



IN VITRO EVALUATION OF AQUEOUS AND ETHANOLIC EXTRACTS OF BARK AND LEAVES OF LANNEA COROMANDELICA FOR ANTIGOUT AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT :

Gout is a metabolic inflammatory disorder associated with hyperuricemia and monosodium urate (MSU) crystal deposition. *Lannea coromandelica* is traditionally used for gout and inflammatory disorders, but comparative evidence for its bark and leaves remains limited. This study evaluated the in-vitro antigout and anti-inflammatory activities of aqueous and ethanolic extracts of bark and leaves. Extracts were screened for phytoconstituents and evaluated for xanthine oxidase inhibition, protein denaturation, MSU crystal-induced protein denaturation, heat-induced haemolysis and HRBC membrane stabilization at 50–800 µg/mL. Allopurinol and diclofenac sodium served as reference standards. All extracts demonstrated concentration-dependent activity. Ethanolic bark extract showed the highest activity among plant extracts, with IC₅₀ values of 116.49 µg/mL for xanthine oxidase inhibition, 101.49 µg/mL for protein denaturation and 109.30 µg/mL for MSU-induced denaturation, followed by ethanolic leaf extract. Phenolics, flavonoids and tannins were detected in all extracts. These findings support the potential antigout and anti-inflammatory properties of *L. coromandelica*, particularly its ethanolic bark extract, warranting further in-vivo and mechanistic studies.

Keywords: *Lannea coromandelica*; Gout; Xanthine oxidase; Anti-inflammatory; Protein denaturation; HRBC membrane stabilization; Medicinal plant

1. INTRODUCTION

Gout is one of the most common inflammatory arthropathies worldwide and results from chronic hyperuricemia, a state in which serum urate concentrations exceed its solubility threshold, leading to the formation and articular deposition of monosodium urate (MSU) crystals. Hyperuricemia arises principally from increased purine turnover and/or impaired renal excretion of uric acid, and xanthine oxidase (XO) occupies a pivotal position in this pathway because it catalyses the terminal oxidative steps that convert hypoxanthine to xanthine and xanthine to uric acid. Deposition of MSU crystals within joints and periarticular tissues activates resident macrophages and synoviocytes through pattern-recognition and inflammasome-linked pathways, culminating in the release of interleukin-1 β and other pro-inflammatory mediators, neutrophil recruitment, and the clinical syndrome of acute gouty arthritis characterised by pain, swelling, erythema and marked functional impairment. Repeated or poorly controlled attacks may progress to chronic tophaceous gout with joint destruction and disability.

The pharmacological management of gout is directed at two complementary goals: lowering serum uric acid through xanthine oxidase inhibitors (allopurinol, febuxostat) or uricosuric agents, and controlling acute inflammation with non-steroidal anti-inflammatory drugs (NSAIDs), colchicine or corticosteroids. Although effective, these agents are associated with well-recognised limitations. Allopurinol may precipitate hypersensitivity reactions, including the potentially fatal allopurinol hypersensitivity syndrome, and requires dose adjustment in renal impairment; NSAIDs carry a risk of gastrointestinal ulceration, cardiovascular events and nephrotoxicity; and colchicine has a narrow therapeutic index with gastrointestinal and haematological toxicity on prolonged use. These constraints, together with issues of cost and long-term tolerability in patients who often have concurrent renal or cardiovascular comorbidity, have sustained interest in safer, plant-derived alternatives that can simultaneously address the enzymatic (uric acid overproduction) and inflammatory (crystal-induced) components of gout.

Screening of traditionally used plants for xanthine oxidase inhibitory activity has proved to be a productive strategy for identifying candidate antigout agents. Kong et al. (2000) demonstrated that several Chinese medicinal plants used in the traditional management of gout possess appreciable xanthine oxidase inhibitory activity, and comparable findings were reported by Nguyen et al. (2004) for a panel of Vietnamese medicinal plants and by Owen and

Johns (1999) for northeastern North American plant remedies traditionally used for gout. Flavonoid constituents in particular have repeatedly been identified as the chemical basis of such activity; Noro et al. (1983), in one of the earliest systematic reports, isolated flavonoid-type xanthine oxidase inhibitors from the flowers and buds of *Daphne genkwa*. Collectively, these studies establish flavonoids, phenolic acids, tannins, alkaloids and terpenoids as plausible mediators of plant-derived xanthine oxidase inhibition and provide a rational basis for the bio-prospecting of traditionally used species, such as *Lannea coromandelica*, for antigout activity. In-vitro assays such as xanthine oxidase inhibition, protein denaturation inhibition and erythrocyte membrane stabilization are widely used first-line tools for such screening because they are inexpensive, reproducible and mechanistically interpretable (Umamaheswari & Chatterjee, 2008), even though they cannot, by themselves, establish in-vivo efficacy.

Lannea coromandelica (Houtt.) Merr. (family Anacardiaceae), locally known as Jingini or Indian ash tree, is a deciduous tree widely distributed across the Indian subcontinent, Sri Lanka and Southeast Asia. Various parts of the plant are used in folk and tribal medicine systems of India and Bangladesh; the stem bark, in particular, is traditionally applied for the treatment of gout, dyspepsia, dysentery, skin eruptions, ulcers and toothache, while the leaves are used for hepatitis, diabetes and inflammatory conditions (Rahman et al., 2016; Rajesh, 2021; Swathi & Lakshman, 2022). This broad ethnomedicinal profile has been reviewed comprehensively by Malu et al. (2024), who catalogued the chemical diversity and biological activities reported across the genus *Lannea*. Prior phytochemical and pharmacological investigations have reported antioxidant, anti-inflammatory, antinociceptive, antidiabetic, antimicrobial and antidiarrhoeal activities for extracts of *L. coromandelica* bark and leaves. Notably, Singh and Singh (1994) demonstrated direct anti-inflammatory activity of a bark extract in rats, providing early in-vivo support for the ethnomedicinal use of the bark in inflammatory conditions, while Islam et al. (2022) confirmed neuropharmacological and antidiabetic effects of the methanolic leaf extract in mice, and Hossen et al. (2026) reported hepatoprotective activity of the bark extract against paracetamol-induced hepatotoxicity in rats. High-performance liquid chromatography analysis of the bark extract has identified gallic acid, (-)-epigallocatechin-3-gallate, catechin, chlorogenic acid and caffeic acid as major polyphenolic constituents, and the extract has been shown to upregulate the Nrf2-heme oxygenase-1 antioxidant pathway in murine macrophages (Alam et al., 2017). Separately, ethyl acetate and methanolic fractions of the leaves have shown high phenolic and flavonoid content with strong free-radical scavenging and hepato/reno-protective antioxidant activity in carbon tetrachloride-intoxicated rats (Kumar & Jain, 2015), while Yun et al. (2014) isolated thirteen chemical constituents, including quercetin and several phenolic acids, from the bark, and Islam et al. (2002) identified a polyflavonoid tannin in the stem bark with zoosporicidal activity, confirming tannins as an abundant and biologically active constituent class in this tissue.

Despite these reports and the explicit ethnomedicinal indication of the bark for gout, a systematic, side-by-side comparison of aqueous and ethanolic extracts of both the bark and the leaves of *L. coromandelica* for antigout activity - encompassing xanthine oxidase inhibition together with complementary models of crystal- and heat-induced inflammatory injury - has not previously been reported. Addressing this gap is important because the choice of plant part and extraction solvent markedly influences both the yield and the qualitative phytochemical profile of an extract, and hence its likely pharmacological potency. The present study was therefore designed to evaluate, in a single comparative framework, the in-vitro antigout and anti-inflammatory activities of aqueous and ethanolic extracts of the bark and leaves of *L. coromandelica*.

The specific objectives of the study were: (i) to prepare aqueous and ethanolic extracts of the bark and leaves of *L. coromandelica* and determine their percentage yield; (ii) to carry out preliminary phytochemical screening of the four extracts; (iii) to evaluate the antigout potential of the extracts through xanthine oxidase inhibition, protein denaturation inhibition and MSU crystal-induced protein denaturation assays; (iv) to evaluate the anti-inflammatory potential of the extracts through heat-induced haemolysis and HRBC membrane stabilization assays; and (v) to compare the activity of the extracts with the standard drugs allopurinol and diclofenac sodium and to identify the extract(s) showing the most consistent activity across the different assays.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh bark and leaves of *Lannea coromandelica* were collected from a natural habitat during the appropriate season and authenticated by a qualified taxonomist; a voucher specimen was deposited in the institutional herbarium for future reference. The plant material was washed thoroughly with running tap water followed by distilled water to remove adhering soil and extraneous matter, shade-dried at room temperature for 10-15 days to minimise degradation of thermolabile constituents, pulverised into a coarse powder using a mechanical grinder, passed through a suitable mesh sieve, and stored in airtight containers protected from light and moisture until extraction.

2.2 Chemicals, Reagents and Instruments

All chemicals and reagents used were of analytical grade. Key reagents included xanthine, xanthine oxidase enzyme, bovine serum albumin (BSA), egg albumin, monosodium urate (MSU), phosphate buffer saline, hyaluronidase, trypsin, allopurinol and diclofenac sodium. Fresh human red blood cells

(HRBC) were obtained from healthy volunteers. Instrumentation comprised a Soxhlet apparatus, rotary vacuum evaporator, UV-Visible spectrophotometer, pH meter, water bath, centrifuge, incubator, hot air oven, analytical balance and magnetic stirrer.

2.3 Preparation of Extracts

Aqueous extracts of the bark and leaves were prepared by cold maceration: accurately weighed powdered material was soaked separately in distilled water (1:10 w/v) for 72 hours at room temperature with intermittent stirring, filtered successively through muslin cloth and Whatman No. 1 filter paper, and concentrated on a water bath below 50 °C. Ethanolic extracts were prepared by continuous hot Soxhlet extraction using ethanol as solvent for 6-8 hours until the siphoning solvent became colourless, followed by concentration under reduced pressure on a rotary vacuum evaporator. All four extracts (bark aqueous, bark ethanolic, leaf aqueous, leaf ethanolic) were dried to constant weight, and the percentage yield was calculated relative to the initial weight of powdered material used. Dried extracts were stored in airtight containers (aqueous extracts in a desiccator; ethanolic extracts at 4 °C) until use. Stock solutions for the in-vitro assays were freshly prepared in distilled water or phosphate buffer and diluted to the required test concentrations (50, 100, 200, 400 and 800 µg/mL).

2.4 Preliminary Phytochemical Screening

Qualitative phytochemical screening of all four extracts was performed using standard established tests described by Trease and Evans (2002) and Harborne (1998): Mayer's, Wagner's, Dragendorff's and Hager's tests for alkaloids; Molisch's, Benedict's and Fehling's tests for carbohydrates; Keller-Killiani and Borntrager's tests for glycosides; the froth/foam test for saponins; Biuret and Ninhydrin tests for proteins and amino acids; ferric chloride and lead acetate tests for phenolics and tannins; Shinoda and alkaline reagent tests for flavonoids; the Salkowski test for terpenoids; the Liebermann-Burchard test for steroids; and the spot test for fixed oils and fats. Results were recorded qualitatively as present (+) or absent (-) based on characteristic colour changes or precipitate formation.

2.5 In-Vitro Antigout Activity

2.5.1 Xanthine Oxidase Inhibitory Assay

Xanthine oxidase inhibitory activity was determined spectrophotometrically following the general principle described for plant-derived xanthine oxidase inhibitors by Noro et al. (1983) and subsequently adapted for extract screening by Kong et al. (2000) and Nguyen et al. (2004). The reaction mixture consisted of 1.0 mL phosphate buffer (0.05 M, pH 7.5), 0.5 mL xanthine solution (0.15 mM) and 0.5 mL of test extract at concentrations of 50-800 µg/mL. The reaction was initiated by adding 0.1 mL of xanthine oxidase enzyme solution (0.1 U/mL), incubated at 25 °C for 15 minutes, and terminated with 1.0 mL of 1 N HCl. Absorbance was read at 290 nm against a reagent blank on a UV-Visible spectrophotometer, with allopurinol (10-100 µg/mL) as the standard. Percentage inhibition and IC₅₀ values were calculated from the concentration-response data.

2.5.2 Inhibition of Protein Denaturation (BSA method)

Inhibition of protein denaturation was assessed using the bovine serum albumin (BSA) method originally described by Mizushima and Kobayashi (1968) and subsequently standardised for plant extract screening by Sakat et al. (2010). The reaction mixture comprised 0.5 mL bovine serum albumin (1% w/v), 0.5 mL phosphate buffer (0.2 M, pH 6.3) and 0.5 mL of test extract (50-800 µg/mL). Mixtures were incubated at 37 °C for 20 minutes and then heated at 70 °C for 10 minutes to induce denaturation. After cooling, turbidity was measured at 660 nm, with diclofenac sodium (50-400 µg/mL) as the standard.

2.5.3 MSU Crystal-Induced Protein Denaturation Assay

This assay adapted the general protein denaturation model of Chandra et al. (2012) to a monosodium urate crystal system in order to more closely simulate the crystal-induced protein injury characteristic of acute gout. Monosodium urate crystals were prepared by dissolving 1 g uric acid in 200 mL of 0.01 N NaOH under heating, adjusting the pH to 7.2, and cooling to 4 °C to allow crystallisation. The reaction mixture consisted of 0.5 mL BSA (1% w/v), 0.5 mL MSU crystal suspension and 0.5 mL of test extract (50-800 µg/mL), incubated at 37 °C for 30 minutes, after which absorbance was measured at 660 nm, with diclofenac sodium as the reference standard.

2.6 In-Vitro Anti-Inflammatory Activity

2.6.1 Heat-Induced Haemolysis Assay

Membrane-stabilizing activity against thermal stress was evaluated according to the principle described by Shinde et al. (1999). Fresh HRBC were washed and a 10% v/v suspension prepared in normal saline. The reaction mixture (1.0 mL HRBC suspension, 1.0 mL phosphate buffer saline pH 7.4, 1.0 mL

extract at 50-800 µg/mL) was incubated at 56 °C for 30 minutes, cooled, centrifuged at 3000 rpm for 10 minutes, and the supernatant absorbance measured at 560 nm, with diclofenac sodium as standard.

2.6.2 HRBC Membrane Stabilization Assay (Hypotonicity-Induced Haemolysis)

Hypotonicity-induced membrane stabilization was assessed using the method described by Oyedapo and Famurewa (1995) and further validated by Anosike et al. (2012). A 10% v/v HRBC suspension was mixed with 1.0 mL hypotonic saline (0.25% NaCl) and 1.0 mL of test extract (50-800 µg/mL), incubated at 37 °C for 30 minutes, centrifuged at 3000 rpm for 10 minutes, and the absorbance of the supernatant measured at 560 nm. Diclofenac sodium served as the reference standard. Percentage membrane stabilization was calculated relative to a control containing distilled water instead of extract.

2.7 Statistical Analysis

All experiments were performed in triplicate (n = 3) and results expressed as mean ± standard error of the mean (SEM). Data were analysed by one-way analysis of variance (ANOVA) at a 95% confidence level; a P-value < 0.05 was considered statistically significant. IC50 values for each assay were estimated from the concentration-response data by regression-based/logarithmic interpolation.

3. RESULTS

3.1 Percentage Yield of Extracts

The percentage yield of the extracts, calculated from 100 g of powdered plant material, followed the order ethanolic leaf (12.40%) > ethanolic bark (10.60%) > aqueous leaf (8.30%) > aqueous bark (7.10%) (Table 1), indicating that ethanol recovered a broader range of extractable constituents than water for both plant parts.

Table 1. Percentage Yield of Bark and Leaf Extracts of *Lannea coromandelica*

Plant Part	Extract	Powder Taken (g)	Extract Obtained (g)	% Yield
Bark	Aqueous	100	7.10	7.10
Bark	Ethanolic	100	10.60	10.60
Leaves	Aqueous	100	8.30	8.30
Leaves	Ethanolic	100	12.40	12.40

3.2 Preliminary Phytochemical Screening

Phenolic compounds/tannins and flavonoids were detected in all four extracts. Alkaloids were present in both bark extracts and the aqueous leaf extract but absent from most alkaloid tests in the ethanolic leaf extract; carbohydrates were detected in all extracts; saponins were confined mainly to the aqueous extracts and the ethanolic leaf extract; terpenoids were detected in the bark extracts and the ethanolic leaf extract; and steroids were detected only in the two ethanolic extracts. Proteins/free amino acids (Biuret test) and fixed oils and fats were not detected in any extract. The results are summarised in Table 2.

Table 2. Preliminary Phytochemical Screening of *Lannea coromandelica* Bark and Leaf Extracts

Phytoconstituent	Test	Bark AQ	Bark ET	Leaf AQ	Leaf ET
Alkaloids	Mayer's / Wagner's / Dragendorff's	+	+	+	–
	Hager's	+	+	+	+
Carbohydrates	Molisch's / Benedict's	+	+	+	+
	Fehling's	+	+	–	+
Glycosides	Keller-Killiani	–	–	–	–
	Boritrager's	+	–	+	+
Saponins	Foam test	–	–	–	–
Proteins/Amino acids	Biuret / Ninhydrin	–/+	–/+	–/+	–/+

Phytoconstituent	Test	Bark AQ	Bark ET	Leaf AQ	Leaf ET
Phenolics & Tannins	Ferric chloride / Lead acetate	+	+	+	+
Flavonoids	Shinoda / Alkaline reagent	+	+	+/-	+
Terpenoids	Salkowski	–	+	–	+
Steroids	Liebermann-Burchard	–	–	–	–
Fixed oils & fats	Spot test	–	–	–	–

3.3 In-Vitro Anticancer Activity

3.3.1 Xanthine Oxidase Inhibitory Activity

All four extracts produced a clear, concentration-dependent increase in xanthine oxidase inhibition over the range 50–800 µg/mL (Table 3). At the highest concentration (800 µg/mL), inhibition was $85.23 \pm 1.98\%$ for bark ethanolic (Bark ET), $78.24 \pm 1.94\%$ for leaf ethanolic (Leaf ET), $75.26 \pm 1.24\%$ for bark aqueous (Bark AQ) and $72.21 \pm 0.67\%$ for leaf aqueous (Leaf AQ) extracts, compared with $94.36 \pm 1.68\%$ for allopurinol. The corresponding IC₅₀ values (Figure 1) were 116.49 µg/mL (Bark ET), 147.32 µg/mL (Leaf ET), 225.09 µg/mL (Bark AQ) and 248.91 µg/mL (Leaf AQ), against 73.12 µg/mL for allopurinol, confirming that the ethanolic extracts, and particularly the bark, possessed the greatest xanthine oxidase inhibitory potency among the plant extracts.

Table 3. Xanthine Oxidase Inhibitory Activity (% Inhibition) of *Lannea coromandelica* Extracts

Conc. (µg/mL)	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Allopurinol
50	18.74±0.91	28.32±1.02	21.26±1.08	26.82±1.64	39.78±1.32
100	39.21±0.64	48.21±1.26	33.54±0.96	43.32±1.33	58.42±1.47
200	46.24±1.56	56.34±1.49	43.28±0.84	55.27±1.52	74.28±1.23
400	68.29±1.73	71.98±1.79	64.57±1.35	72.32±0.98	87.62±1.04
800	75.26±1.24	85.23±1.98	72.21±0.67	78.24±1.94	94.36±1.68

Data expressed as mean ± SEM (n = 3); one-way ANOVA, P < 0.05.

IC₅₀ (µg/mL): Bark AQ = 225.09; Bark ET = 116.49; Leaf AQ = 248.91; Leaf ET = 147.32; Allopurinol = 73.12.

3.3.2 Inhibition of Protein Denaturation

All extracts showed progressively increasing inhibition of BSA denaturation with rising concentration (Table 4). At 800 µg/mL, inhibition reached $90.84 \pm 1.84\%$ (Bark ET), $86.84 \pm 1.92\%$ (Leaf ET), $78.31 \pm 1.42\%$ (Bark AQ) and $74.64 \pm 1.09\%$ (Leaf AQ), compared with $96.27 \pm 1.94\%$ for diclofenac sodium. IC₅₀ values were 101.49 µg/mL (Bark ET), 115.01 µg/mL (Leaf ET), 177.72 µg/mL (Leaf AQ) and 209.59 µg/mL (Bark AQ), versus 67.30 µg/mL for diclofenac, again identifying Bark ET as the most potent plant extract.

Table 4. Inhibition of Protein Denaturation (BSA Method) by *Lannea coromandelica* Extracts

Conc. (µg/mL)	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Diclofenac
50	26.54±0.74	34.58±1.03	23.42±0.84	29.49±0.86	42.84±1.09
100	34.63±1.27	49.64±0.94	37.68±1.13	47.63±1.24	59.54±0.87
200	48.77±0.95	66.48±1.24	52.53±1.07	59.38±1.54	75.74±1.42
400	66.98±0.67	81.62±1.09	69.17±1.64	78.27±1.47	88.52±1.47
800	78.31±1.42	90.84±1.84	74.64±1.09	86.84±1.92	96.27±1.94

Data expressed as mean ± SEM (n = 3); one-way ANOVA, P < 0.05.

IC₅₀ (µg/mL): Bark AQ = 209.59; Bark ET = 101.49; Leaf AQ = 177.72; Leaf ET = 115.01; Diclofenac = 67.30.

3.3.3 MSU Crystal-Induced Protein Denaturation

In the MSU crystal-induced denaturation assay, inhibition again increased in a concentration-dependent manner (Table 5). At 800 µg/mL, Bark ET showed the highest inhibition among the plant extracts ($88.09 \pm 1.43\%$), followed by Leaf ET ($81.92 \pm 1.98\%$), Bark AQ ($70.64 \pm 1.92\%$) and Leaf AQ ($69.82 \pm 1.33\%$), compared with $93.66 \pm 1.33\%$ for diclofenac. IC₅₀ values were 109.30 µg/mL (Bark ET), 126.87 µg/mL (Leaf ET), 276.88 µg/mL (Bark AQ) and 290.51 µg/mL (Leaf AQ), against 71.67 µg/mL for diclofenac, mirroring the pattern seen in the general BSA denaturation assay.

Table 5. MSU Crystal-Induced Protein Denaturation Inhibition by *Lannea coromandelica* Extracts

Conc. (µg/mL)	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Diclofenac
50	19.74±0.94	32.28±1.12	18.34±1.61	26.66±1.12	42.63±1.28
100	30.76±1.21	48.17±1.08	29.86±1.94	44.28±1.28	56.82±1.44
200	44.28±1.27	62.43±1.33	43.72±1.77	60.94±1.34	72.45±1.62
400	56.47±1.98	78.87±1.74	55.38±1.93	76.43±1.26	84.64±1.27
800	70.64±1.92	88.09±1.43	69.82±1.33	81.92±1.98	93.66±1.33

Data expressed as mean ± SEM (n = 3); one-way ANOVA, P < 0.05.

IC₅₀ (µg/mL): Bark AQ = 276.88; Bark ET = 109.30; Leaf AQ = 290.51; Leaf ET = 126.87; Diclofenac = 71.67.

3.4 In-Vitro Anti-Inflammatory Activity

3.4.1 Heat-Induced Haemolysis Inhibition

All extracts inhibited heat-induced haemolysis in a concentration-dependent manner (Table 6). At 800 µg/mL, Leaf ET showed the highest inhibition among the plant extracts ($91.48 \pm 1.38\%$), followed by Bark ET ($88.23 \pm 1.98\%$), Bark AQ ($81.38 \pm 1.71\%$) and Leaf AQ ($78.38 \pm 1.86\%$), compared with $94.32 \pm 1.86\%$ for diclofenac. IC₅₀ values were 106.16 µg/mL (Bark ET), 109.86 µg/mL (Leaf ET), 160.73 µg/mL (Bark AQ) and 176.22 µg/mL (Leaf AQ), against 72.75 µg/mL for diclofenac.

Table 6. Heat-Induced Haemolysis Inhibition by *Lannea coromandelica* Extracts

Conc. (µg/mL)	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Diclofenac
50	25.63±1.18	33.27±1.12	23.82±0.98	32.34±1.08	44.21±1.12
100	39.32±0.98	48.62±1.33	38.72±1.07	46.63±1.33	58.34±1.92
200	54.92±1.63	64.62±1.82	52.52±1.33	71.46±1.27	76.51±1.74
400	68.81±1.33	77.98±1.34	68.23±1.24	84.35±1.46	88.19±1.82
800	81.38±1.71	88.23±1.98	78.38±1.86	91.48±1.38	94.32±1.86

Data expressed as mean ± SEM (n = 3); one-way ANOVA, P < 0.05.

IC₅₀ (µg/mL): Bark AQ = 160.73; Bark ET = 106.16; Leaf AQ = 176.22; Leaf ET = 109.86; Diclofenac = 72.75.

3.4.2 HRBC Membrane Stabilization Assay

Membrane stabilization increased progressively with concentration in all extracts (Table 7). At 800 µg/mL, Bark ET produced the highest stabilization among the plant extracts ($90.97 \pm 1.74\%$), followed by Leaf ET ($88.42 \pm 1.72\%$), Bark AQ ($83.23 \pm 1.64\%$) and Leaf AQ ($80.81 \pm 1.94\%$), compared with $96.52 \pm 1.67\%$ for diclofenac. IC₅₀ values were 106.90 µg/mL (Bark ET), 114.22 µg/mL (Leaf ET), 145.82 µg/mL (Bark AQ) and 159.51 µg/mL (Leaf AQ), against 64.89 µg/mL for diclofenac.

Table 7. HRBC Membrane Stabilization Activity of *Lannea coromandelica* Extracts

Conc. (µg/mL)	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Diclofenac
50	29.82±1.21	38.94±1.07	26.41±1.13	35.32±0.98	48.57±1.54
100	42.21±1.34	48.86±1.26	38.85±1.84	44.86±1.33	65.63±1.32

Conc. (µg/mL)	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Diclofenac
200	62.54±1.67	67.95±1.41	62.21±1.67	64.52±1.52	78.64±1.74
400	74.52±1.89	80.48±1.77	71.23±1.75	79.21±1.64	89.89±1.33
800	83.23±1.64	90.97±1.74	80.81±1.94	88.42±1.72	96.52±1.67

Data expressed as mean ± SEM (n = 3); one-way ANOVA, P < 0.05.

IC₅₀ (µg/mL): Bark AQ = 145.82; Bark ET = 106.90; Leaf AQ = 159.51; Leaf ET = 114.22; Diclofenac = 64.89.

3.5 Summary of IC₅₀ Values

Across all five in-vitro assays, the ethanolic bark extract consistently returned the lowest IC₅₀ value among the four plant extracts, followed by the ethanolic leaf extract, with the corresponding aqueous extracts showing the weakest activity in every assay (Table 8), although all plant extracts remained less potent than the respective reference standards.

Table 8. Comparative IC₅₀ Values (µg/mL) of *Lannea coromandelica* Extracts and Standard Drugs

Assay	Bark AQ	Bark ET	Leaf AQ	Leaf ET	Standard
Xanthine oxidase inhibition	225.09	116.49	248.91	147.32	73.12 (Allopurinol)
Protein denaturation inhibition	209.59	101.49	177.72	115.01	67.30 (Diclofenac)
MSU crystal-induced denaturation	276.88	109.30	290.51	126.87	71.67 (Diclofenac)
Heat-induced haemolysis inhibition	160.73	106.16	176.22	109.86	72.75 (Diclofenac)
HRBC membrane stabilization	145.82	106.90	159.51	114.22	64.89 (Diclofenac)

4. DISCUSSION

The present study evaluated, for the first time in a single comparative design, the in-vitro antigout and anti-inflammatory activities of aqueous and ethanolic extracts of the bark and leaves of *Lannea coromandelica*. Five complementary in-vitro assays were employed: xanthine oxidase inhibition, which targets the terminal enzymatic step of uric acid biosynthesis; protein denaturation inhibition and MSU crystal-induced protein denaturation, which model the inflammatory injury associated with urate crystal exposure; and heat-induced haemolysis and HRBC membrane stabilization, which are established surrogates for lysosomal membrane protection during inflammation (Oyedapo & Famurewa, 1995; Shinde et al., 1999). Across all five assays, a consistent pattern emerged: activity increased with concentration in every extract, the ethanolic extracts outperformed their aqueous counterparts, and the ethanolic bark extract was the most active of the four plant extracts, though none matched the potency of the standard drugs allopurinol and diclofenac sodium.

The higher percentage yield obtained with ethanol (10.60-12.40%) relative to water (7.10-8.30%) is consistent with the ability of a solvent of intermediate polarity to recover a broader spectrum of polar and moderately polar phytoconstituents, including several phenolic and flavonoid compounds, than water alone. A comparable solvent-dependent effect on extractive efficiency and antioxidant potency has previously been reported for *L. coromandelica* leaves, where an ethyl acetate fraction of a crude methanolic extract showed markedly higher total phenolic and flavonoid content and stronger free-radical scavenging and in-vivo hepato/reno-protective antioxidant activity than the corresponding aqueous fraction (Kumar & Jain, 2015). Extraction yield alone, however, does not equate to pharmacological potency, since biological activity depends on the nature and concentration of the active constituents recovered rather than on the total mass of extractive obtained.

Preliminary phytochemical screening detected phenolic compounds, tannins and flavonoids in all four extracts, which is of particular relevance to the present findings. Flavonoids and phenolic acids are well documented to inhibit xanthine oxidase, in some cases by chelating the molybdenum cofactor or competing at the substrate-binding pocket of the enzyme, as demonstrated for flavonoid constituents of *Daphne genkwa* (Noro et al., 1983) and for multiple plant species screened by Kong et al. (2000) and Nguyen et al. (2004), and are also recognised for antioxidant and membrane-stabilizing properties that can limit protein denaturation and lysosomal membrane injury during inflammation. High-performance liquid chromatography of *L. coromandelica* bark extract has previously identified gallic acid, (-)-epigallocatechin-3-gallate, catechin, chlorogenic acid and caffeic acid as major polyphenols, and this extract has been shown to activate the Nrf2-heme oxygenase-1 antioxidant pathway in murine macrophages, providing independent chemical support for the phenolic-rich composition inferred from the qualitative screening performed here (Alam et al., 2017). Similarly, Yun et al.

(2014) isolated quercetin and several phenolic acids from the bark, reinforcing the likelihood that phenolic and flavonoid constituents underlie the biological activities observed. Tannins, detected in all four extracts, are also capable of binding proteins and biological membranes and may contribute directly to the protein-denaturation-inhibitory and membrane-stabilizing effects observed; a polyflavonoid tannin has been specifically identified in *L. coromandelica* stem bark by Islam et al. (2002), lending direct chemical support to this mechanistic interpretation. Terpenoids and steroids, detected selectively in the ethanolic extracts, may provide additional, though as yet uncharacterised, contributions to the overall activity profile, a chemical diversity also emphasised in the recent reviews of this species by Swathi and Lakshman (2022) and Malu et al. (2024). Because preliminary screening is only qualitative, however, it cannot establish which individual constituent, or combination of constituents, is responsible for a given biological effect; the observed activities are therefore best interpreted as arising from the combined or synergistic action of multiple phytoconstituents rather than from a single active principle.

In the xanthine oxidase inhibition assay, the ethanolic bark extract achieved the lowest IC₅₀ (116.49 µg/mL) among the four plant extracts, indicating the greatest capacity to suppress the enzymatic step responsible for uric acid generation, a mechanism directly analogous to that of allopurinol (IC₅₀ 73.12 µg/mL). This level of activity is broadly comparable to that reported for other traditionally used antigout plants: Kong et al. (2000) and Owen and Johns (1999) similarly found that ethanolic or hydro-alcoholic extracts of gout remedies produced moderate, sub-allopurinol xanthine oxidase inhibition, supporting the plausibility of a genuine, if modest, urate-lowering mechanism for *L. coromandelica* bark. This finding is also consistent with the traditional use of the bark, rather than the leaf, for the treatment of gout in tribal medicine systems of the Indian subcontinent and Bangladesh (Rahman et al., 2016; Rajesh, 2021). The complementary protein denaturation and MSU crystal-induced denaturation assays extended this observation by demonstrating that the same ethanolic bark extract also limited protein damage under both generalised (heat-induced BSA denaturation) and gout-specific (MSU crystal-associated) conditions, with IC₅₀ values of 101.49 and 109.30 µg/mL, respectively - values that approached, though did not match, those of diclofenac sodium (67.30 and 71.67 µg/mL). Because monosodium urate crystals are known to activate synoviocytes and denature adjacent proteins during acute gouty flares, activity in this assay is mechanistically relevant to the inflammatory component of gout, over and above simple urate lowering; the direct anti-inflammatory activity of a *L. coromandelica* bark extract reported in vivo by Singh and Singh (1994) lends independent support to this interpretation.

The heat-induced haemolysis and HRBC membrane stabilization assays provided convergent evidence of membrane-protective activity, again favouring the ethanolic extracts (Bark ET IC₅₀ 106.16 and 106.90 µg/mL, respectively) over the aqueous extracts (Bark AQ IC₅₀ 160.73 and 145.82 µg/mL). Because the erythrocyte membrane shares structural features with the lysosomal membrane, protection against heat- and hypotonicity-induced haemolysis is widely accepted as indicative of an ability to prevent the release of lysosomal proteolytic enzymes that amplify tissue injury during inflammation (Oyedapo & Famurewa, 1995; Shinde et al., 1999; Anosike et al., 2012). The consistency of this membrane-stabilizing effect across two independent stress models (thermal and osmotic) strengthens the inference that the extracts, and particularly the ethanolic bark extract, possess genuine anti-inflammatory potential rather than an assay-specific artefact.

Taken together, the concordant ranking of extracts across all five assays - ethanolic bark > ethanolic leaf > aqueous bark > aqueous leaf, in every case less active than the corresponding standard - supports a coherent, dual antigout mechanism for *L. coromandelica*, combining modest inhibition of uric acid formation (via xanthine oxidase) with protection against crystal- and heat/osmotic-induced inflammatory injury. This pattern is broadly consistent with, and extends, previous reports describing antioxidant, anti-inflammatory, antidiabetic, neuropharmacological and hepatoprotective activity for extracts of this plant (Alam et al., 2017; Islam et al., 2022; Kumar & Jain, 2015; Rahman et al., 2016; Singh & Singh, 1994; Hossen et al., 2026), but the present investigation is, to our knowledge, the first to evaluate xanthine oxidase inhibition specifically, and to do so alongside multiple inflammation-related endpoints, in a single side-by-side comparison of bark and leaf, aqueous and ethanolic extracts.

The study has several limitations that should be acknowledged. First, all findings are derived from in-vitro biochemical and erythrocyte-based models; they demonstrate mechanistic plausibility but cannot establish that the extracts would lower serum uric acid or exert anti-inflammatory efficacy in vivo, a caveat that applies equally to the plant-derived xanthine oxidase inhibitors screened by Nguyen et al. (2004) and Owen and Johns (1999). Second, the phytochemical screening performed was qualitative rather than quantitative, so the precise concentration of phenolics, flavonoids and tannins in each extract, and their individual contribution to the observed activities, remains to be determined by chromatographic and spectroscopic methods (e.g., HPLC/HPTLC quantification, isolation-guided bioassay), of the type used by Alam et al. (2017) and Yun et al. (2014). Third, potential cytotoxicity of the extracts toward the erythrocytes and other cells used in these assays was not separately assessed, which should be addressed in follow-up work. Future studies should therefore include quantitative phytochemical and chromatographic profiling, isolation and structural characterisation of the responsible constituents, enzyme kinetic studies to define the mode of xanthine oxidase inhibition, cytokine and cell-based inflammatory models, and appropriate in-vivo evaluation (e.g., potassium oxonate-induced hyperuricemia and MSU crystal-induced paw oedema models, building on the in-vivo anti-inflammatory model of Singh and Singh, 1994) to substantiate the therapeutic relevance of the present in-vitro findings.

5. CONCLUSION

Aqueous and ethanolic extracts of the bark and leaves of *Lannea coromandelica* exhibited concentration-dependent antigout and anti-inflammatory activity across five complementary in-vitro assays: xanthine oxidase inhibition, protein denaturation inhibition, MSU crystal-induced protein denaturation, heat-induced haemolysis inhibition and HRBC membrane stabilization. The ethanolic bark extract consistently showed the lowest IC₅₀ values among the four plant extracts in every assay, followed by the ethanolic leaf extract, with both aqueous extracts showing comparatively weaker activity; none of the plant extracts, however, matched the potency of the standard drugs allopurinol and diclofenac sodium. These effects are plausibly linked to the phenolic, flavonoid and tannin constituents detected on preliminary phytochemical screening and previously characterised in this species. The findings provide scientific evidence in support of the traditional use of *L. coromandelica* bark for gout and inflammatory conditions and identify the ethanolic bark extract as the most promising candidate for further bioactivity-guided isolation, mechanistic and in-vivo investigation.

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