

In Vitro Evaluation of the Anti-Inflammatory Activity of Petroleum Ether Extract of *Tectona grandis* Linn. (Bark)

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Abstract – *Tectona grandis* Linn. f. (Lamiaceae), commonly known as teak, is traditionally used in folk and Ayurvedic medicine as an astringent and anti-inflammatory agent. The present study was undertaken to evaluate the in vitro anti-inflammatory activity of the petroleum ether extract of *Tectona grandis* bark, obtained by successive Soxhlet extraction, using the protein (albumin) denaturation inhibition assay. Preliminary phytochemical screening of the petroleum ether extract revealed the presence of steroids, terpenoids, quinones, glycosides and fixed oils, while polar constituents such as flavonoids, tannins and saponins were largely absent. The extract exhibited concentration-dependent inhibition of bovine serum albumin denaturation over the range of 200–1000 µg/mL, with percentage inhibition increasing from 12.88 ± 0.17% to 64.42 ± 0.84%, compared with 18.97 ± 0.09% to 94.85 ± 0.45% for the standard drug diclofenac sodium at equivalent concentrations. Although the extract showed lower potency than the standard at every concentration tested, the clear dose-dependent trend confirms genuine anti-inflammatory potential, which may be attributed to the non-polar phytoconstituents (steroids, terpenoids and quinones) detected in the extract. These findings provide preliminary in vitro evidence supporting the traditional use of *Tectona grandis* bark in inflammatory conditions and warrant further investigation through membrane-stabilisation assays, in vivo models and bioactivity-guided isolation of the responsible constituents.

Key Words: *Tectona grandis*, Anti-inflammatory activity, Petroleum ether extract, Protein denaturation, In vitro evaluation, Phytochemical screening.

I. INTRODUCTION

Herbal medicines have formed the foundation of healthcare systems throughout the world for centuries, with the World Health Organization estimating that nearly 80% of the global population relies, at least in part, on traditional plant-based remedies for primary healthcare. Medicinal plants owe their therapeutic potential to a wide array of naturally occurring phytochemicals — flavonoids, tannins, phenolics, alkaloids, terpenoids and glycosides — many of which possess antioxidant, antimicrobial and anti-inflammatory properties.

Inflammation is a complex protective response of the body to harmful stimuli such as pathogens, tissue injury or irritants, characterised by redness, heat, swelling, pain and loss of function. While inflammation is essential for tissue repair, its

excessive or prolonged form contributes to numerous chronic diseases, including rheumatoid arthritis, atherosclerosis and inflammatory bowel disease. Denaturation of tissue proteins is a well-documented cause of inflammatory and rheumatic conditions, and agents capable of inhibiting this denaturation are considered potential anti-inflammatory candidates — a principle that underlies the widely used in vitro albumin denaturation inhibition assay.

Tectona grandis Linn. f. (family Lamiaceae), commonly known as teak, is a large deciduous tree native to South and Southeast Asia and cultivated extensively across peninsular India, including the Dharmapuri region of Tamil Nadu. Beyond its economic importance as a timber species, various parts of the plant — particularly the bark — have long been used in Ayurvedic and folk medicine as an astringent and anti-inflammatory agent for the treatment of wounds, diarrhoea and hepatic disorders. Earlier pharmacological studies have reported antinociceptive, anti-inflammatory, antidiabetic, hepatoprotective and antimicrobial activities in aqueous, ethanolic and methanolic extracts of the plant; however, the non-polar (petroleum ether) fraction of the bark, which is expected to be enriched in steroids, terpenoids and quinones, remains comparatively unexplored for anti-inflammatory activity. The present study was therefore designed to prepare the petroleum ether extract of *Tectona grandis* bark, screen it for preliminary phytoconstituents, and evaluate its in vitro anti-inflammatory potential using the protein denaturation inhibition assay, in comparison with the standard NSAID diclofenac sodium.

II. REVIEW OF LITERATURE

Bhangale et al. (2010) reported significant anti-inflammatory and analgesic activity of the aqueous bark extract of *Tectona grandis* in the carrageenan-induced paw oedema model, supporting its traditional medicinal use. Singh et al. (2021), in a comprehensive review, summarised evidence that bark, leaf, root and wood extracts of the plant contain flavonoids, phenolics, quinones, steroids and terpenoids responsible for antioxidant, analgesic, antimicrobial and anti-inflammatory activities. Han et al. (2023) demonstrated that methanolic extracts and petroleum ether/ethyl acetate fractions of *Tectona grandis* suppressed pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) in LPS-stimulated RAW 264.7 macrophages, while Parawansah et al. (2025) confirmed dose-dependent reduction of TNF-α levels using combined in vivo and in silico approaches. Jaybhaye et al. (2020) additionally

reported that the ethanolic bark extract reduced airway inflammation in an anti-asthmatic model. Collectively, these studies establish a strong pharmacological rationale for evaluating the anti-inflammatory potential of *Tectona grandis* bark, and highlight a gap in the literature regarding its non-polar (petroleum ether) fraction, which the present study addresses.

III. AIM AND OBJECTIVES

The aim of the present study was to evaluate the in vitro anti-inflammatory activity of the petroleum ether extract of *Tectona grandis* bark using a validated biochemical assay model. The specific objectives were: (i) to collect and authenticate *Tectona grandis* bark and prepare the petroleum ether extract by Soxhlet extraction; (ii) to determine the percentage yield and carry out preliminary phytochemical screening of the extract; (iii) to evaluate the in vitro anti-inflammatory activity using the protein (albumin) denaturation inhibition assay at graded concentrations; and (iv) to compare the activity of the extract with the standard NSAID diclofenac sodium.

IV. MATERIALS AND METHODS

A. Plant Material Collection and Authentication

Fresh bark of *Tectona grandis* Linn. f. was collected from Dharmapuri district, Tamil Nadu, and authenticated. The bark was washed thoroughly with tap water followed by distilled water, shade-dried at room temperature, powdered using a mechanical grinder, and stored in an airtight container until further use.

B. Extraction

Approximately 20 g of the coarse dried bark powder was subjected to continuous hot extraction in a Soxhlet apparatus using petroleum ether (60–80°C) as solvent for 6–8 hours, until the siphoned solvent appeared colourless. The extract was concentrated under reduced pressure using a rotary vacuum evaporator at below 40°C, dried, weighed, and the percentage yield was calculated. The dried extract was stored at 4°C in an airtight container until further analysis.

C. Preliminary Phytochemical Screening

The petroleum ether extract was subjected to standard qualitative chemical tests to detect the major classes of phytoconstituents — alkaloids (Mayer's, Wagner's and Dragendorff's tests), flavonoids (Shinoda test), tannins and phenolics (ferric chloride test), saponins (foam test), glycosides (Keller–Killiani test), steroids and terpenoids (Salkowski test), carbohydrates (Molisch's and Benedict's tests), proteins (Biuret test), quinones (sulfuric acid test) and fixed oils (spot test), following the standard procedures of Harborne and Kokate.

D. In Vitro Anti-Inflammatory Activity – Protein Denaturation Inhibition Assay

The reaction mixture (5 mL) consisted of 0.2 mL of 1% w/v bovine serum albumin, 2.8 mL of phosphate-buffered saline (pH 6.4), and 2 mL of the test extract at concentrations of 200, 400, 600, 800 and 1000 µg/mL, prepared in a minimal volume

of DMSO/Tween-80 and made up with buffer. A control tube contained vehicle in place of extract, and a standard tube contained diclofenac sodium at equivalent concentrations. All mixtures were incubated at $37 \pm 2^\circ\text{C}$ for 15–20 minutes, then heated at $70\text{--}75^\circ\text{C}$ for 5 minutes. After cooling, turbidity was measured spectrophotometrically at 660 nm against a blank. The percentage inhibition of protein denaturation was calculated as:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample or standard. All experiments were performed in triplicate.

V. RESULTS

The petroleum ether extraction of *Tectona grandis* bark yielded 15.88 g from 20 g of dried powder, corresponding to a percentage yield of 79.4% (Table 1).

Table 1: Percentage Yield of Petroleum Ether Extract

S.No.	Solvent	Wt. of Dried Bark (g)	Wt. of Extract (g)	% Yield
1	Petroleum ether	20	15.88	79.4

Preliminary phytochemical screening of the petroleum ether extract revealed the presence of glycosides, steroids, terpenoids, quinones and fixed oils/fats, while alkaloids, flavonoids, tannins, phenolic compounds, saponins and carbohydrates were absent — consistent with the non-polar nature of the solvent (Table 2).

Table 2: Preliminary Phytochemical Screening of Petroleum Ether Extract

Phytochemical Constituent	Petroleum Ether Extract
Alkaloids	–
Flavonoids	–
Tannins	–
Phenolic compounds	–
Saponins	–
Glycosides	+
Steroids	+
Terpenoids	+
Carbohydrates	–
Proteins	–
Quinones	+
Fixed oils and fats	+

The extract exhibited concentration-dependent inhibition of bovine serum albumin denaturation across the tested range of 200–1000 µg/mL. Inhibition increased from $12.88 \pm 0.17\%$ at 200 µg/mL to $64.42 \pm 0.84\%$ at 1000 µg/mL, compared with $18.97 \pm 0.09\%$ to $94.85 \pm 0.45\%$ for the standard diclofenac sodium at the same concentrations (Table 3, Fig. 1). The

standard showed consistently higher inhibition than the extract at every concentration tested, though both followed a clear linear dose-response relationship, with small standard deviations ($\leq \pm 1\%$) indicating good reproducibility.

Table 3: In Vitro Anti-Inflammatory Activity (Protein Denaturation Inhibition) of Petroleum Ether Extract of *Tectona grandis* Bark

Concentration ($\mu\text{g/mL}$)	Sample Inhibition (%)	Standard (Diclofenac) Inhibition (%)
200	12.88 $\pm$ 0.17	18.97 $\pm$ 0.09
400	25.77 $\pm$ 0.34	37.94 $\pm$ 0.18
600	38.65 $\pm$ 0.50	56.91 $\pm$ 0.27
800	51.54 $\pm$ 0.67	75.88 $\pm$ 0.36
1000	64.42 $\pm$ 0.84	94.85 $\pm$ 0.45

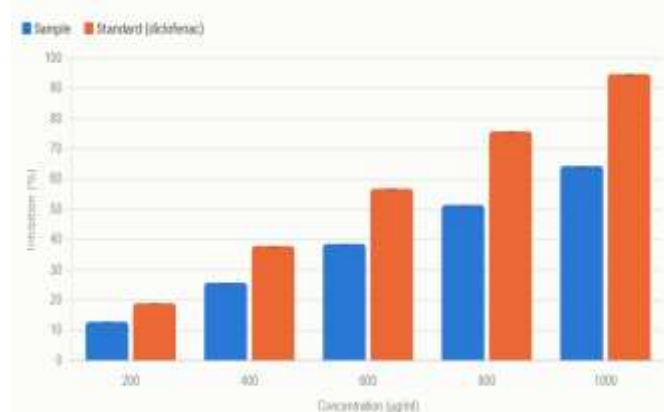


Fig. 1: Percentage inhibition of protein denaturation by petroleum ether extract of *Tectona grandis* bark and standard diclofenac sodium.

VI. DISCUSSION

The albumin denaturation inhibition assay is a widely used in vitro model for screening anti-inflammatory potential, based on the principle that denaturation of tissue proteins is a well-documented cause of inflammatory and rheumatic diseases; agents that prevent such denaturation are therefore regarded as candidate anti-inflammatory agents. In the present study, both the petroleum ether extract and the standard drug diclofenac sodium exhibited a clear concentration-dependent increase in percentage inhibition of protein denaturation across the 200–1000 $\mu\text{g/mL}$ range, consistent with the typical pharmacological behaviour of anti-inflammatory agents in this assay.

Although diclofenac showed higher inhibition than the extract at every concentration tested — for instance, 18.97% versus 12.88% at 200 $\mu\text{g/mL}$, and 94.85% versus 64.42% at 1000 $\mu\text{g/mL}$ — the extract nonetheless demonstrated measurable and reproducible protein-stabilising activity. This activity may be attributed to the non-polar phytoconstituents detected in the extract, namely steroids, terpenoids and quinones, which are known to stabilise protein conformation through hydrogen bonding and hydrophobic interactions, thereby preventing heat-induced unfolding — a mechanism analogous to that proposed for NSAIDs such as diclofenac. The absence of polar constituents such as flavonoids and tannins in

this fraction suggests that the observed activity is largely independent of these classically anti-inflammatory polyphenolic compounds, pointing instead to a lipophilic-constituent-mediated mechanism that merits further isolation and characterisation.

VII. CONCLUSION

The petroleum ether extract of *Tectona grandis* bark exhibited concentration-dependent in vitro anti-inflammatory activity, as evidenced by inhibition of bovine serum albumin denaturation ranging from 12.88% to 64.42% over 200–1000 $\mu\text{g/mL}$. Although less potent than the standard diclofenac sodium at all tested concentrations, the consistent dose-response relationship confirms genuine anti-inflammatory potential in the extract, likely mediated by its steroid, terpenoid and quinone constituents. These findings lend scientific support to the traditional use of teak bark in inflammatory conditions and warrant further studies — including membrane-stabilisation assays, in vivo models, and bioactivity-guided isolation — to fully characterise the responsible constituents and mechanism of action.

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