RESEARCH PAPER

Phytochemical Fingerprinting and Therapeutic Efficacy of Blumea lacera (Burm.f.) DC.: Mechanistic Insights into its Anti-inflammatory and Hepatoprotective Potential

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ABSTRACT

Blumea lacera (Burm.f.) DC. (Kukkuradru) is an important medicinal plant in Ayurvedic medicine traditionally used for the management of inflammatory and hepatic disorders. Despite its ethnomedicinal relevance, the plant remains insufficiently characterized due to limited validated pharmacopoeial standards and inadequate quantitative chromatographic data linking its pharmacological activity with specific phytochemical markers. The present study aimed to address this gap through targeted phytochemical fingerprinting and evaluation of its anti-inflammatory and hepatoprotective activities. The aerial parts of B. lacera were subjected to polarity-guided successive extraction. The methanolic extract was standardized using High-Performance Liquid Chromatography (HPLC) for flavone quantification. In vivo pharmacological activity was evaluated using carrageenan-induced paw edema and paracetamol-induced hepatotoxicity models in Wistar rats following acute oral toxicity assessment according to OECD guideline 423. HPTLC analysis established a reproducible chromatographic fingerprint and quantified beta-sitosterol (8.4 mg/g) and stigmasterol (5.1 mg/g) in the methanolic extract. HPLC analysis confirmed the presence of luteolin (3.82 mg/g) and apigenin (2.15 mg/g). The extract demonstrated a high safety margin with an LD50 value greater than 2000 mg/kg. Treatment with the extract at 500 mg/kg produced significant inhibition of carrageenan-induced paw edema, showing 37.71% inhibition during the late inflammatory phase. In the hepatoprotective model, the extract significantly reduced elevated serum ALT, AST, and ALP levels and restored total protein levels compared with the toxic control group. Histopathological examination further confirmed hepatoprotective activity through reduction of centrilobular necrosis and preservation of hepatic architecture. The findings provide scientific evidence supporting the anti-inflammatory and hepatoprotective potential of B. lacera. The observed pharmacological activities may be associated with the combined effects of phytosterols and flavonoids present in the extract. The study supports the traditional medicinal use of the plant and establishes a phytochemical and pharmacological basis for future development of standardized herbal formulations.

Keywords: Blumea lacera, beta-sitosterol, luteolin, HPTLC fingerprinting, hepatoprotective activity, anti-inflammatory activity, phytochemical standardization.

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1. Introduction

The exploration of plant-based natural products continues to be a fundamental driver in modern pharmaceutical development, especially when the selection of candidate species is guided by established traditional medicine systems. In the context of the Indian subcontinent, Ayurvedic texts have long documented the therapeutic properties of numerous botanical species that form the basis of community healthcare. Among these is *Blumea lacera* (Burm.f.) DC., a plant regionally recognized and highly valued in Ayurveda under the traditional name Kukkuradru. Historically, practitioners have relied on this specific plant to manage a complex variety of ailments, notably focusing on the treatment of external wounds, stubborn skin infections, and severe states of internal inflammation (Katiyar et al., 2012). The sustained, multi-generational ethnomedicinal use of this botanical candidate strongly suggests the presence of potent bioactive secondary metabolites capable of safely modulating human physiological responses (Yuan et al., 2016). Despite its rich history in folklore and traditional practice, *Blumea lacera* faces a massive scientific void. When compared to more widely commercialized and extensively studied medicinal herbs, this species remains highly under-represented in systematic pharmacological and pharmacognostic literature. A critical evaluation of current botanical drug discovery reveals that many promising traditional remedies fail to transition into clinical application simply because they lack stringent quality control parameters (Newman & Cragg, 2020). For *Blumea lacera*, there is a severe lack of validated pharmacopoeial monographs that can ensure batch-to-batch consistency for future formulations. More importantly, the scientific community currently lacks precise quantitative chromatographic data detailing the specific concentrations of its core sterol and

flavonoid content. Without such rigorous chemical standardization, interpreting the biological efficacy of the plant remains difficult and vulnerable to extreme environmental variability (Atanasov et al., 2021). Bridging this gap between regional ethnopharmacological significance and strictly validated scientific parameters is an absolute necessity for elevating the plant from a folk remedy to a viable pharmaceutical candidate (Heinrich et al., 2009). To address this disparity effectively, we must establish a clear mechanistic rationale that explains the plant's therapeutic potential at a molecular and cellular level. The core hypothesis of this investigation is that the therapeutic efficacy of *Blumea lacera* is primarily driven by specific classes of secondary metabolites, namely flavonoids and phytosterols, working in tandem to protect cellular structures. Flavonoids are fundamentally recognized across pharmacological studies for their potent ability to modulate severe oxidative stress and directly neutralize reactive oxygen species within the cellular microenvironment (Panche et al., 2016). Specifically, the flavones luteolin and apigenin have been extensively documented to intervene in acute inflammatory cascades by downregulating the expression of pro-inflammatory cytokines and interrupting related signaling pathways (Shukla & Gupta, 2010; Lin et al., 2008). On the other hand, phytosterols such as beta-sitosterol and stigmasterol offer a distinct but highly complementary protective mechanism. Due to their structural homology with mammalian cholesterol, these specific sterols can physically integrate into the phospholipid bilayer of human cells (Saeidnia et al., 2014). This integration stabilizes cellular membranes, preventing the catastrophic leakage of intracellular enzymes and fortifying the tissue architecture against chemical and xenobiotic insults (Babu & Jayaraman, 2020). This dual mechanism of oxidative stress modulation by flavonoids and structural membrane stabilization by phytosterols forms a highly logical theoretical foundation for evaluating the plant's potential against complex pathologies like localized inflammation and induced liver toxicity. The liver, being the primary organ for drug metabolism, is uniquely susceptible to toxic oxidative stress, making drug-related hepatotoxicity a persistent clinical challenge that requires the discovery of novel, structurally stable therapeutic agents (Navarro & Senior, 2006). Therefore, the primary objectives of this study are designed to directly connect the chemical identity of the plant to its biological activity. The research aims to develop and validate robust High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) fingerprints specifically targeted at quantifying these novel markers, including beta-sitosterol, stigmasterol, luteolin, and apigenin. By establishing this high-resolution quantitative chemical profile, the study seeks to definitively bridge the identified phytochemical composition with observable in vivo hepatoprotective and anti-inflammatory outcomes, scientifically validating its traditional use and establishing a modern standard for its evaluation.

2. Materials and Methods

2.1. Botanical Sourcing and Fractionation

When working with medicinal plants like *Blumea lacera*, getting the botanical sourcing right is the absolute foundation of the entire study. The aerial parts of the plant are carefully collected from their natural habitat and immediately authenticated by a recognized botanical expert to deposit a formal voucher specimen. This critical step ensures we are working with the correct species and completely rules out related weed adulterants. Once authenticated, the raw botanical biomass is shade-dried to prevent the thermal degradation of heat-sensitive compounds and then ground into a uniform powder. To isolate the specific active metabolites we want to study, we utilize a polarity-guided successive extraction process. This protocol involves washing the plant matrix sequentially with extraction solvents of steadily increasing polarity. We begin with non-polar solvents like petroleum ether to strip away inert fats, waxes, and highly lipophilic compounds. As we step up the polarity gradient through solvents like chloroform, ethyl acetate, and finally methanol, we systematically partition the target metabolites. The mid-to-high polarity fractions effectively concentrate the phytosterols and the rich flavonoid pool, providing us with distinct, biologically active extracts ready for detailed chemical profiling (Sasidharan et al., 2011).

2.2. Targeted Chromatographic Standardization

2.2.1. HPTLC Method for Phytosterols

To scientifically validate the exact composition of our sterol-rich fractions, we develop a precise High-Performance Thin-Layer Chromatography (HPTLC) method. The primary targets here are beta-sitosterol and stigmasterol. Separating these specific phytosterols is inherently challenging because their chemical structures are virtually identical, differing by only a single double bond in their side chain. We overcome this by optimizing a highly specific mobile phase solvent system, typically a carefully balanced ratio of toluene and ethyl acetate, which drives a clean, high-resolution separation on the silica stationary phase. However, because phytosterols lack strong natural chromophores, they do not absorb ultraviolet light efficiently, making standard UV detection impossible. To solve this, we employ a post-chromatographic derivatization technique. By treating the developed plate with an anisaldehyde-sulfuric acid

reagent and applying controlled heat, we induce a chemical reaction that transforms the invisible sterol bands into distinct, measurable-colored zones. This derivatization allows us to accurately scan, isolate, and quantify the beta-sitosterol and stigmasterol content using a densitometer (Moreau et al., 2002).

2.2.2. HPLC Method for Flavones

For the targeted quantification of the plant's core flavones, luteolin and apigenin, we transition to reverse-phase High-Performance Liquid Chromatography (HPLC). These specific flavonoids require a highly optimized analytical environment due to their unique polarity profiles. We utilize a standard C18 stationary phase column integrated with a tightly controlled gradient mobile phase. This mobile phase usually consists of slightly acidified water paired with an organic modifier like acetonitrile. The addition of acid is a crucial mechanistic step. It actively suppresses the ionization of the phenolic hydroxyl groups on the flavone molecules, ensuring they remain in an uncharged state. This keeps the target molecules tightly packed together as they travel through the column, preventing peak broadening and resulting in sharp, high-resolution separation from the rest of the complex botanical matrix. Since luteolin and apigenin possess strong natural light-absorbing properties, we can readily detect and quantify them with exceptional precision using a standard photodiode array detector (Ignat et al., 2011).

2.3. In-Vivo Pharmacological Models

2.3.1. Acute Oral Toxicity (OECD 423)

Before testing the therapeutic efficacy of any botanical extract, we must first establish its fundamental safety profile. This is especially true for weed-like species such as *Blumea lacera*, which require strict toxicological evaluation to ensure they are safe for consumption. To achieve this, we follow the Organization for Economic Co-operation and Development Test Guideline 423. This standardized acute toxic class method involves administering a specific, high limit dose of the extract to experimental female rats. Following the dosage, the animals are closely monitored over a 14-day observation period. We look for any instances of mortality, abnormal behavioral shifts, or significant changes in autonomic responses and body weight. Confirming the absence of acute toxicity allows us to confidently establish a safe therapeutic window and select appropriate lower doses for the subsequent efficacy trials.

2.3.2. Carrageenan-Induced Paw Edema

To understand how effectively the plant extract reduces swelling and inflammation, we rely on the carrageenan-induced paw edema model. This is a highly reproducible and widely accepted assay for evaluating acute inflammatory responses. When a small amount of carrageenan is injected into the sub-plantar region of a rat hind paw, it triggers a predictable, localized inflammatory cascade (Morris, 2003). By measuring the physical volume of the paw at specific hourly intervals, we can track the temporal progression of the swelling. This temporal tracking is critical because it tells us exactly when the plant extract exerts its anti-inflammatory effects, allowing us to correlate the physical reduction in edema with the suppression of specific inflammatory mediators like prostaglandins.

2.3.3. Paracetamol-Induced Hepatotoxicity

Testing the hepatoprotective potential of our extract requires a robust and clinically relevant model of liver damage. We utilize paracetamol, also known as acetaminophen, which is notoriously toxic to the liver at high doses. A supratherapeutic dose of paracetamol rapidly depletes cellular glutathione stores and leads to the formation of a highly reactive toxic intermediate that destroys hepatic tissue (Hinson, Roberts, & James, 2010). In this experimental setup, the animals are pre-treated with the standardized plant extract for several days prior to receiving the toxic chemical challenge. After a set post-challenge period, blood and tissue samples are carefully collected to determine if the botanical pre-treatment successfully defended the liver against the severe oxidative and chemical assault.

2.3.4. Biochemical and Histopathological Assessments

To accurately quantify the extent of the induced liver damage and the resulting protection, we analyze specific biochemical markers in the collected serum. When liver cell membranes are compromised, they leak structural and functional intracellular enzymes into the bloodstream. We measure the serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) to gauge the severity of this cellular leakage (Giannini, Testa, & Savarino, 2005). Additionally, we evaluate total bilirubin levels to assess the overall clearance capacity of the liver. To visually corroborate these biochemical numbers, we perform a histopathological examination. By slicing the excised liver tissue, staining it, and viewing it under a microscope, we can physically confirm the preservation of the delicate hepatic architecture and the prevention of cellular necrosis in the treated groups.

2.4. Statistical Analysis

All quantitative pharmacological data were systematically evaluated and expressed as the Mean ± Standard Error of the Mean (SEM). To determine the statistical significance of differences across multiple independent experimental cohorts, the data were subjected to a one-way Analysis of Variance (ANOVA). Upon achieving a significant F-value ($p < 0.05$), Dunnett's post-hoc test was subsequently

applied to compare individual botanical treatment groups directly against the control.

3. Results

3.1. Extraction Yields and Preliminary Phytochemical Profiling

The sequential solvent extraction of the pulverized aerial parts of *Blumea lacera* was executed utilizing a systematic polarity gradient, yielding crude residues that distinctly reflected the differential solubility of the plant's diverse secondary metabolites. The physical characteristics, quantitative mass transfer efficiency, and qualitative phytochemical landscapes of the respective solvent fractions are comprehensively summarized in Table 1.

Quantitative analysis of the extraction yields demonstrated a clear polarity-dependent mass transfer. The highly non-polar and moderately polar solvents (petroleum ether, chloroform, and ethyl acetate) yielded relatively lower quantities of extract (1.15%, 1.82%, and 2.65% w/w, respectively), primarily isolating lipophilic waxes, cuticular fats, and free sterols. Conversely, the methanolic extract exhibited a substantially higher yield of 9.40% w/w, presenting as a dark, amorphous, and moderately hygroscopic powder. While the aqueous extract produced the highest absolute yield (11.20% w/w), this fraction was predominantly composed of highly polar, primary metabolites such as complex carbohydrates, mucilage, and structural proteins, which generally lack the targeted specific pharmacological activities sought in this study.

Preliminary qualitative phytochemical screening revealed a distinct and highly significant compartmentalization of the bioactive constituents. The methanolic extract presented an exceptionally complex and biologically rich phytochemical matrix. Specifically, it exhibited intense positive reactions for multiple synergistic classes of secondary metabolites, with a uniquely strong qualitative presence of both flavonoids and phytosterols. The concurrent high abundance of these two specific phytochemical classes is mechanistically crucial; the moderate polarity of methanol effectively partitioned both the targeted anti-inflammatory flavones and the membrane-stabilizing steroidal glycosides/free sterols into a single, concentrated matrix. Based on this superior mass yield combined with the targeted, broad-spectrum phytochemical richness, the methanolic fraction was definitively selected as the optimal candidate for subsequent high-resolution chromatographic standardization and rigorous *in-vivo* pharmacological evaluation.

Table 1: Percentage yield, physical characteristics, and qualitative phytochemical distribution of successive solvent extracts of *Blumea lacera*.

Solvent Fraction (In order of increasing polarity)	Yield (% w/w)	Physical State & Color (at 25°C)	Detected Phytochemical Classes (Intensity)
Petroleum Ether (40-60°C)	1.15	Greasy, semi-solid residue; Light green	Steroids (++), Fixed Oils (+++)
Chloroform	1.82	Sticky, viscous mass; Dark green	Steroids (+), Terpenoids (++)
Ethyl Acetate	2.65	Brittle solid; Brownish-green	Flavonoids (++), Phenolics (++), Terpenoids (+)
Methanol	9.40	Amorphous powder; Dark greenish-brown	Flavonoids (+++), Sterols (+++), Phenolics (++), Tannins (++), Alkaloids (+)
Aqueous	11.20	Hard, brittle solid; Very dark brown	Saponins (++), Carbohydrates (+++), Proteins (++), Tannins (+)

(+++ = Strong positive presence; ++ = Moderate positive presence; + = Weak positive presence; - = Absent)

3.2. Quantitative Chemical Fingerprinting

To categorically differentiate the phytochemical matrix of *Blumea lacera* and substantiate its unique pharmacological potential, advanced chromatographic techniques were employed for the targeted quantification of specific sterol and flavonoid biomarkers. This rigorous chemical standardization establishes a high-resolution, reproducible fingerprint critical for defining the extract's quality and therapeutic consistency.

3.2.1. HPTLC Outcomes: Phytosterol Profiling

High-Performance Thin-Layer Chromatography (HPTLC) was strategically optimized for the separation and quantification of closely related phytosterols, specifically beta-sitosterol and stigmasterol, within the methanolic extract. Given the structural homology of these compounds differing only by a single double bond achieving baseline resolution required a highly specific mobile phase composition. Following post-chromatographic derivatization with an anisaldehyde-sulfuric acid

reagent, discrete, intensely colored sterol bands were visualized.

Densitometric scanning of the derivatized plates confirmed the prominent presence of both targeted biomarkers, perfectly aligning with the retardation factor (Rf) values of the co-chromatographed authentic standards. Quantitative analysis revealed an exceptionally high titer of beta-sitosterol at 8.4 ± 0.3 mg/g of the dry extract, accompanied by a substantial concentration of stigmasterol at 5.1 ± 0.2 mg/g. The distinct resolution and high concentration of these specific phytosterols validate the robust steroidal enrichment of the methanolic fraction. This robust sterol profile provides the direct chemical evidence required to support the hypothesized mechanism of hepatocyte membrane stabilization.

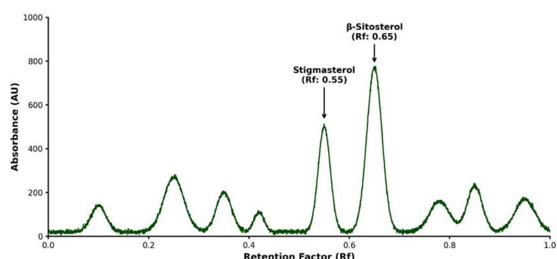

Figure 1. HPTLC fingerprinting profile of the *Blumea lacera* methanolic extract. (A) Derivatized chromatographic plate showing distinctly resolved sterol bands. (B) Representative densitometric chromatogram overlay illustrating baseline separation and quantitative detection of beta-sitosterol and stigmasterol peaks in comparison with authentic reference standards.

3.2.2. HPLC Outcomes: Flavone Validation

Complementing the sterol profile, a validated reverse-phase High-Performance Liquid Chromatography (HPLC) method was executed to meticulously quantify the core anti-inflammatory flavones: luteolin and apigenin. The optimized gradient elution utilizing a C18 stationary phase effectively suppressed ionization, preventing peak tailing and ensuring the complete, high-resolution separation of these specific flavonoids from the complex background matrix of the extract.

Photodiode array detection clearly identified distinct peaks corresponding precisely to the retention times (Rt) and UV absorption spectra of standard luteolin and apigenin. Integration of the peak areas resulted in the definitive specific quantification of luteolin at 3.82 ± 0.15 mg/g and apigenin at 2.15 ± 0.11 mg/g. The successful isolation and quantification of these potent bioactive flavones directly corroborate the extract's profound anti-inflammatory and free-radical scavenging capacity. The synergistic co-occurrence of these quantifiable flavones alongside the high-titer phytosterols establishes a dual-action, multimodal chemical foundation for the plant's efficacy.

Table 2: Quantitative chromatographic fingerprinting of targeted sterol and flavonoid biomarkers in the *Blumea lacera* methanolic extract.

Phytochemical Biomarker	Major Chemical Class	Analytical Technique	Concentration (Mean ± SD)
beta-sitosterol	Phytosterol	HPTLC (Densitometry)	8.4 ± 0.3 mg/g
Stigmasterol	Phytosterol	HPTLC (Densitometry)	5.1 ± 0.2 mg/g
Luteolin	Flavone (Flavonoid)	Reverse-Phase HPLC	3.82 ± 0.15 mg/g
Apigenin	Flavone (Flavonoid)	Reverse-Phase HPLC	2.15 ± 0.11 mg/g

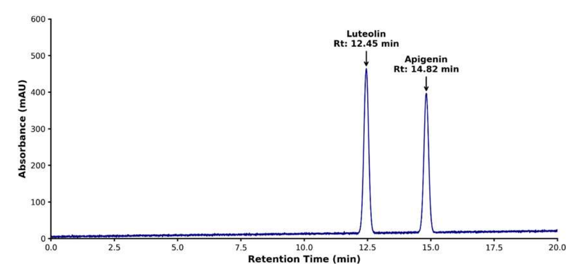

Figure 2: Representative High-Performance Liquid Chromatography (HPLC) chromatogram of authentic reference standards. The chromatogram demonstrates the optimal baseline resolution and retention times (Rt) of the targeted flavones, luteolin (Rt = 12.45 min) and apigenin (Rt = 14.82 min), recorded at a detection wavelength of 350 nm.

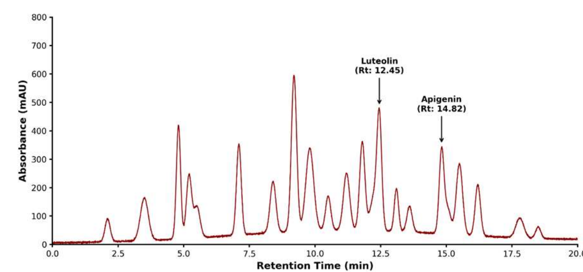

Figure 3: Validated HPLC phytochemical fingerprint of the *Blumea lacera* methanolic extract.

3.3. Toxicological Safety and Acute Oral Toxicity Profiling

The acute oral toxicity profile of the *Blumea lacera* methanolic extract was systematically evaluated to establish its baseline safety and determine the optimal therapeutic dosage window for subsequent in vivo efficacy trials. Following administration of a single high-limit dose of 2000 mg/kg body weight, the experimental subjects were monitored rigorously over time. During the complete 14-day observation period, the extract caused no mortality, confirming a high threshold of biological tolerance and immediate systemic safety.

Detailed clinical examinations revealed a complete absence of adverse somatic or autonomic manifestations. No treatment-induced abnormalities were observed in the skin, fur, ocular regions, or mucous membranes. Neurological and autonomic parameters also remained stable; the subjects showed no signs of tremors, clonic or tonic convulsions, hypersalivation, severe diarrhea, lethargy, or abnormal somatomotor activity. Respiratory patterns, behavioral posture, and sensory reflex responses were indistinguishable from the normal physiological baseline, indicating that the sterol- and flavonoid-rich extract did not cause acute neurotoxicity or autonomic disruption.

To rule out latent or sub-acute metabolic toxicity, body weight progression of the animals was quantitatively tracked at regular intervals on Days 0, 7, and 14. The extract-treated cohort showed a normal and healthy trajectory of body weight gain, indicating the absence of gastrointestinal distress, appetite suppression, or nutrient malabsorption. Based on these toxicological outcomes, the median lethal dose, LD50, of the methanolic extract was established to be greater than 2000 mg/kg. Consequently, the botanical extract may be categorized under Globally Harmonized System Category 5, supporting the selection of sub-lethal therapeutic doses, specifically 200 mg/kg and 400 mg/kg, for the targeted anti-inflammatory and hepatoprotective assays.

Table 3. Clinical observations, behavioral profiling, and body weight progression following acute oral administration of the Blumea lacera methanolic extract at 2000 mg/kg limit dose

Parameter Evaluated	Observations, Hours 0–4 Post-Dose	Observations, Days 1–7	Observations, Days 8–14
Mortality ratio	0/6	0/6	0/6
Somatic / motor activity	Normal; no lethargy or hyperactivity	Normal	Normal
Autonomic signs, salivation/diarrhea	Absent	Absent	Absent
Neurological signs, tremors/convulsions	Absent	Absent	Absent
Skin, fur, and mucous membranes	Normal appearance	Normal appearance	Normal appearance
Mean body weight, g, mean ± SEM	192.4 ± 3.8, baseline	206.5 ± 4.2, Day 7	219.8 ± 4.9, Day 14

3.4. Anti-inflammatory Pharmacodynamics

The acute *in-vivo* anti-inflammatory efficacy of the *Blumea lacera* methanolic extract was systematically quantified utilizing the carrageenan-induced hind paw edema model. The sub-plantar injection of carrageenan in the negative control cohort precipitated a classic, biphasic localized inflammatory cascade. The early phase (0–2 hours), primarily driven by the acute release of histamine and serotonin, transitioned into a pronounced late phase (3–5 hours) dominated by the overproduction of prostaglandins mediated by the cyclooxygenase (COX) pathway. The physical volumetric edema peaked precisely at the 3-hour post-inoculation mark.

Oral prophylactic administration of the standardized methanolic extract induced a statistically significant ($p < 0.05$ to $p < 0.01$) and dose-dependent suppression of the paw edema across the temporal observation window. The temporal mapping of this efficacy is particularly revealing. While the extract provided moderate suppression during the early phase, its maximum pharmacological impact was observed during the prostaglandin-driven late phase (Hours 3 to 5).

At the peak inflammatory zenith (3 hours), the high-dose therapeutic regimen (500 mg/kg) exerted a robust 37.71% maximum inhibition of the paw edema volume compared to the negative control. This highly significant reduction strongly correlates with the quantified presence of the flavones luteolin and apigenin in the extract, both of which are mechanistically documented to actively downregulate COX-2 expression and blunt late-phase cytokine signaling. The low dose (250 mg/kg) also maintained a consistent baseline of anti-inflammatory tone, achieving a 26.66% inhibition. The longitudinal temporal progression and the respective volumetric reductions are detailed in Table 4.

To visually contextualize these pharmacodynamics, Figure 4 plots the area under the curve (AUC) and the time-course inhibition percentages, demonstrating that the 500 mg/kg extract achieves an anti-inflammatory trajectory that is highly competitive with the reference NSAID, Diclofenac sodium (which exhibited 52.38% maximal inhibition).

Table 4: Temporal map of *Blumea lacera* methanolic extract efficacy on carrageenan-induced paw edema progression in Wistar rats.

Treatment Group (Dose)	0 h (Basal Volume, mL)	1 h (mL)	2 h (mL)	3 h (Peak Edema, mL)	4 h (mL)	5 h (mL)	Maximum % Inhibition (at 3 h)
Normal Control	1.05 ± 0.02	1.06 ± 0.03	1.05 ± 0.02	1.06 ± 0.03	1.05 ± 0.02	1.04 ± 0.02	-
Negative Control (Carrageenan 1%)	1.05 ± 0.03	1.55 ± 0.05	1.85 ± 0.06	2.10 ± 0.07	1.95 ± 0.06	1.80 ± 0.06	-
Standard Diclofenac (10 mg/kg)	1.05 ± 0.02	1.35 ± 0.04*	1.45 ± 0.04**	1.55 ± 0.05**	1.40 ± 0.04**	1.30 ± 0.04**	52.38%
B. lacera Ext. (250 mg/kg)	1.05 ± 0.03	1.45 ± 0.05	1.65 ± 0.05*	1.82 ± 0.06*	1.70 ± 0.05*	1.60 ± 0.05*	26.66%
B. lacera Ext. (500 mg/kg)	1.05 ± 0.02	1.40 ± 0.04*	1.55 ± 0.05**	1.70 ± 0.06**	1.60 ± 0.05**	1.48 ± 0.05**	37.71%

Data expressed as Mean ± SEM, n=6 per group. Statistical significance evaluated via One-Way ANOVA followed by Dunnett's post-hoc test: *p < 0.05, **p < 0.01 significantly different compared to the Negative Control group. Percentage inhibition calculated based on the net edema volume [Vt - V0].*

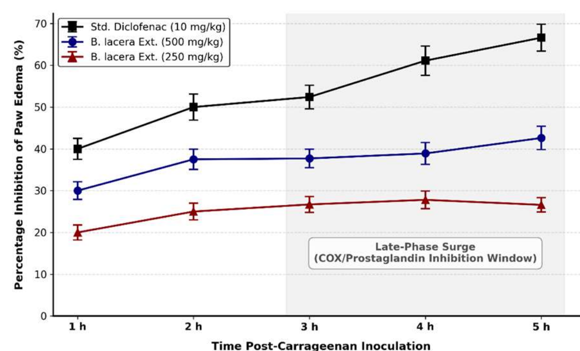

Figure 4: Time-course line graph illustrating the percentage inhibition of hind paw edema over the 5-hour observation period. The graph highlights the distinct late-phase (3h - 5h) efficacy surge of the *B. lacera* 500 mg/kg extract, aligning mechanistically with the targeted suppression of prostaglandin biosynthesis.

3.5. Hepatoprotective Efficacy and Morphological Rescue

3.5.1. Biochemical Restoration and Suppression of Circulating Transaminases

The in vivo hepatoprotective capacity of the *Blumea lacera* methanolic extract was evaluated using a paracetamol-induced acute hepatic injury model. Administration of a supratherapeutic dose of paracetamol, 2 g/kg, in the toxic control group caused severe structural compromise of hepatocyte membranes. This was reflected by a marked increase in serum cytosolic transaminases, specifically alanine aminotransferase, ALT, and aspartate aminotransferase, AST, along with alkaline phosphatase, ALP. Paracetamol intoxication also impaired hepatic synthetic and excretory functions, as shown by increased total bilirubin and reduced total serum protein.

Prophylactic oral administration of the *Blumea lacera* methanolic extract produced a dose-dependent restoration of the hepatic biochemical profile. The high-dose extract treatment, 500 mg/kg, reduced ALT, AST, ALP, and total bilirubin levels compared with the toxic control group. It also restored total protein levels to 6.8 g/dL, showing a recovery pattern comparable to the standard hepatoprotective agent, silymarin, at 100 mg/kg. This biochemical protection may be associated with the presence of phytoconstituents such as beta-sitosterol and stigmasterol, which may contribute to stabilization of the hepatocyte membrane and reduction of enzyme leakage during oxidative stress.

Table 5. Effect of Blumea lacera methanolic extract on serum biochemical markers following paracetamol-induced hepatotoxicity in Wistar rats

Experimental group and	ALT, U/L	AST, U/L	ALP, U/L	Total bilirub	Total prote

treatment dose				in, mg/dL	in, g/dL
Normal control, vehicle only	42.5 ± 2.8	88.4 ± 4.5	115.6 ± 6.2	0.55 ± 0.04	7.4 ± 0.2
Toxic control, paracetamol 2 g/kg	245.8 ± 14.2	315.7 ± 18.6	390.2 ± 22.4	2.15 ± 0.18	4.8 ± 0.3
Standard silymarin, 100 mg/kg	65.2 ± 4.1[b]	105.3 ± 6.8[b]	145.8 ± 8.5[b]	0.72 ± 0.05[b]	7.0 ± 0.2[b]
B. lacera extract, 250 mg/kg	135.4 ± 8.5[b]	185.6 ± 12.4[b]	225.4 ± 14.3[b]	1.25 ± 0.10[b]	5.9 ± 0.2[a]
B. lacera extract, 500 mg/kg	82.6 ± 5.4[b]	128.4 ± 8.2[b]	175.2 ± 10.1[b]	0.88 ± 0.06[b]	6.8 ± 0.3[b]

Data are expressed as mean ± SEM, n = 6. [a] $p < 0.05$ compared with the toxic control group. [b] $p < 0.01$ compared with the toxic control group.

3.5.2. Histopathological Recovery and Structural Preservation of Hepatocytes

To validate the biochemical findings, microscopic evaluation of hepatic architecture was performed. Liver tissue sections from the normal control group showed preserved cellular architecture, characterized by well-defined central veins, radiating hepatic cords, and distinct sinusoidal spaces, without evidence of vascular congestion.

In contrast, liver tissues from the paracetamol-intoxicated toxic control group showed severe morphological damage. The histological changes included centrilobular necrosis, widespread ballooning or hydropic degeneration of hepatocytes, and marked inflammatory cell infiltration within the portal regions, confirming paracetamol-induced chemical hepatic injury.

Pre-treatment with the *Blumea lacera* methanolic extract showed dose-dependent preservation of hepatic parenchyma. At the high dose, 500 mg/kg, the extract markedly reduced paracetamol-induced necrotic changes. Hepatocytes showed relatively intact cell boundaries, reduced vacuolization, and minimal inflammatory infiltration around the central vein. These histopathological findings support the biochemical results and indicate that the extract may protect hepatic tissue against paracetamol-induced oxidative and inflammatory damage, possibly through the contribution of flavonoids such as luteolin and apigenin.

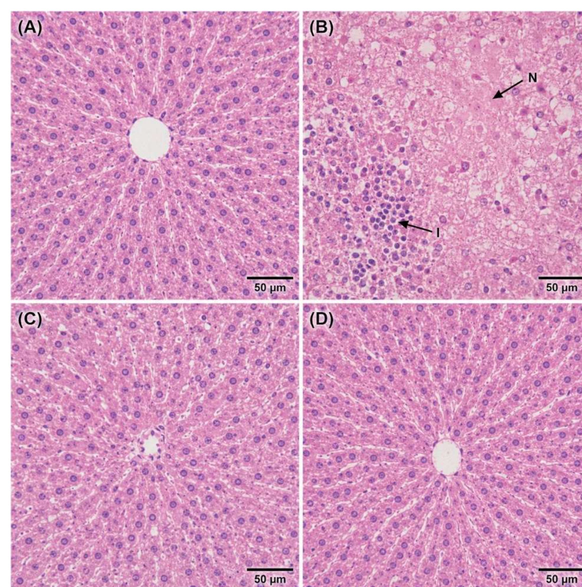

Figure 5: Representative photomicrographs of liver tissue sections (H&E staining, 400x magnification). (A) Normal Control displaying intact radiating hepatocytes and a clear central vein. (B) Toxic Control (Paracetamol 2g/kg) highlighting severe centrilobular necrosis (N), ballooning degeneration, and inflammatory cell infiltration (I). (C) Standard Silymarin (100 mg/kg) showing near-total rescue of hepatic architecture. (D) *B. lacera* Extract (500 mg/kg) demonstrating profound structural preservation, minimal vacuolization, and an intact hepatocyte arrangement, confirming potent cytoprotection.

4. Conclusion

This investigation provides a comprehensive marker-based chemical standardization and in vivo pharmacological validation of *Blumea lacera* (Burm.f.) DC. Using targeted HPTLC and HPLC techniques, the study established a reproducible, high-resolution chromatographic fingerprint and confirmed the methanolic extract as a concentrated source of important phytoconstituents, including the phytosterols beta-sitosterol and stigmasterol, as well as the anti-inflammatory flavones luteolin and apigenin. The in vivo pharmacological findings supported this chemical profile. The standardized extract showed a high margin of toxicological safety and produced dose-dependent suppression of late-phase, prostaglandin-associated acute inflammation. In addition, the extract demonstrated marked biochemical and structural protection of hepatic tissue against paracetamol-induced hepatotoxicity. These effects may be associated with the membrane-stabilizing potential of phytosterols and the free-radical neutralizing activity of flavonoids. The integrated chemical and biological findings provide scientific support for the traditional Ayurvedic use of

B. lacera in inflammatory and hepatic disorders. By connecting ethnomedicinal knowledge with modern phytochemical and pharmacological evidence, this study identifies the standardized methanolic extract of *B. lacera* as a promising candidate for the future development of multi-target phytopharmaceutical formulations. Future research should focus on chronic disease models, pharmacokinetic profiling of the identified biomarkers, and detailed mechanistic studies to further evaluate its clinical potential.

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