyperuricemia and acute joint inflammation, which is primarily driven by xanthine oxidase enzyme responsible for uric acid production and inflammation-associated membrane damages. The present study was aimed to comparatively evaluate the *in-vitro* biochemical xanthine oxidase (XO) inhibitory activity and anti-inflammatory (membrane stabilizing) potential of ethanolic extracts of *Drynaria quercifolia* (L.)J.sm and *Curcuma aeruginosa* Roxb. rhizomes which is ethnobotanically used against rheumatism. XO inhibition was evaluated spectrophotometrically, while the anti-inflammatory activity was evaluated using hypotonic and heat-induced erythrocyte membrane stabilization assays, with febuxostat and diclofenac used as standard drugs. Both extracts exhibited concentration-dependent XO inhibition. *C. aeruginosa* showed an IC_{50} value of 4.2±0.4 µg/mL, which was comparable to febuxostat (3.5±0.5 µg/mL), whereas *D. quercifolia* exhibited a higher IC_{50} value of 18.0±0.7 µg/mL, indicating moderate enzyme inhibitory activity. In the hypotonic solution-induced hemolysis assay, *D. quercifolia* and *C. aeruginosa* resulted as 64.3% and 67.0% of membrane stabilization, which is compared with 82.4% for diclofenac. In the heat-induced hemolysis activity, *D. quercifolia* exhibited an IC_{50} of 132 µg/mL which shows stronger membrane protection whereas, *C. aeruginosa* has IC_{50} of 207.0 µg/mL and diclofenac of 83.8 µg/mL. The findings demonstrates a complementary dual biochemical profile, wherein *C. aeruginosa* exhibits stronger xanthine oxidase inhibitory activity, while *D. quercifolia* shows relatively greater membrane stabilizing potential. The combined enzyme-level and membrane-level protection supports the preliminary *in-vitro* anti-gout potential of both the rhizomes ethanolic extracts. Further *in-vivo* and molecular studies are required to substantiate their therapeutic significance.

Keywords: Xanthine oxidase inhibition, membrane stabilization, *Drynaria quercifolia*, *Curcuma aeruginosa*, anti-inflammatory activity, Gout

Introduction
Gout is a metabolic inflammatory disorder arising from dysregulation of purine metabolism, characterized by sustained hyperuricemia and deposition of monosodium urate crystals (MSU) in joints and peri-articular tissues [1, 2]. The biochemical basis of hyperuricemia is primarily governed by the enzyme xanthine oxidase (XO), which catalyzes the sequential oxidation of hypoxanthine to xanthine and xanthine to uric acid [3, 4]. Beyond its role in urate biosynthesis, XO is also a significant enzymatic source of reactive oxygen species (ROS), thereby functioning as a biochemical link between hyperuricemia, oxidative stress and inflammation [5, 6]. Excessive XO activity thus contributes simultaneously to uric acid accumulation and amplification of redox-mediated inflammatory damage, establishing XO as a central therapeutic target in gout pathophysiology [4, 6].
Synthetic XO inhibitors such as allopurinol and febuxostat remain the main medications of urate-lowering therapy. Despite their clinical effectiveness, long-term administration of these drugs is associated with adverse reactions including hypersensitivity syndromes, hepatic dysfunction, renal complications and cardiovascular risks [7, 8]. These limitations leads towards the necessity to identify alternative XO inhibitors that can provide enzyme

suppression together with additional anti-inflammatory and cytoprotective benefits. In this context, naturally occurring phytochemicals are being increasingly explored as multi-target agents capable of modulating enzyme activity, oxidative stress and inflammation in a concerted manner [9, 10].

In clinical practice, the two major pathogenic components of gout are managed using two distinct drug classes. Long-term control of hyperuricemia is achieved using xanthine oxidase inhibitors such as febuxostat, a selective non-purine XO inhibitor widely prescribed in gout patients.[11, 12] In contrast, the acute inflammatory manifestations of gout flares are primarily treated using non-steroidal anti-inflammatory drugs (NSAIDs), among which diclofenac is one of the most frequently prescribed agents for rapid suppression of pain and inflammation due to its potent cyclooxygenase inhibition and lysosomal membrane-stabilizing action[13, 14]. Thus, febuxostat represents the pharmacological standard for urate-lowering therapy, whereas diclofenac represents the standard for acute anti-inflammatory control in gout.

Inflammation constitutes the second major pathogenic axis of gout, particularly during acute gout flares. Monosodium urate crystals activate neutrophils and macrophages, triggering the release of lysosomal enzymes, proteases, and pro-inflammatory mediators that culminate in severe tissue injury and pain [2, 15]. Stabilization of lysosomal membranes is therefore a critical anti-inflammatory strategy, as it prevents enzyme leakage and limits cellular auto-digestion. The erythrocyte membrane closely resembles the lysosomal membrane in structural organization and stress-induced destabilization and erythrocyte membrane stabilization assays are widely employed as reliable *in-vitro* indicators of membrane-protective and anti-inflammatory potential [16-18].

Medicinal plants are rich sources of bioactive secondary metabolites such as phenolics, flavonoids, terpenoids and curcuminoids, many of which exhibit proven xanthine oxidase inhibitory, antioxidant and membrane-stabilizing activities [9, 10, 19]. Unlike synthetic single-target drugs, plant extracts contain multiple phytochemicals that can act simultaneously on different pathological pathways. Such multi-target activity is particularly valuable in gout, where both uric acid overproduction and inflammation-associated oxidative and membrane damage contribute to disease progression. Therefore, plant-based therapeutics offer a rational approach for the integrated management of the metabolic and inflammatory components of gout.

Drynaria quercifolia (L.) J. Sm (Polypodiaceae) and *Curcuma aeruginosa* Roxb. (Zingiberaceae) are rhizomatous medicinal plants extensively employed in traditional medicine for disorders associated with inflammation, musculoskeletal pain and joint dysfunction.

D. quercifolia is widely used for bone healing, arthritis and joint-related ailments and is known to contain abundant flavonoids, triterpenoids and phenolic acids linked to membrane stabilization and anti-inflammatory activity [20, 21]. *C. aeruginosa* is traditionally used in the management of swelling, pain and inflammatory conditions and is reported to be rich in terpenoids, curcuminoids and polyphenolic compounds associated with enzymatic inhibition and redox modulation [22, 23]. In both species, the rhizome serves as the principal reservoir of these bioactive constituents.

The selection of ethanol as the extraction solvent and the prioritization of these two rhizomes were further supported by our earlier comparative phytochemical and antioxidant investigation, which demonstrated that the ethanolic extracts of both *Drynaria quercifolia* and *Curcuma aeruginosa* possessed the highest phenolic and flavonoid contents along with superior radical scavenging and reducing capacities, establishing their strong redox relevance to gout-associated oxidative stress [24].

Although *Curcuma aeruginosa* and *Drynaria quercifolia* possess well-recognized ethno medicinal relevance in inflammatory disorders, systematic comparative evaluation of their ethanol extracts for xanthine oxidase inhibition together with membrane-stabilizing activity under standardized experimental conditions remains limited. Since effective gout management requires simultaneous suppression of uric acid biosynthesis and control of inflammation-associated membrane destabilization, a dual mechanistic investigation is scientifically warranted, which forms the basis of the present study.

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Drynaria quercifolia (L.) J. Sm. rhizomes were obtained from the Kolli Hills region of Tamil Nadu, India, whereas *Curcuma aeruginosa* Roxb. rhizomes were sourced from the Beltola Bazaar herbal market, Guwahati, Assam. Both plant materials were botanically idenfied by the Botanical Survey of India, Southern Circle, Coimbatore, Tamilnadu. Authenticated voucher specimens were preserved in the institutional herbarium for future reference. The same authenticated plant materials were used in our earlier phytochemical and antioxidant investigation and in the present study to ensure experimental consistency.

2.2 Extraction

The shade-dried rhizomes of *Drynaria quercifolia* and *Curcuma aeruginosa* were separately pulverized into coarse powder. The powdered samples were individually subjected to Soxhlet extraction using ethanol as the solvent. The extraction was continued until complete exhaustion of the plant material. The resulting ethanol extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator (Yamato BO410, Japan), followed by drying to constant weight. The dried crude ethanol slurry were weighed to determine percentage yield and stored in airtight containers at 4 ° C until further use. Stock solutions were prepared at a concentration of 1 mg/mL for subsequent xanthine oxidase inhibition and membrane stabilization assays.

2.3 *In vitro* Xanthine Oxidase Inhibitory Activity

The *in-vitro* xanthine oxidase (XO) inhibitory activity of the ethanol extracts of *Curcuma aeruginosa* (ECA) and *Drynaria quercifolia* (EDQ) was evaluated using a UV-spectrophotometric method with minor modifications as described by Umamaheswari *et al* [25]. Phosphate buffer (pH 7.5) was prepared by dissolving 6.8 g of potassium dihydrogen orthophosphate and 1.56 g of sodium hydroxide in 900 mL of distilled water, adjusting the pH with sodium hydroxide, and making up the final volume to 1000 mL. The XO working solution (0.1 U/mL) was prepared by diluting 1 mL of enzyme stock solution (1 U/mL) with 9 mL of phosphate buffer. The substrate solution was prepared by dissolving 0.0056 g of xanthine in 200 mL of phosphate buffer. A 1 N HCl solution was prepared by diluting 8.28

mL of concentrated hydrochloric acid to 100 mL with distilled water. The reaction mixture consisted of 1.0 mL of test solution at the required concentration, 1.5 mL of phosphate buffer (pH 7.5), and 0.05 mL of XO enzyme solution (0.1 U/mL). The mixture was pre-incubated at 25 °C for 15 min. The reaction was initiated by the addition of 1.0 mL of substrate solution (150 µM xanthine) and incubated at 25 °C for 30 min. The reaction was terminated by the addition of 0.5 mL of 1 N HCl, and the absorbance was measured at 290 nm using a UV-Visible spectrophotometer. A preliminary dose range finding experiment (1-200 µg/mL) was performed for each ethanol extract. Initial single-point screening indicated that the *Curcuma aeruginosa* ethanol extract inhibited XO at lower concentrations than the *Drynaria quercifolia* ethanol extract. Based on these observations, the concentration ranges were selected for the detailed assay i.e, 1.25-80 µg/mL for *C. aeruginosa*, 5-320 µg/mL for *D. quercifolia* and 1-32 µg/mL for febuxostat. All the experiments were performed in triplicate.

The percentage inhibition of xanthine oxidase activity was calculated using the formula:

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

where, $A_{control}$ is the absorbance of the reaction mixture without extract (vehicle control) and A_{sample} is the absorbance in the presence of the test extract or standard. IC_{50} values were estimated by graphical interpolation from plots of percentage inhibition versus concentration.

2.4 *In vitro* Anti-Inflammatory Activity

2.4.1 Membrane Stabilization Assay

a. Hypotonic Solution-Induced Hemolysis

Alsever's solution was prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in distilled water and sterilized as described by Shinde *et al.* (1999)[26]. Fresh blood was collected from Sprague Dawley rats through retro-orbital plexus puncture under ethical approval and immediately mixed with an equal volume of sterilized Alsever's solution. The blood suspension was centrifuged at 3000 rpm for 10 min, and the packed erythrocytes were washed three times with isosaline (0.9% NaCl, pH 7.2). A 10% (v/v) erythrocyte suspension was prepared using isosaline. The reaction mixture (final volume 4.5 ml) consisted of 1 ml phosphate buffer (pH 7.4), 2 ml hypotonic saline (0.45% NaCl), 1 ml plant extract (1 mg/ml), and 0.5 ml erythrocyte suspension. Diclofenac sodium was used as the standard drug. The control contained all reagents except plant extract, while phosphate buffer served as the blank. The mixtures were incubated at 37 °C for 30 min and centrifuged. The absorbance of the hemoglobin released in the supernatant was measured at 560 nm using a UV-Visible spectrophotometer. The percentage of membrane stabilization was calculated using the formula:

$$\text{\% Inhibition} = \frac{A\ Control - A\ Sample}{A\ Control} \times 100$$

Where $A_{Control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample.

b. Heat-Induced Hemolysis

The heat-induced hemolysis assay was carried out according to Shinde *et al.* (1999) [26] with slight modifications as reported by Henneh *et al.* (2018)[27]. The reaction mixture (2 ml) consisted of 1 ml of 10% erythrocyte suspension and 1 ml of plant extract (1 mg/ml), which was gently mixed. The positive control contained 1 ml RBC suspension and 1 ml diclofenac sodium at concentrations of 10, 30, and 100 µg/ml, while the negative control consisted of 1 ml RBC suspension and 1 ml normal saline. All treatments were performed in triplicate. The mixtures were heated at 56 °C for 30 min, allowed to cool to room temperature, and centrifuged at 2500 rpm for 10 min. The absorbance of the supernatant was measured at 560 nm using a UV-Visible spectrophotometer (Shimadzu) as a measure of hemolysis. The percentage inhibition of hemolysis was calculated using the formula:

$$\text{\% Inhibition} = \frac{A\ Control - A\ Sample}{A\ Control} \times 100$$

Where $A_{Control}$ is the absorbance of the control and A_{sample} is the absorbance of the sample.

2.5 Statistical analysis

All the experiments were carried out in triplicate and the results are expressed as mean±standard deviation (SD) (n = 3). The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 *In-vitro* Xanthine Oxidase (XO) Inhibitory Activity

The ethanol extracts of *Drynaria quercifolia* and *Curcuma aeruginosa* exhibited a clear concentration-dependent inhibition of xanthine oxidase (Table 1A; Fig. 1). *C. aeruginosa* produced 41.67±0.01% inhibition at 1.25 µg/mL and the inhibitory effect increased progressively to 75.63±0.01% at 80 µg/mL. In contrast, *D. quercifolia* showed relatively lower inhibition at the initial concentration (9.53±0.06% at 5 µg/mL), with a gradual increase to 90.36±0.0350% inhibition at 320 µg/mL. The standard drug febuxostat showed a steeper inhibitory response within a lower concentration range, producing 25.92±0.02% inhibition at 1 µg/mL and increasing to 89.56±0.01% at 32 µg/mL. The IC_{50} values from the concentration-inhibition curves, were

18.0±0.7 µg/mL for *D. quercifolia*, 4.2±0.4 µg/mL for *C. aeruginosa* and 3.5±0.5 µg/mL for febuxostat (Table 1B).

Based on IC_{50} values, *C. aeruginosa* exhibited approximately 4.3-fold greater XO inhibitory potency than *D. quercifolia* and showed inhibitory activity comparable to the standard drug. *D. quercifolia* required substantially higher concentrations to achieve equivalent levels of enzyme inhibition. Overall, both ethanol extracts demonstrated dose-dependent XO inhibitory behavior, with *C. aeruginosa* showing stronger inhibition at lower concentrations, while

D. quercifolia displayed a more gradual inhibitory profile across a wider concentration range.

Table 1A: Xanthine Oxidase Inhibitory Activity of *Drynaria quercifolia*, *Curcuma aeruginosa* Extracts and Standard Febuxostat

D. quercifolia		*C. aeruginosa*		Febuxostat	
Concentration (µg/mL)	**% Inhibition**	**Concentration (µg/mL)**	**% Inhibition**	**Concentration (µg/mL)**	**% Inhibition**
5	9.53±0.06	1.25	41.67±0.01	1	25.92±0.02
10	36.18±0.01	2.50	47.50±0.02	2	38.90±0.00
20	65.47±0.00	5.00	51.47±0.02	4	57.33±0.01
40	73.76±0.01	10.0	56.60±0.00	8	72.57±0.01
80	78.85±0.01	20.0	66.26±0.01	16	84.13±0.01
160	86.09±0.02	40.0	73.66±0.01	32	89.56±0.00
320	90.36±0.04	80.0	75.63±0.01	-	-

Values are expressed as mean ± SD (n = 3).

Table 1B: IC_{50} values for xanthine oxidase inhibition

Sample	IC_{50} (µg/mL)
D. quercifolia	18.0±0.7
C. aeruginosa	4.2±0.4
Febuxostat	3.5±0.5

IC_{50} represents the concentration required to inhibit 50% of xanthine oxidase activity. Values are expressed as mean ± SD of triplicate determinations (n = 3).

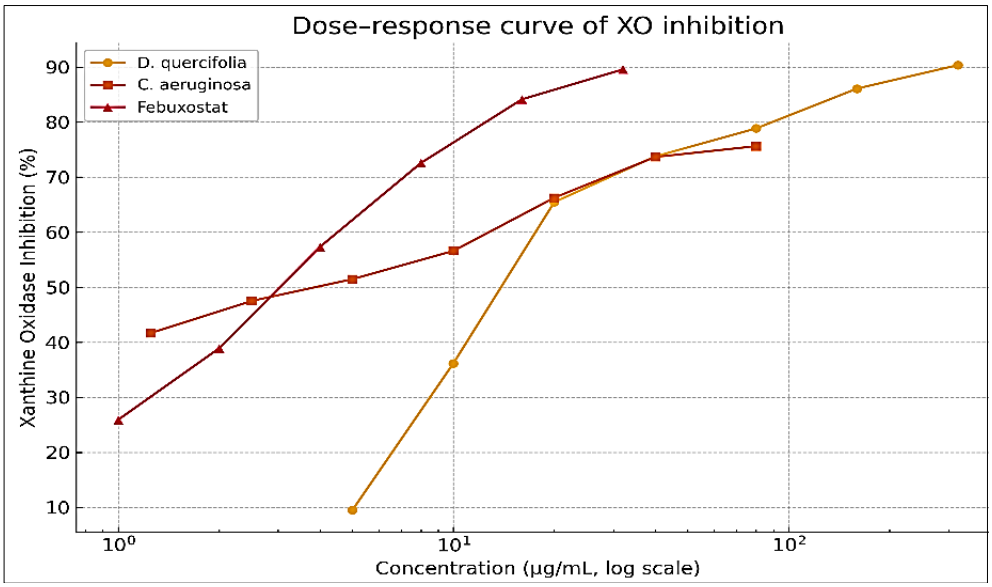

Fig 1: Dose-dependent xanthine oxidase inhibitory activity of *Drynaria quercifolia* (EDQ) and *Curcuma aeruginosa* (ECA) extracts compared with the standard drug Febuxostat.

3.2 *In-vitro* membrane stabilizing activity (Table-3)

3.2.1 Hypotonic Solution-Induced Membrane Stabilization

In the hypotonicity-induced hemolysis assay, both ethanol extracts exhibited moderate membrane stabilizing activity (Table 3; Fig. 2A). *D. quercifolia* produced 64.3% membrane stabilization, while *C. aeruginosa* showed 67% membrane stabilization at the tested concentration. The standard drug diclofenac exhibited a higher membrane protective effect with 82.4% inhibition of hemolysis. The membrane stabilizing activities of both plant extracts were lower than that of diclofenac, while the protective effects of *C. aeruginosa* and *D. quercifolia* were comparable to each other.

Table 3: *In-vitro* membrane stabilizing activity of ethanol extracts of *Drynaria quercifolia* and *Curcuma aeruginosa*

Ethanol extracts of Sample	Hypotonic Solution- induced %	Heat induced Haemolysis IC_{50} (µg/mL)
D. quercifolia	64.3 ± 0.2^{c}	132
C. aeruginosa	67.0 ± 0.4^{b}	207
Diclofenac	82.4 ± 1.4^{a}	83.8

Values are expressed as mean ± SD (n = 3) for hypotonic solution-induced membrane stabilization. One-way ANOVA followed by Tukey's post-hoc test ($p<0.05$). IC_{50} values for heat-induced hemolysis represent the concentration required to inhibit 50% hemolysis.

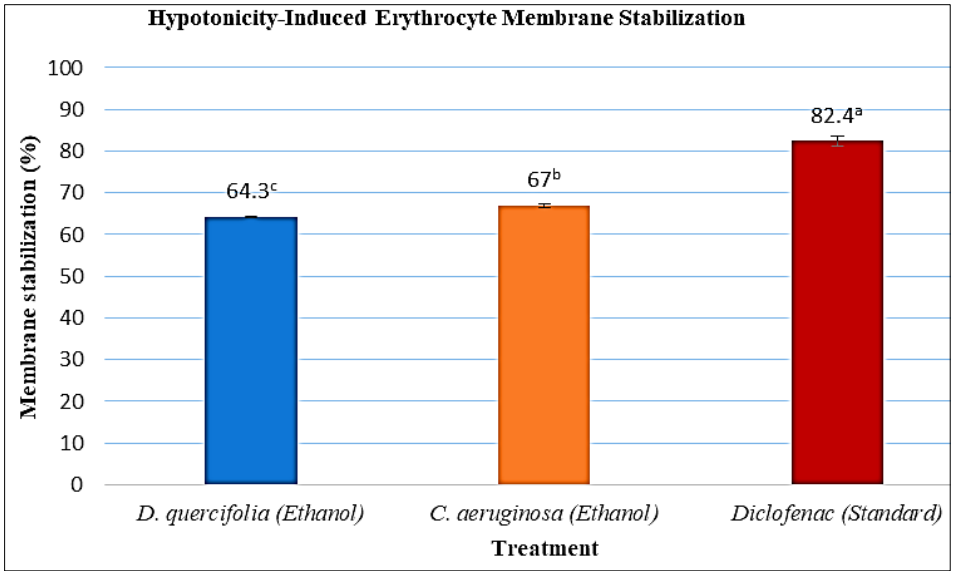

Fig 2 A: Hypotonic solution-induced membrane stabilization by ethanol extracts of *Drynaria quercifolia* and *Curcuma aeruginosa* compared with diclofenac.

3.2.2 Heat-Induced Hemolysis

In the heat-induced hemolysis model, the membrane stabilizing potency of the samples was expressed as IC_{50} values (Table 3; Fig. 2B). The IC_{50} values were 132 µg/mL for *D. quercifolia*, 207 µg/mL for *C. aeruginosa* and 83.8 µg/mL for diclofenac. Diclofenac demonstrated significantly greater membrane protection than both ethanol extracts. Among the plant extracts, *D. quercifolia* exhibited a significantly lower IC_{50} value than *C. aeruginosa*, indicating a relatively higher membrane protective efficiency of *D. quercifolia* under thermal stress conditions.

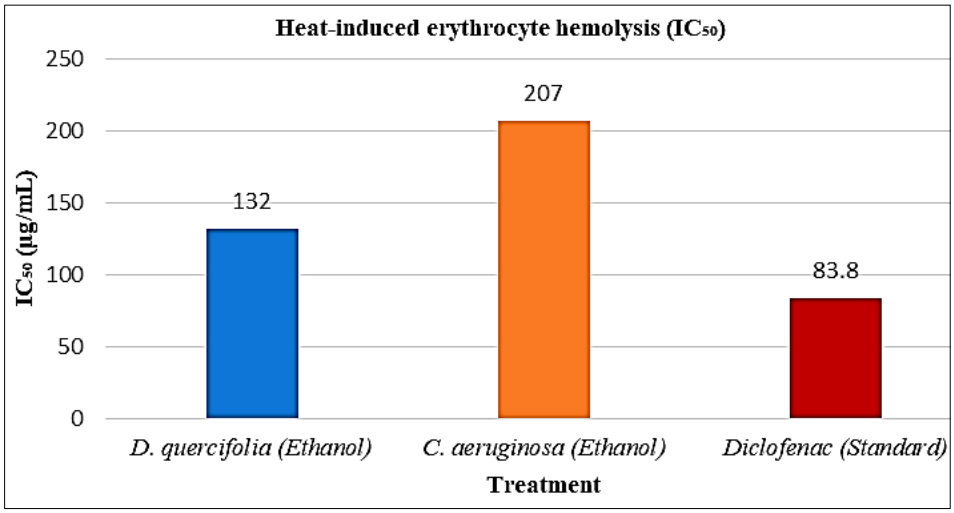

Fig 2B: Heat-induced hemolysis inhibitory activity (IC_{50}) of ethanol extracts of *Drynaria quercifolia* and *Curcuma aeruginosa* compared with diclofenac

4. Discussion

Hyperuricemia is the primary biochemical abnormality underlying gout and is characterized by increased production or reduced excretion of uric acid, leading to monosodium urate crystal deposition in joints and subsequent inflammatory responses. Xanthine oxidase (XO) catalyzes the terminal steps of purine metabolism, converting hypoxanthine to xanthine and xanthine to uric acid; hence, inhibition of this enzyme remains a central therapeutic target in gout management. Alongside urate overproduction, inflammatory membrane destabilization and lysosomal enzyme release play a pivotal role in the progression of acute gouty inflammation [28].

In the present investigation, ethanol extracts of *Curcuma aeruginosa* and *Drynaria quercifolia* were evaluated for their in-vitro anti-gout and anti-inflammatory potential using XO inhibition and erythrocyte membrane stabilization models, respectively, following a dual-mechanistic experimental strategy similar to previously reported anti-gout screening approaches [29, 30]. Both extracts exhibited dose-dependent inhibition of XO activity, confirming their ability to interfere with uric acid biosynthesis. The lower IC_{50} value of *C. aeruginosa* compared with *D. quercifolia* indicates a higher affinity of *C. aeruginosa* phytoconstituents toward the XO active site. The inhibitory potency of *C. aeruginosa* was comparable to that of the standard drug febuxostat, suggesting the presence of highly active XO-modulating compounds in its ethanol extract.

Plant-derived phenolics and flavonoids are well documented as natural XO inhibitors due to their ability to interact with the molybdenum-pterin active center of the enzyme, thereby suppressing uric acid formation. The comparatively stronger

XO inhibition observed with *C. aeruginosa* in the present study may therefore be attributed to its rich content of curcuminoids, terpenoids and polyphenolic compounds. The pronounced antioxidant potential and high phenolic and flavonoid content of both rhizomes reported in our earlier study [24] further mechanistically support the enzyme-level inhibitory effects observed in the current investigation.

Inflammation-mediated membrane destabilization represents another fundamental pathological event in gout, where lysosomal membrane rupture leads to the release of proteases and amplification of tissue injury. Since the erythrocyte membrane closely resembles the lysosomal membrane in both structure and susceptibility to stress-induced damage, membrane stabilization assays are widely employed as surrogate in-vitro models for evaluating anti-inflammatory activity. In the hypotonicity-induced hemolysis model, both *C. aeruginosa* and *D. quercifolia* demonstrated moderate membrane-protective effects at the tested concentration, whereas diclofenac exhibited the highest stabilization.

Diclofenac was used as the reference standard in the membrane stabilization assays because non-steroidal anti-inflammatory drugs (NSAIDs) constitute the first-line pharmacological treatment for acute gout flares. Diclofenac exerts its anti-inflammatory action primarily through cyclooxygenase (COX) inhibition and suppression of prostaglandin synthesis and also exhibits direct lysosomal and erythrocyte membrane-stabilizing effects, thereby preventing the release of tissue-damaging proteolytic enzymes during acute inflammation. Major international treatment guidelines, including those issued by the American College of Rheumatology and EULAR, recommend NSAIDs such as diclofenac for the rapid control of pain and inflammation in acute gouty arthritis [11, 12]. Thus, the superior membrane-stabilizing activity of diclofenac in the present in-vitro models provides a clinically relevant pharmacological benchmark for interpreting the membrane-protective effects of the plant extracts.

Under thermal stress conditions in the heat-induced hemolysis model, *D. quercifolia* exhibited a lower IC_{50} value than *C. aeruginosa*, indicating comparatively stronger membrane-protective efficiency of *D. quercifolia* at elevated temperatures. Heat-induced hemolysis primarily results from protein denaturation and oxidative membrane damage. The superior thermal membrane stabilization exhibited by *D. quercifolia* suggests the presence of thermostable flavonoids and triterpenoids with lipid-peroxidation-inhibitory and cytoprotective properties. Previous phytochemical studies on *D. quercifolia* reporting flavonoids and terpenoid derivatives associated with membrane stabilization further support this observation [20, 24].

Overall, the present findings reveal a complementary dual biological profile between the two rhizomes. *Curcuma aeruginosa* exhibited stronger XO inhibitory efficacy, indicating greater potential for suppressing uric acid biosynthesis, whereas *Drynaria quercifolia* displayed relatively superior membrane-stabilizing efficiency under heat-induced stress, suggesting stronger protection against inflammation-mediated membrane damage. This dual biochemical behavior closely parallels the combined clinical use of xanthine oxidase inhibitors such as febuxostat for urate-lowering therapy and NSAIDs such as diclofenac for acute inflammatory control in gout. Such complementary dual targeting is highly relevant for a disease that simultaneously involves urate overproduction and inflammation-driven cellular injury.

5. Conclusion

The ethanol extracts of *Curcuma aeruginosa* and *Drynaria quercifolia* demonstrated a complementary dual anti-gout action through significant xanthine oxidase inhibition and erythrocyte membrane stabilization. *C. aeruginosa* exhibited stronger urate-lowering potential via superior XO inhibition, whereas *D. quercifolia* showed comparatively higher membrane-protective anti-inflammatory activity under stress conditions. Together, these *in-vitro* findings indicate their potential to target both metabolic and inflammatory components of gout, warranting further *in-vivo* and molecular validation.

6. Acknowledgments

The authors acknowledge the financial support provided by the Tamil Nadu State Council for Science and Technology (TNSCST) through the Research Funding for Research Scholars in Colleges (RFRS) scheme, which facilitated the successful completion of this study. The authors also sincerely acknowledge the Department of Pharmacology, Sri Ramakrishna College of Pharmacy, for providing the necessary laboratory facilities to carry out the xanthine oxidase inhibition assay.

7. Ethical Considerations

All experimental procedures involving rat blood were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of KMCRET (Approval No.: IAEC-KMCRET/ReRc/Ph.D/117/2024). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

8. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

9. Authors contribution

Deepa M. A. conceived and supervised the study. Narthanaa S. performed the experimental work and drafted the manuscript. Pugalenthi M. critically reviewed and corrected the manuscript. The other authors helped for the final version of the manuscript correction.

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