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# Anti-inflammatory potential of root extracts of *Gmelina arborea*roxb

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**Abstract**
The anti-inflammatory potential of different root extracts of *Gmelina arborea*Roxb. was investigated using the in-vitro human red blood cell (HRBC) membrane stabilization method. Petroleum ether extract, Chloroform, Ethyl acetate extract, methanol, and aqueous extracts were evaluated at concentrations of 200 and 400 µg/ml, with Diclofenac (50 and 100 µg/ml) as the standard. Among the extracts, the methanol extract demonstrated the most significant (**p < 0.01) anti-inflammatory activity, stabilizing the HRBC membrane effectively. These findings suggest that the methanol extract of *G. arborea* may serve as a promising candidate for developing anti-inflammatory agents. The HRBC membrane stabilization method is an established model for assessing the efficacy of potential anti-inflammatory agents by preventing hemolysis under hypotonic conditions. The stability provided by plant extracts can be attributed to the inhibition of lysosomal enzymes responsible for tissue damage and inflammation. *G. arborea*, traditionally used in Ayurvedic and Unani medicine, has been reported to possess multiple pharmacological properties, including antioxidant, antimicrobial, and hepatoprotective activities. The significant anti-inflammatory effects observed in the methanol extract may be due to the presence of bioactive phytochemicals like flavonoids, lignans, and alkaloids, which have been documented for their anti-inflammatory and membrane-stabilizing properties. The study highlights the potential of *G. arborea* as an alternative to synthetic anti-inflammatory agents, which often carry the risk of adverse effects. Further in-vivo studies and bioactive compound isolation are recommended to substantiate these findings and explore the underlying mechanisms of action.

**Keywords:** *Gmelina arborea*Roxb., anti-inflammatory, human red blood cell membrane stabilization method

## Introduction

*Gmelina arborea*Roxb. (Lamiaceae) is a deciduous tree known by various vernacular names such as Gambhari (Hindi), Kashmarya (Sanskrit), White teak, Kashmir tree, and Gmelina (English). This versatile tree is predominantly found in tropical and subtropical regions of South Asia, including India, Sri Lanka, and Myanmar, and has been extensively utilized in traditional Ayurvedic, Unani, and folk medicine for centuries (Rekha *et al.,* 2021). The therapeutic versatility of *G. arborea* can be attributed to its rich phytochemical profile, comprising bioactive compounds like lignans (arboreol, isoarboreol, arborone), flavonoids (luteolin), iridoid alkaloids, hentriacontanol, and gmelanone (Punita *et al.,* 2013). These phytochemicals are predominantly concentrated in the heartwood, leaves, and roots of the plant.

The root bark of *G. arborea* is particularly significant due to its traditional applications in treating various inflammatory disorders, digestive problems, and nervous system ailments. In Ayurvedic formulations, it is utilized to manage conditions associated with Vata and Pitta imbalances, suggesting its efficacy in inflammation control and tissue repair. The plant's ethnomedicinal value has been validated by modern pharmacological studies, highlighting its potential as an antioxidant, antimicrobial, hepatoprotective, cardioprotective, and anti-inflammatory agent (Kumar *et al.,* 2021).

Inflammation is an essential physiological response to harmful stimuli like pathogens, damaged cells, and irritants. Although acute inflammation is a protective mechanism, its prolonged state, termed chronic inflammation, can contribute to the pathogenesis of several degenerative diseases, including cardiovascular disorders, neurodegenerative conditions, autoimmune diseases, metabolic syndromes, and cancers (Mariotti *et al.,* 2004; Wang *et al.,* 2022). The inflammatory process is regulated by a myriad of mediators like cytokines (TNF-α, IL-1, IL-6), eicosanoids (prostaglandins, leukotrienes), histamines, and bradykinins, which orchestrate a complex network of cellular signaling pathways (Kumar *et al.,* 2013). Uncontrolled release of these mediators can lead to excessive immune responses, causing tissue damage and disease progression.

Conventional anti-inflammatory therapies, including nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, are widely used to alleviate inflammatory symptoms. However, their long-term use is associated with significant adverse effects, such as gastrointestinal toxicity, renal impairment, and cardiovascular complications (Vane *et al.,* 1995) [10]. Therefore, there is an increasing demand for safer and more effective anti-inflammatory agents derived from natural sources. Plants like *G. arborea* have garnered scientific interest for their potential to modulate inflammatory responses through multi-target mechanisms while minimizing side effects (Saleem *et al.,* 2020).

The anti-inflammatory potential of *G. arborea* can be largely attributed to its ability to inhibit pro-inflammatory enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), reducing the synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes (Rekha *et al.,* 2021). Additionally, its flavonoid and lignan components exhibit membrane-stabilizing properties, protecting erythrocytes from hemolysis and lysosomal enzyme release, which are pivotal in chronic inflammation (Wang *et al.,* 2022). Studies have demonstrated the radical scavenging activity of its phenolic constituents, contributing to its antioxidative and anti-inflammatory potential (Punita *et al.,* 2013).

Given the promising traditional and scientific evidence, this study aimed to evaluate the in-vitro anti-inflammatory potential of various root extracts of *G. arborea* using the HRBC membrane stabilization method. The method simulates the stabilization of lysosomal membranes, whose rupture leads to the release of hydrolytic enzymes and subsequent inflammation. By stabilizing these membranes, plant extracts can mitigate inflammatory responses and offer therapeutic benefits for inflammatory diseases (Kumar *et al.,* 2012). The present investigation also seeks to bridge the gap between traditional applications and scientific validation, providing a basis for further in-vivo studies and the potential development of phytopharmaceuticals from *G. arborea*.

## Material and Methods

### Plant Material

The roots of *Gmelina arborea* Roxb. were collected during the peak blooming season from areas around Jamnagar. A herbarium specimen was prepared and submitted to the Pharmacognosy Museum of I.P.G.T. & R.A., Jamnagar, with Herbarium No. 6262, for future reference. The collected roots were shade-dried and coarsely powdered. The powdered material was subjected to continuous extraction in a Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate, methanol, and water as solvents.

*In-vitro Anti-inflammatory Activity*

The human red blood cell (HRBC) membrane stabilization method, as described by Kumar et al. (2013), was employed to assess the anti-inflammatory potential of the extracts. Blood was collected from a healthy human volunteer who had not taken any NSAIDs for at least 14 days before the experiment. The blood was mixed with an equal volume of Alsever's solution (containing 2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) and centrifuged at 3000 rpm. The packed cells obtained were washed three times with isosaline (0.85% NaCl) and then diluted to a 10% v/v suspension using the same solution.

Various concentrations of the extracts (200 and 400 µg/ml) were prepared using distilled water. To each test concentration, 1 ml of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of the HRBC suspension were added. The samples were incubated at 37°C for 30 minutes and then centrifuged at 3000 rpm for 20 minutes. The hemoglobin content of the supernatant was measured using a UV spectrophotometer at 560 nm. Diclofenac (50 and 100 µg/ml) was used as the standard drug, and a control sample was prepared without extracts or standard drugs.

The percentage of HRBC membrane stabilization (protection) was calculated using the following formula (Kumar *et al.,* 2012) [3]:

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100$$

### Statistical Analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Dunnett's *t*-test, with $p$ values < 0.05 considered significant

## Results & Discussion

The results are presented in Table 1 as follows:

**Table 1:** *In-vitro* Anti-inflammatory Activity of Root Extracts of *Gmelina arborea*Roxb.

| S. No. | Groups | Concentration (µg/ml) | % Protection (Mean ± S.E.M) |
|---|---|---|---|
| 1 | Control | - | - |
| 2 | Petroleum ether extract | 200 | 40.24 ± 1.11* |
| | | 400 | 51.00 ± 1.45** |
| 3 | Chloroform extract | 200 | 61.12 ± 1.30** |
| | | 400 | 68.11 ± 2.42* |
| 4 | Ethyl acetate extract | 200 | 50.50 ± 2.20** |
| | | 400 | 61.40 ± 0.45* |
| 5 | Methanol extract | 200 | 65.30 ± 1.5** |
| | | 400 | 70.54 ± 1.27** |
| 6 | Aqueous extract | 200 | 52.40 ± 1.21** |
| | | 400 | 59.42 ± 2.44** |
| 7 | Diclofenac | 50 | 71.30 ± 1.50** |
| | | 100 | 79.45 ± 1.65** |

*The results are expressed as mean ± S.E.M [n=4]. *p < 0.01 compared to the control group.

The petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts of the root of *Gmelina arborea*were evaluated for in-vitro anti-inflammatory activity using the HRBC membrane stabilization method. The anti-inflammatory effect was concentration-dependent, as the activity increased with a corresponding increase in the extract concentration. Among the extracts, the methanolic and chloroform extracts at a concentration of 400 µg/ml exhibited the highest membrane stabilization, demonstrating 70.54% and 68.11% protection, respectively, with significant effects ($p < 0.01$) compared to the HRBCs in a hypotonic solution. All results were compared with the standard drug, diclofenac.

The roots of *Gmelina arborea* contain various bioactive phytochemicals known to possess membrane-stabilizing properties that prevent the lysis of erythrocyte membranes caused by hypotonic stress. Since the erythrocyte membrane is analogous to the lysosomal membrane, stabilizing it suggests a potential for stabilizing lysosomal membranes as well (Ramprasath, 2004) [8]. Lysosomal membrane stabilization prevents the release of hydrolytic enzymes that contribute to inflammatory responses and tissue damage (Murugasan *et al.,* 1981) [6]. NSAIDs are known to stabilize membranes, enhancing their anti-inflammatory efficacy. Although the precise mechanism of membrane stabilization by the extracts remains unclear, it may inhibit the osmotic loss of intracellular fluid and electrolytes, which could lead to hypotonic hemolysis. Rajendran et al. (2008) [7] proposed that the extract might suppress pathways that increase the efflux of these components. The methanol extract exhibited significant anti-inflammatory activity at 400 µg/ml, demonstrating strong potential for anti-inflammatory

effects. The in-vitro results obtained for the methanolic extract are in alignment with its established traditional uses.

## Conclusion

The methanol and chloroform extracts of *Gmelina arborea* demonstrated significant membrane stabilization effects by inhibiting hypotonicity-induced lysis of erythrocytes. Stabilization of the lysosomal membrane plays a crucial role in limiting inflammatory responses by preventing the release of lysosomal constituents from activated neutrophils, such as proteases and bacterial enzymes, which contribute to further tissue inflammation and damage (Murugasan *et al.*, 1981 [6]; Smith, 2004) [9]. Considering the significant in-vitro anti-inflammatory activity observed in the methanol and chloroform extracts, likely due to their rich bioactive phytoconstituents, these extracts could be selected for in-vivo evaluation for anti-inflammatory potential. The methanolic and chloroform extracts exhibited 70.54% and 68.11% protection, respectively, at a concentration of 400 µg/ml, whereas the standard drug, diclofenac, showed 79.45% protection at 100 µg/ml. Based on these findings, it can be concluded that the methanol and chloroform extracts of *Gmelina arborea* possess considerable anti-inflammatory activity, potentially offering a safer alternative to conventional NSAIDs.

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