
PHYTOCHEMICAL CHARACTERIZATION AND ACUTE TOXICUTY EVALUATION OF *LAGERSTROEMIA OVALIFOLIA* LEAVE EXTRACT

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ABSTRACT

Lagerstroemia ovalifolia Teijsm. & Binn is a plant native to Indonesia that is used empirically in traditional medicine; however, little scientific data has been published regarding its standardisation and safety. This study aimed to phytochemical characterization and analyse the safety of the *Lagerstroemia ovalifolia* leaf extract through in vivo acute toxicity testing. The extract was obtained via percolation with 70% ethanol for 3 hours, yielding a recovery rate of 12.47%. Organoleptically, the extract was viscous, blackish-green in colour, had a characteristic odour and a bitter taste, with water-soluble and ethanol-soluble compound contents of 7% and 12%, respectively. Phytochemical screening yielded positive results for flavonoids, tannins, terpenoids, phenols, saponins and alkaloids; UPLC profiling detected compounds with m/z 302 (presumed to be quercetin) and m/z 448 (presumed to be luteolin O-7 glycoside). Non-specific parameters and microbial contamination were within the required limits. The acute toxicity test revealed no mortality, toxic symptoms or significant changes in organ weight, with an estimated LD₅₀ value of >5,000 mg/kg body weight, categorized as practically non-toxic according to the acute toxicity classification (Hodge & Sterner, 1943; OECD, 2001). The results of the study indicate that benger leaf extract meets most standardization parameters and exhibits no acute toxicity at the tested doses, thus holding potential for further development as a raw material for herbal medicine.

KEYWORDS

Lagerstroemia ovalifolia, percolation, characterization, UPLC, acute toxicity



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INTRODUCTION

Indonesia possesses abundant natural resources, including medicinal plants with the potential to be developed as raw materials for herbal medicines. This potential represents a strategic asset in supporting the resilience and self-reliance of the national pharmaceutical industry. As science and technology advance, research capacity is key to realising mastery over potential local natural resources; one such plant with potential for medicinal use is *Lagerstroemia*. The genus *Lagerstroemia* belongs to the family *Lythraceae*; this plant is rich in secondary metabolites, including flavonoids, terpenoids, phenols, tannins and alkaloids, which have been reported to contribute to various pharmacological activities, such as lowering blood sugar levels, as well as anti-obesity, anti-cancer, antiviral, antimicrobial, antioxidant and anti-inflammatory effects (Yue et al., 2024) . One of its species is *Lagerstroemia ovalifolia* Teijsm. & Binn, locally known as ‘benger’. This species has received relatively limited scientific attention and is distributed across several Asian regions, including Indonesia, Malaysia, Thailand, the Philippines, Myanmar, India and China, and has been empirically utilised in traditional medicine. Despite its potential as a source of local natural materials, information regarding the quality characteristics, chemical profile and safety of *L. ovalifolia* leaf extracts remains very limited; this lack of information is one aspect that needs to be addressed in the development of ‘benger’ as a standardised raw material for herbal medicines .(Samang et al., 2025)

Standardisation is a crucial stage in the development of natural materials; it ensures that the processes guaranteeing the final product (medicines, extracts or extract-based products) meet specific parameter values that remain constant across different batches, thereby providing a basis for quality control in subsequent development stages. Standardisation parameters include specific parameters relating to identity and chemical characteristics, as well as non-specific parameters relating to physicochemical quality and safety. In the development of natural medicinal raw materials, quality requirements may refer to the Indonesian Herbal Pharmacopoeia and the Indonesian National Agency of Drug and Food Control (BPOM RI) guidelines. (BPOM RI, 2023; Kemenkes RI, 2022) In addition to quality aspects and chemical characteristics, safety evaluation is a crucial part of the development of medicinal plants. Empirical use of plants does not directly guarantee safety across various dose levels. In vivo acute toxicity testing is required to obtain preliminary information regarding the potential for systemic toxic effects following the administration of the extract at a specific dose. Based on this, this study aims to characterise *L. ovalifolia* leaf extract through the standardisation of specific and non-specific parameters, analyse the compound profile using UPLC, and evaluate acute toxicity in vivo in female ddY strain mice. It is hoped that this study will provide scientific data on the quality, chemical characteristics and acute safety of the benger leaf extract, serving as a basis for its further development as a raw material for herbal medicines.

RESEARCH METHOD

Metode

This study is a laboratory-based experimental study aimed at evaluating the quality parameters of Benger leaf (*Lagerstroemia ovalifolia* Teijsm. & Binn.) extract using the percolation extraction technique. Collection and identification of plant material,

preparation of crude extracts, extraction, testing of specific and non-specific extract parameters, compound profiling using UPLC, and in vivo acute toxicity testing on female ddY strain mice

Sample

The sample for this study consisted of fresh, green Benger leaves (*Lagerstroemia ovalifolia* Teijsm. & Binn.), obtained from the Central Lampung region of Lampung.

Research location

The research was carried out at the Pharmaceutical Biology Laboratory and the Pharmacology Laboratory, Universitas Indonesia Maju, Jl. Harapan Lenteng Agung, Jagakarsa, South Jakarta. It was also carried out at the LABTIB BRIN laboratory.

Research Equipment

Apparatus: furnace, autoclave (GEA), laminar air flow cabinet, drying oven (Faithfull), incubator (TranaLAB), rotary evaporator (AeLAB), evaporator (Buchi Rotavapor R-220), UPLC-PDA system (Waters Acquity UPLC) with UPLC C18 column, atomic absorption spectrophotometer (AAS) (Biobase), set of percolation apparatus (Duran), water bath (RIO), UV steriliser (B-One), electric hob (Maspion), blender (Miyako, Kirin), digital balance (Fujitsu), an analytical balance (Sartorius), a desiccator (Duran), a magnetic stirrer, an ELISA reader, a refrigerator, as well as glassware and volumetric equipment (measuring flasks, Erlenmeyer flasks, beakers, shallow weighing bottles, porcelain crucibles, pycnometers, test tubes, porcelain dishes, Petri dishes, No. 40 mesh sieves, funnels, UPLC vials, microscope slides) made from Pyrex, micropipettes (Dragonlab), PTFE syringe filters (0.2 µm), droppers, Bunsen burners, thermometers (Krisbow), stirring rods, spatulas, horn spoons, filter paper, tissues, and aluminium foil.

Materials: percolation extract, 70% ethanol, 96% ethanol, distilled water, sodium hydroxide (NaOH), potassium iodide (KI), sodium chloride (NaCl), sodium sulphate (Na₂SO₄), magnesium (Mg), chloroform (CHCl₃), methanol, ferric chloride (FeCl₃), hydrochloric acid (HCl), nitric acid (HNO₃), amyl alcohol, lead(II) nitrate (Pb(NO₃)₂), cadmium(II) nitrate (Cd(NO₃)₂), mercury(II) nitrate (Hg(NO₃)₂), sulphuric acid (H₂SO₄), Wagner's, Dragendorff's and Mayer's reagents, 0.9% NaCl solution, plate count agar (PCA), potato dextrose agar (PDA), medium-protein mouse feed, and individual cage bases.

Test animals: 30 female ddY strain mice, 6 weeks old, meeting the criteria of being adult and healthy, having never given birth, not currently pregnant, with a minimum body weight of 20 grams and a weight variation of ≤20%. Ethics approval No: 106/KE.03/SK/07/2026

Extraction using the Percolation Method

The extraction method employed was percolation, carried out over a period of 3 hours with a solvent ratio of 1:15 using 70% ethanol. The resulting concentrated extract was further concentrated using a rotary evaporator. The percolate was evaporated using a rotary evaporator at a temperature of 40°C until a concentrated extract was obtained, which was then weighed to calculate the yield.

$$\% \text{ Rendemen} = \frac{\text{Weight of Concentrated Extract}}{\text{Weight of the Raw Herbal Material Powder}} \times 100 \%$$

Testing of Specific Parameters (BPOM RI, 2023)

Extract Identification and Organoleptic Assessment

The identification of the extract is described objectively, including the name of the extract, the name of the plant (Indonesian and Latin), and the part of the plant used. The organoleptic assessment is carried out visually and includes shape, odour, colour and taste.

Concentrations of Water- and Ethanol-Soluble Compounds

One gram of the extract was macerated in 100 mL of water saturated with chloroform (water solubility test) or 96 per cent ethanol (ethanol solubility test) for 24 hours; it was shaken during the first 6 hours and then left to stand for 18 hours. A 20 mL portion of the filtrate was evaporated to dryness in a porcelain dish and the residue was heated at 105°C until constant weight was reached (Kemenkes RI, 2022; Rachma & Saptawati, 2022)

$$\text{Concentration of ethanol-soluble compounds} = \frac{W_{2(g)} - W_{0(g)}}{W_1(g)} \times 100\%$$

(W₀ = weight of the cup; W₁ = extract weight; W₂ = (weight of the dish + residue))

Phytochemical Screening (Test Tube Assays)

Qualitative tests were carried out for alkaloids (Mayer's, Wagner's and Dragendorff's reagents), flavonoids (Mg powder + concentrated HCl + amyl alcohol), tannins (1% FeCl₃), terpenoids (chloroform + concentrated H₂SO₄), phenols (5% FeCl₃), and saponins (foam test + 2N HCl), with positive results indicated by a colour change or a characteristic precipitate specific to each class of compound

Phytochemical Profiling

The extract samples obtained via the percolation method were dissolved in methanol for LC/MS and filtered using a 0.45 µm Millipore membrane. A 2 µL aliquot of the filtrate was injected into the UPLC system using an autosampler. Analysis was carried out in full-scan mode within the m/z range of 200-800 using a mass detector (MS). Compound identification was based on retention time (Rt), molecular mass (m/z) and MS/MS fragments, and annotation was performed by comparing these results against databases such as KEGG and LipidMaps to determine the identities of the major compounds present in the extract. (Chawla & Ranjan, 2016)

Non-Specific Parameters (BPOM RI, 2023; Kemenkes RI, 2022)

Loss on Drying, Specific Gravity and Moisture Content

Drying loss is determined by heating 1 gram of extract at 105°C until a constant weight is reached. Specific gravity is determined using a pycnometer at 25°C with a 5% extract in 96% ethanol. Moisture content is determined by gradual drying at 105°C until the difference between two consecutive weighings is ≤0.25%.

Total and Acid-Insoluble Ash Content

The total ash content is determined by incinerating 2 grams of the extract in a furnace at 600°C until a constant weight is reached. The acid-insoluble ash content is determined by boiling the ash from the incineration in dilute HCl, filtering the residue, and then incinerating it again (Kemenkes RI, 2022; Rachma & Saptawati, 2022)

Heavy Metal Contamination

Qualitative tests for Pb, Cd and Hg were carried out on samples obtained by wet digestion (concentrated HNO₃) using colour reagents specific to each metal. Quantitative

analyses for Pb and Cd were carried out using an atomic absorption spectrophotometer at wavelengths of 217 nm (Pb) and 228.8 nm (Cd), with calibration curves derived from a series of standard solutions and chemically digested samples (Syarif et al., 2022)

Microbial Contamination

The Total Plate Count (TPC) is determined using the serial dilution method (10^{-1} to 10^{-5}) on PCA medium, incubated at 37°C for 24-48 hours. The Yeast and Mould Count (YMC) is determined by serial dilution (10^{-1} to 10^{-3}) on PDA medium, incubated at 25°C for 5-7 days. The number of colonies is counted and multiplied by the dilution factor.

In vivo toxicity testing (Sargent et al., 2010)(BPOM RI, 2022)

Grouping of Test Animals

The grouping of test animals in the acute toxicity test was carried out in accordance with BPOM 2022 guidelines. The test animals were divided into six groups (five dose groups plus one control group), each comprising five female mice in accordance with Federer's formula. The highest dose was 15 g/kg body weight; the control group was administered 0.5% sodium CMC. The mice were housed in polycarbonate cages (5 per cage) lined with wood shavings, individually marked, and cage hygiene was maintained (litter changed 2–3 times per week). Each test animal in the cages was individually marked to facilitate observation, data collection, and to minimise errors in the research and data collection. The allocation of test animals for the acute toxicity test and the corresponding dose calculations are shown in Table 1.

Table 1. Acute Toxicity Test Dose Calculation

Group	Equivalent dose in mice	
	Dose (mg/20 g BW)	Equivalent Dose (mg/kg BW)
Dose 1	9	312,5
Dose II	18	625
Dose III	36	1,250
Dose IV	71	2,500
Dose V	143	5,000

Dose calculations for experimental animals were converted from humans to rats and from rats to mice. (BPOM RI, 2022) Mice were fasted for 12-16 hours (water was still provided), then administered the test sample orally in a 0.5% sodium carCageymethylcellulose (CMC-Na) suspension (0.5 ml/20 g body weight) using a blunt feeding tube. Food was reintroduced 1-2 hours after treatment. Toxicity observations were carried out at 30, 60 and 120 minutes, on day 1, and continued daily until day 14.

Weighing and Body Weight Monitoring

The mice were fasted, weighed, and then administered a single oral dose of the test sample via a gastric tube. Food was reintroduced 1-2 hours after administration. Body weight was recorded at the start of the study and monitored daily for 14 days.

Observation of Toxic Symptoms

This was carried out at 30, 60 and 120 minutes, then at 24 hours, and continued daily for 14 days. Symptoms observed included motor activity, central and autonomic nervous system function, digestive function, and changes to the skin and fur.

Mortality Observation

Mice were observed for 14 days to record mortality. If an animal died before rigor mortis set in, a post-mortem examination was carried out to examine for organ abnormalities.

Dissection and Macroscopic Examination of Organs

Carried out on mice that had died during the observation period or after day 14. Following neck dislocation and a 16-18-hour fast, the animals were dissected and the organs (liver, kidneys, spleen, lungs, heart) were removed, cleaned, weighed and examined macroscopically.

The extraction results using the percolation method yielded a recovery rate of 12.47 per cent. This value meets the quality requirements of the Indonesian Herbal Pharmacopoeia (FHI), 2nd edition (2022), namely not less than 10% (>10%). A yield that meets this standard indicates optimal efficiency in the extraction of compounds, meaning the extract can proceed to testing for specific and non-specific parameters.

Specific Parameters of the Extract.

The testing of specific parameters aims to determine the identity, organoleptic characteristics, and solubility profile of the compounds contained in the benger leaf extract (*Lagerstroemia ovalifolia* Teijsm. & Binn.), consisting of three tests: extract identity, organoleptic evaluation, and solubility in water and ethanol. The results of the evaluation are presented in Table 2.

RESULT AND DISCUSSION

Extract yield

Tabel 1. Extract yield.

Extraction Method	Simplicia (g)	Amount of solvent (g)	Percolation extract	Rendemen (%)
Percolation	150 gram	1500 mL	18,7 gram	12,47 %

The extraction results using the percolation method yielded a recovery rate of 12.47%, indicating efficient recovery of extractable compounds from the sample. The extract was subsequently subjected to testing for specific and non-specific parameters.

Specific Parameters of the Extract

The testing of specific parameters aimed to determine the identity, organoleptic characteristics and solubility profile of the compounds in the benger leaf extract (*Lagerstroemia ovalifolia* Teijsm. & Binn.). The results of the evaluation are presented in Table 2.

Table 2. Test Results for Specific Parameters of Benger Leaf Extract

Parameters	Results	General Requirement (FHI, 2022)
Identity	<i>L. ovalifolia</i> Teijsm. & Binn. (Leaves)	Suitable
Organoleptic	Thick, blackish-green, with a distinctive smell and a bitter taste	-
Water Solubility	7%	≤ 100%
Ethanol Solubility	12%	≤ 100%

The concentrated extract of benger leaves showed a higher concentration of ethanol-soluble compounds (12%) than water-soluble compounds (7%). This indicates that the polarity of 70% ethanol is effective in extracting secondary metabolites, as its nature as

a semi-polar/universal solvent with a non-polar ethyl group attracting non-polar compounds (steroids, terpenoids), whilst the polar hydroxyl group attracts polar compounds (flavonoids, alkaloids, saponins, tannins). The values for these two parameters comply with the FHI 2022 standard requirements.

Phytochemical Screening

Table 3. Results of Phytochemical Screening of Leaf Extract.

Parameter	Reagent	Requirements (FHI, 2022)
Alkaloid	Mayer	Negative (-)
	Wagner	Positif (+)
	Dragendorff	Positif (+)
Flavonoid	Mg + HCl + Amilalkohol	Positif (+)
Tanin	FeCl ₃ 1%	Positif (+)
Terpenoid	Kloroform + H ₂ SO ₄	Positif (+)
Phenol	FeCl ₃ 5%	Positif (+)
Saponin	Aquades Panas	Positif (+)

The results of the analysis showed that the extract tested positive for flavonoids, tannins, terpenoids, phenols, saponins and alkaloids. In the alkaloid test, Mayer's reagent gave a negative result, whilst Wagner's and Dragendorff's reagents gave positive results. This difference in sensitivity is influenced by the specific structural characteristics of the alkaloids; consequently, the presence of alkaloids was still confirmed as positive as the minimum requirement of at least two positive reagents was met.

Tabel 4. Putative identification of the main compounds detected in the extract of *Lagerstroemia ovalifolia* leaves and their reported pharmacological activities based on the UPLC base peak m/z data

No	RT (min)	Base peak m/z	Prediction of compounds and pharmacological benefits	Pharmacological Benefits	Refs.
1	3.70	307.20	Putative phenolic compound (unidentified)	Antioxidant	-
2	3.91	617.27	2-Linoleoyl-3-oleoyl-sn-glycerol, C ₃₉ H ₇₀ O ₅	Antioxidant	Present study (LC-HRMS)
3	4.22	511.240	Caffeoyl tartaric acid derivative, C ₂₄ H ₁₆ O ₁₃	Antioxidant, anti-inflammatory, neuroprotection	(Kim et al., 2020)
4	4.55	301.080	Ellagic acid, C ₁₄ H ₆ O ₈ , or Quercetin, C ₁₅ H ₁₀ O ₇	Potent antioxidant, anti-inflammatory, and cardioprotective	(Li et al., 2024) Present study (LC-HRMS)
5	4.83	395.100	3-O-methylellagic acid 4'-sulfate, C ₁₅ H ₈ O ₁₁ S	Antioxidant, antidiabetic, cognitive enhancer	-
6	5.07	433.19	Quercetin-3-O-β-L-xyloside, C ₂₀ H ₁₈ O ₁₁	Antioxidant, anti-inflammatory	(Kim et al., 2020)
7	5.28	447.24	Quercetin 3-O-β-D-rhamnoside, C ₂₁ H ₂₀ O ₁₁ or 3'-O-methyl-4-O-β-D-xylopyranosyl ellagic acid, C ₂₀ H ₁₆ O ₁₂	Anti-inflammatory, antioxidant	(Li et al., 2024)(Kim et al., 2020)
8	5.78	431.23	Kaempferol-3-O-α-L-rhamnoside, C ₂₁ H ₂₀ O ₁₀	Anti-inflammatory, antioxidant	(Kim et al., 2020)

From a phytochemical perspective, Benger leaf LOE displays a characteristic metabolite profile enriched in polyphenolic compounds, predominantly quercetin-type

flavonoids, alongside diverse phenolic acid and quinic acid derivatives. This compositional pattern aligns with previous findings for *Lagerstroemia* species, including *L. speciosa* and *L. indica*, which have likewise been reported to contain quercetin and its glycosides, luteolin, as well as ellagitannins and gallotannins as principal constituents. (Sai Saraswathi et al., 2017) The detection of these compounds in Bengel leaf extract suggests that *L. ovalifolia* exhibits a phytochemical profile consistent with that typically reported for the genus *Lagerstroemia*, characterized by a composite presence of flavonoids and phenolic acids. These classes of metabolites are known to contribute substantially to antioxidant and anti-inflammatory activities.

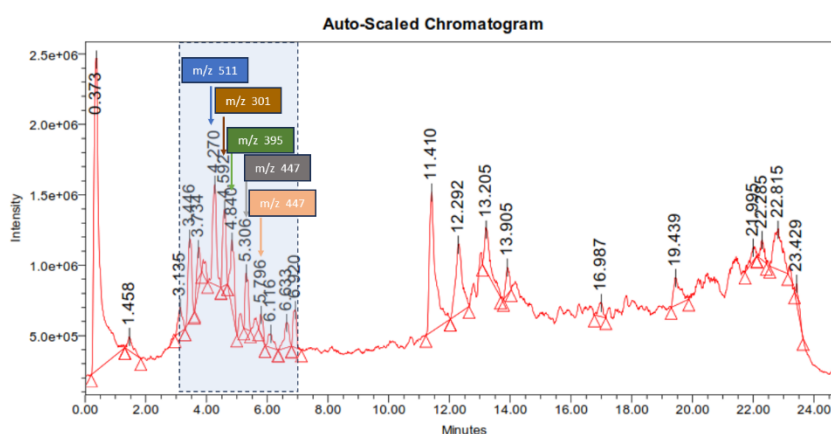


Figure. 2. UPLC chromatogram of the percolation extract of benger leaves (*Lagerstroemia ovalifolia* Teijsm. & Binn.), showing major peaks with corresponding retention times (minutes). The highlighted region (RT 3–7 min) indicates peaks tentatively identified as flavonoid compounds based on their m/z values

Non-Specific Parameters

Tests for non-specific parameters were carried out to determine the level of cleanliness, purity and stability of the extract (Table 5).

Table 5. Test Results for Non-Specific Parameters of the Extract of Bengel Leaves

Parameter	Result	Requirement (FHI, 2022)	Description
Drying Shrinkage	11,6%	≤ 10%	not Compliant
Specific Gravity	0,886 g/mL	-	-
Moisture content	8%	≤ 10%	Compliant
Total Ash Content	4,74%	≤ 16,6%	Compliant
Acid-Insoluble Ash Content	0,03%	≤ 2,30%	Compliant
Solvent Residue	0,98 g/mL	≤ 1	Compliant

Most of the non-specific parameters met the FHI (2022) standards. The low values for total ash content (4.74 per cent) and acid-insoluble ash (0.03 per cent) indicate minimal contamination from external inorganic minerals (sand/soil). The residual solvent value (0.98 g/mL) also indicates adequate evaporation efficiency.

However, the drying loss (11.6 per cent) exceeded the threshold (≤ 10 per cent). This high value is influenced by the hygroscopic nature of the extract during storage, as well as residual solvent and water that did not evaporate completely during the evaporation process. This needs to be optimised to prevent the degradation of active compounds and microbial growth during long-term storage

Heavy Metal and Microbial Contamination

Safety testing was carried out to ensure that the extract was free from harmful contaminants (Table 7 and table 8).

Table 7. Results of Heavy Metal

Parameters	Result	Requirement (BPOM RI, 2019)	Description
Lead (Pb)	7,75 mg/kg	≤ 10 mg/kg	Compliant
Cadmium (Cd)	Not detected	≤ 0,3 mg/kg	Compliant

Table 8. Results of Microbial Contamination Testing

Parameters	Result	Requirement (PERKA BPOM No. 32/2019)	Description
ALT	0 colony /g	Raw material categories (BPOM No. 32/2019)	Compliant
AKK	0 colony/g		Compliant

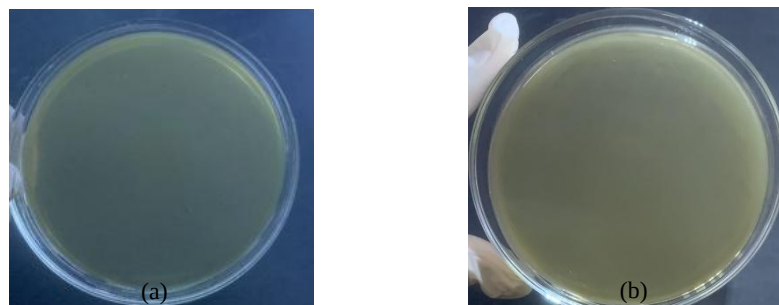


Figure 1. Appearance of the test plates for microbial contamination testing of the percolation extract of benger leaves (*Lagerstroemia ovalifolia* Teijsm. & Binn.): (a) Total Plate Count (ALT) test; (b) Yeast and Mould Count (AKK) test.

Heavy Metals: The extract has been found to be free from contamination by the toxic metals Pb and Cd, as the levels are below the maximum limits set by the Indonesian Food and Drug Authority (BPOM RI, 2019). Microbial Contamination: The Total Plate Count (TPC) and Yeast and Mould Count (YMC) are subject to specific quality standards for the category of herbal raw material extracts/simplicia, as set out in BPOM Regulation No. 32 of 2019.

Results of in vivo tests on female ddY strain mice

During the 14-day observation period, feed intake was calculated as the difference between the amount of feed provided and the amount remaining.

Table 8. Feed Consumption by Test Animals (g)

Cage	Day													
	Feed Consumption (g)													
I	29.40	69.20	78.02	76.84	76.53	90.08	98.15	91.24	80.22	47.41	64.70	74.52	88.76	89.83
II	22.59	55.10	56.27	56.23	53.82	75.18	59.70	77.77	65.50	13.30	26.36	72.74	82.54	67.84
III	37.02	43.70	87.93	83.04	72.82	70.82	68.96	95.00	85.96	33.96	70.89	60.92	63.72	93.44
IV	26.06	46.20	87.65	87.04	72.54	70.23	86.00	99.92	84.11	28.27	73.89	70.54	99.37	96.74
V	26.04	44.50	87.45	44.90	43.62	41.12	40.40	80.28	82.30	21.74	38.52	44.04	50.14	53.02
VI	11.36	20.40	45.50	42.53	25.18	26.18	40.64	45.84	62.93	26.57	43.98	58.94	78.31	75.32

The results show that the groups receiving the highest doses (groups V and VI) experienced a consistent decrease in feed consumption compared to the control group and the low-dose groups (I-IV), especially in the middle of the observation period (Days 4-9). This indicates a physiological impact of the test substance, considering that changes in feed consumption and body weight can be early indicators of toxic effects. Data from the low-dose group indicate that the administration of the test substance up to 1,250 mg/kgBW is still within safe limits in terms of feed consumption.

Body Weight Monitoring

No group showed a body weight reduction exceeding 10% of the initial value throughout the observation period, indicating no clinically significant toxic effect on growth despite the reduced feed intake observed in the high-dose groups.

Table 9. Mean Body Weight of Mice per Group (g).

Day	Cage I	Cage II	Cage III	Cage IV	Cage V	Cage VI
1	29.04	27.12	28.58	26.72	26.52	28.22
2	28.98	27.16	28.88	26.64	26.42	28.20
3	29.38	27.88	28.74	27.18	27.20	25.82
4	30.22	28.31	29.46	27.72	26.22	24.94
5	31.00	28.72	30.11	28.40	26.77	25.92
6	30.88	28.90	30.18	26.97	26.48	25.77
7	30.82	28.48	30.18	28.56	27.22	25.35
8	30.75	28.30	29.86	28.32	27.14	25.44
9	31.04	28.06	30.20	28.88	27.24	25.72
10	31.16	27.86	30.32	28.80	26.92	25.74
11	31.06	27.60	30.20	28.90	26.82	25.90
12	31.02	27.84	30.48	29.26	27.46	26.10
13	30.74	27.58	29.72	29.06	26.98	24.72
14	30.41	27.16	29.96	28.98	27.28	26.38

Throughout the trial, various clinical parameters indicative of toxic effects such as tremors, sedation, motor disturbances, convulsions, and responses from the autonomic and central nervous systems were monitored. During the observations, which were carried out three times a day until day 14, no toxicological clinical symptoms were found in mice from any of the treatment groups receiving the highest dose of 5,000 mg/kg body weight. Based on the observation parameters covering the central nervous system, the autonomic nervous system, respiratory function, digestion and macroscopic findings, all observation scores were zero (0). This indicates that oral administration of the test substance did not induce any clinically observable acute toxic responses, whether neurological, functional or physiological. The absence of symptoms such as sedation, tremor, mydriasis, diarrhoea or changes in skin colour indicates that the test substance did not exhibit any phenotypically evident acute toxic effects in the test animals at the maximum dose administered. (Ahmed et al., 2022)

Table 10. Summary of Clinical Toxic Sign Evaluation

Parameter	Result
Central nervous system (sedation, motor activity, convulsion, tremor)	Not observed
Autonomic nervous system (miosis, mydriasis, salivation, urination)	Not observed
Respiratory rate	Normal
Heart rate	Normal
Digestive system (diarrhea, constipation)	Not observed
Macroscopic findings (piloerection, skin color change)	Not observed

Mortality

Mice were monitored daily for mortality throughout the 14-day observation period across all treatment groups, including the highest dose (5,000 mg/kg body weight). No mortality was observed in any group during the study period, consistent with the absence of clinical toxic signs and supporting an estimated LD₅₀ greater than 5,000 mg/kg body weight.

Organ weight measurements

Measurements of the main organ weights were taken at the end of the observation period, covering the liver, kidneys, heart, lungs and spleen. The mean and standard deviation for each group are shown in the following table

Table 11. Mean Organ Weight of Mice (g)

Group	Liver ± SD	Kidney ± SD	Heart ± SD	Lung ± SD	Spleen ± SD
Dosage 1	1,360 ± 0,185	0,304 ± 0,036	0,144 ± 0,009	0,224 ± 0,084	0,212 ± 0,077
Dosage 2	1,468 ± 0,199	0,332 ± 0,033	0,136 ± 0,009	0,200 ± 0,024	0,204 ± 0,017
Dosage 3	1,648 ± 0,445	0,340 ± 0,060	0,136 ± 0,009	0,164 ± 0,026	0,172 ± 0,018
Dosage 4	1,384 ± 0,217	0,324 ± 0,033	0,132 ± 0,011	0,152 ± 0,023	0,176 ± 0,052
Dosage 5	1,392 ± 0,191	0,308 ± 0,039	0,112 ± 0,030	0,212 ± 0,073	0,192 ± 0,076

It can be concluded from the observations that there were no significant differences between the groups in terms of organ weight, although the highest-dose group showed a higher average heart weight and greater variation in spleen weight. Major metabolic organs such as the liver and kidneys remained within physiological limits, with no indication of pathological enlargement or shrinkage.

CONCLUSION

The percolation extract of benger leaves (*Lagerstroemia ovalifolia* Teijsm. & Binn.) meets most quality standards for specific and non-specific parameters, with a yield of 12.47%, moisture content of 8%, total ash content of 4.74%, solvent residue of 0.98 g/mL, and is free from toxic heavy metal contaminants (Pb and Cd). Phytochemical screening and UPLC profiling confirmed the presence of active secondary metabolites, including flavonoids, tannins, terpenoids, phenols, and saponins. However, two parameters did not meet the required standards: drying shrinkage (11.6%, exceeding the ≤10% limit) and yeast and mould count (0 colony/g, exceeding the <10 colony/g limit), indicating the need for further optimisation of the drying process and microbial control during extraction and storage.

The results of in vivo acute toxicity testing indicate that the extract is relatively safe, with an estimated LD₅₀ value of >5,000 mg/kg body weight. Doses up to 5,000 mg/kg body weight did not induce mortality, clinical signs of toxicity, or abnormal organ weights, although a reduction in feed intake was observed in the high-dose group. Overall, the benger leaf extract shows a promising safety and quality profile as a raw material for standardised herbal medicine, with drying and microbial parameters requiring further refinement before application in medicinal formulations.

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