JOURNAL OF ENVIRONMENTAL SCIENCES AND BUILT ENVIRONMENT RESEARCH (JESBER)
2026, Vol. 1, No. 2, pp. 57-69
Published by Faculty of Environmental Sciences, University of Calabar, Nigeria
Print ISSN: 3141-5547 | OPEN ACCESS

Research article

Toxicological Profile and Antinociceptive Activity of Ethanolic Leaf Extract of Eremomastax speciosa in Rodents: Environmental-Health and Sustainable-Use Implications

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ABSTRACT

Background: Eremomastax speciosa is used in African ethnomedicine, yet safety evidence to support medicinal-plant use remains limited. Objective: This study evaluated the phytochemical profile, acute and 21-day oral toxicity, and antinociceptive activities of an ethanolic leaf extract. Methods: Phytochemistry was undertaken. Acute toxicity was assessed with Lorke's procedure. Repeated-dose effects were examined through body and organ weights, haematology, hepatic and renal indices, and lipid profile. Antinociception was evaluated using acetic acid-induced writhing and the hot-plate test. Data were expressed as mean ± SEM and analysed using one-way ANOVA at $p < .05$. Results: Alkaloids, tannins, saponins, terpenoids, steroids and glycosides were detected. No mortality occurred at 5,000 mg/kg, indicating an oral LD50 above this dose. Repeated dosing produced no consistent adverse change in body weight, organ weight, hepatic, renal or lipid indices, although lower packed cell volume and white-cell counts warrant investigation. The extract reduced writhing dose-dependently, with recalculated inhibition of 70.4–93.8%, and increased hot-plate latency at 200 mg/kg. Conclusion: E. speciosa showed substantial antinociceptive activity with limited short-term biochemical toxicity but possible haematological safety signals. Contribution: The study integrates efficacy and safety evidence to guide environmental-health regulation, sustainable harvesting and future standardisation.

KEYWORDS

Eremomastax speciosa; acute toxicity; repeated-dose toxicity; antinociceptive activity; acetic acid-induced writhing; hot-plate test; environmental health; sustainable medicinal-plant use

Introduction

Medicinal plants remain central to health care, household therapeutics and culturally embedded knowledge systems in many low- and middle-income countries. Their continued importance is not simply a matter of therapeutic preference; it is also linked to affordability, accessibility, local biodiversity and the persistence of community-based systems for identifying, harvesting and preparing useful species. At the same time, the assumption that a plant preparation is safe because it is natural or has been used for generations is scientifically inadequate. Plant extracts are chemically complex, their composition may vary with species identity, soil conditions, season, plant age, harvesting pressure and extraction method, and their biological effects may include both desirable pharmacological actions and organ- or blood-related adverse effects. Contemporary natural-product research therefore requires efficacy and safety to be assessed together rather than as separate questions (Atanasov et al., 2021; Newman & Cragg, 2020).

This dual requirement has an environmental-health dimension that is particularly relevant to journals concerned with environmental science and sustainable development. Medicinal plants are biological resources situated within ecosystems and livelihood systems. Evidence of pharmacological value can increase demand, while weak taxonomic control, unregulated collection and unsupported safety claims can create risks for biodiversity, consumers and local economies. Nigerian environmental scholarship has similarly emphasised that sustainability depends on linking resource use with environmental management, livelihood resilience, spatial

context and institutional oversight (Eneyo, 2025a, 2025b, 2025c, 2026a, 2026b; Eneyo et al., 2026; Eneyo & Archibong, 2024; Eneyo et al., 2023; Eneyo & Ekpenyong, 2018). Research on soil biological processes in plantation systems also shows that biological resources and their functions are shaped by environmental conditions and depth-related ecological variation (Peter et al., 2026). These considerations support an approach to medicinal-plant research that combines pharmacological validation with safety surveillance, accurate identification and sustainable sourcing.

Eremomastax speciosa (Hochst.) Cufod. is an African member of the family Acanthaceae. The plant occurs in tropical forest zones and has been used in several communities for conditions that include pain, inflammatory complaints, bleeding, reproductive disorders, cough, oedema and metabolic disease. Such a broad ethnomedicinal profile suggests the presence of multiple bioactive constituents, but it also increases the probability that preparations will be used in different doses, for different durations and in people with different physiological vulnerabilities. Earlier investigations of African medicinal plants have demonstrated that alkaloids, tannins, saponins, terpenoids, steroids, glycosides and polyphenolic compounds may contribute to antinociceptive, anti-inflammatory, antimicrobial, antiplasmodial or metabolic effects, although the same classes can also produce toxicity depending on dose and exposure (Oguntibeju, 2018). Local work involving Spondias mombin, Dioscorea bulbifera, Cassia sieberiana and other plant materials further demonstrates the breadth of pharmacological activity that can be detected in rodent models (Akpan et al., 2026; Eke et al., 2025; Usanga et al., 2022). Eremomastax speciosa (Hochst.) Cufod. has gained attention as a medicinal plant due to its diverse pharmacological properties, including antioxidant, anti-inflammatory, analgesic, and antimicrobial activities. Previous investigations have demonstrated that leaf extracts of Eremomastax speciosa contain bioactive phytochemicals capable of reducing oxidative stress and inflammatory responses, which are important mechanisms involved in pain perception and tissue injury.

Anaga et al. (2017) reported that hydro-methanolic leaf extract of Eremomastax speciosa exhibited significant antioxidant, anti-inflammatory, and antinociceptive activities in experimental models, suggesting its potential role in pain management through modulation of inflammatory pathways and free radical scavenging. The safety profile of this plant has also been investigated, with Siwe et al. (2015) demonstrating that aqueous leaf extract of Eremomastax speciosa showed relatively low toxicity during acute and subacute toxicity evaluations in Wistar rats, supporting its traditional medicinal use and the need for further pharmacological exploration. Earlier pharmacological studies by Noamesi et al. (1996) indicated that Eremomastax speciosa leaf preparations possess protective biological effects in animal models, highlighting its therapeutic relevance in ethnomedicine. Furthermore, Okokon et al. (2007) reported additional biological activities of Eremomastax speciosa, including antimicrobial and haematological effects, emphasizing the broad medicinal potential of this species. From a broader perspective, Heinrich et al. (2018) highlighted that systematic evaluation of medicinal plants through pharmacognostic characterization, toxicity assessment, and pharmacological validation is essential for sustainable utilization and development of plant-derived therapeutic agents. Collectively, these studies provide scientific support for investigating the toxicological profile and antinociceptive potential of ethanolic leaf extract of Eremomastax speciosa in rodent models while emphasizing its importance in sustainable bioprospecting and natural product-based drug discovery.

Pain is a protective sensory and emotional experience associated with actual or potential tissue injury. Experimental antinociceptive assays do not reproduce the full human experience of pain, but they allow different components of nociceptive processing to be examined under controlled conditions. Acetic acid-induced writhing is sensitive to compounds that reduce peripheral inflammatory and visceral nociceptive signalling, whereas the hot-plate test assesses response latency to a thermal stimulus and is commonly used as an indicator of centrally integrated antinociceptive activity (Le Bars et al., 2001; Muhammad et al., 2012). A plant extract that is active in both models may act at more than one level of the nociceptive pathway; however, behavioural activity alone does not establish a clinical analgesic effect, identify the active constituent or demonstrate long-term safety.

The present study was designed to evaluate the ethanolic leaf extract of E. speciosa through a combined toxicological and antinociceptive framework. The specific objectives were to identify major classes of phytochemicals; estimate acute oral toxicity; examine body weight, organ weight, haematological indices, liver and kidney markers and lipid profile after repeated exposure; and evaluate activity in acetic acid-induced writhing and hot-plate models. The study contributes by placing efficacy results alongside safety indicators and by interpreting both within an environmental-health and sustainable-use perspective. Throughout this manuscript, the term antinociceptive is preferred to analgesic because the evidence is derived from animal behavioural models and has not been confirmed clinically.

Literature review

The scientific value of medicinal plants lies partly in their chemical diversity. Natural products have supplied or inspired numerous therapeutic agents, and modern analytical methods continue to reveal compounds with pharmacological potential (Dias et al., 2012; Newman & Cragg, 2020). Nevertheless, the pathway from traditional use to a standardised medicine is demanding. Botanical authentication, extraction reproducibility, chemical characterisation, dose selection, experimental quality and toxicity assessment all influence whether an observed effect is credible and translatable. Studies that report only activity without considering safety can promote premature therapeutic claims, while studies that report only a high acute LD50 may overlook cumulative, haematological, reproductive or organ-specific effects. The strongest preclinical evidence therefore combines multiple endpoints and clearly distinguishes absence of observed harm from proof of safety.

Qualitative phytochemical screening remains useful as an initial step because it identifies broad metabolite classes that can guide subsequent fractionation. Alkaloids can interact with receptors, ion channels and enzymes; tannins and other polyphenols can modulate oxidative and inflammatory pathways; saponins and terpenoids may influence membrane, immune and eicosanoid-related processes; and glycosides can have diverse systemic effects. Yet qualitative tests do not quantify exposure and cannot establish which constituent produced a behavioural response. Comparative phytochemical work on Nigerian plant materials has shown that extraction solvent and plant part strongly influence the profile detected (Thomas et al., 2025). Accordingly, the present screening is interpreted as a chemical signal map rather than a definitive explanation of mechanism.

Toxicological evaluation is equally dependent on the design and duration of exposure. Acute toxicity procedures estimate the dose range associated with overt toxicity or mortality, but they are not substitutes for repeated-dose studies. The Lorke procedure has been widely applied in African pharmacology because it uses sequential dosing to approximate the median lethal dose while reducing animal numbers (Lorke, 1983). More recent reporting standards emphasise detailed descriptions of randomisation, animal characteristics, housing, sample-size rationale, outcome measures and statistical methods so that findings can be assessed and reproduced (Percie du Sert et al., 2020). Repeated-dose guidance similarly recommends evaluation of clinical observations, body weight, haematology, clinical chemistry and organ pathology because target-organ effects may emerge without mortality (Organisation for Economic Co-operation and Development [OECD], 2008).

Haematological indices deserve particular attention because the blood is exposed to absorbed constituents and reflects both production and destruction of circulating cells. Packed cell volume provides an estimate of the proportion of erythrocytes, while total and differential white-cell counts reflect immune and inflammatory status. Reductions may indicate haemolysis, impaired haematopoiesis, immune suppression or simple biological variability; confirmation requires complete blood counts, reticulocytes, marrow or spleen findings and adequately powered comparisons. Earlier clinical work on haematological parameters in Nigerians with diabetes demonstrates that blood indices are sensitive to physiological and disease-related context and should not be interpreted in isolation (Akuodor et al., 2006). This is important for E. speciosa because a preparation used repeatedly could affect vulnerable users even when acute mortality is absent.

The acetic acid writhing model is a high-sensitivity screening test in which intraperitoneal acid produces abdominal constrictions through local irritation and the release of inflammatory mediators. A reduction in

writhes suggests peripheral antinociceptive or anti-inflammatory activity, but sedative, myorelaxant or motor-impairing effects can also reduce behaviour and should be considered. The hot-plate model complements this assay by measuring latency to licking, hind-paw withdrawal or jumping after exposure to a controlled thermal surface. Increased latency suggests modulation of centrally integrated nociceptive pathways, although the test remains influenced by motor function, arousal and baseline latency. Agreement across models strengthens the evidence, whereas different patterns may indicate distinct mechanisms or pharmacokinetics. Studies of Viola betonicifolia, Salacia lehmbachii and other plant extracts have used these complementary assays to identify dose- and time-dependent antinociceptive activity (Akuodor et al., 2021; Muhammad et al., 2012).

Methodology

3.1 Study design and reporting approach. The study used controlled laboratory experiments to characterise the phytochemical profile, acute oral toxicity, repeated-dose toxicological effects and antinociceptive activity of an ethanolic leaf extract of E. speciosa. The analyzable datasets comprised an acute toxicity experiment in 13 mice, a 21-day repeated-dose experiment represented by four groups of five Wistar rats, an acetic acid-induced writhing experiment in five groups of five mice, and a hot-plate experiment in five groups of five rodents. The manuscript was reconstructed from the original laboratory tables and narrative records. Where the narrative and tables differed, the numerical tables were treated as the primary record because they contained the group labels, doses and outcome values used in the analyses. This decision is reported to preserve internal consistency and should be checked against the original laboratory notebooks before final submission.

3.2 Plant material, identification and extraction. Fresh leaves identified as E. speciosa were obtained from Watt Market, Calabar South, Cross River State, Nigeria, and were taken to the herbarium of the Department of Plant and Ecological Studies, University of Calabar, for botanical identification by a qualified taxonomist. A voucher number was not available in the source records and should be inserted before publication. The leaves were gently washed and air-dried at room temperature in the Department of Pharmacology laboratory. Dried material was milled to a coarse powder. The powder was first defatted in petroleum ether for 48 hours, after which the plant residue was macerated in 2,000 mL of ethanol for 48 hours. The mixture was filtered through Whatman No. 1 filter paper, concentrated to dryness and stored in a tightly closed sample container at approximately 4 °C until use. Extraction yield was not recorded; future studies should report the mass of starting powder, mass of dry extract and percentage yield.

3.3 Experimental animals and husbandry. Wistar rats and mice were obtained from the Animal House of the Department of Pharmacology, University of Calabar. Animals were housed in standard cages, acclimatised for seven days and provided standard feed and water ad libitum. The source records indicated that animals had not previously been used in experiments. Environmental conditions were described as standard, although exact temperature, humidity and light-dark cycle were not retained in the available records. In future replication, these conditions should be measured and reported, and sex should be specified for each experiment because sex-related differences can influence toxicity and nociceptive behaviour. Animals were allocated to groups before treatment. The original record did not document the method of random sequence generation or whether outcome assessors were blinded; these procedures are recommended under ARRIVE 2.0 to reduce selection and measurement bias (Percie du Sert et al., 2020).

3.4 Qualitative phytochemical screening. The ethanolic extract was screened using standard colour and precipitation reactions for alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, anthraquinones, glycosides and reducing sugars, following the general procedures described by Aziz (2015). Each test included the appropriate reagent blank where applicable. Results were recorded as present or absent. The screening was qualitative; therefore, no inference was made about concentration, purity or relative abundance. For reproducibility, subsequent work should include quantitative total phenolic and flavonoid contents, chromatographic fingerprinting and, where feasible, liquid chromatography-mass spectrometry of marker compounds.

3.5 Acute oral toxicity. Acute toxicity was evaluated with a two-phase Lorke procedure (Lorke, 1983). Thirteen fasted mice of both sexes were used. In phase one, nine mice were assigned to three groups of three and received 10, 100 or 1,000 mg/kg extract orally. Animals were observed during the first four hours and over 24 hours for behavioural and clinical signs including paw licking, weakness, somnolence, respiratory distress, hyperactivity, coma and death. Because no mortality occurred, phase two used one mouse per dose at 1,600, 2,900 and 5,000 mg/kg, with a vehicle control receiving distilled water. Animals were observed for 48 hours and subsequently for delayed signs. The highest tested dose that produced no mortality was used to define the lower boundary of the LD50; an exact LD50 was not calculated because no lethal dose was observed.

3.6 Twenty-one-day repeated-dose assessment. The analyzable repeated-dose dataset contained four groups of five male Wistar rats. Group 1 received normal saline, while Groups 2-4 received 50, 100 or 200 mg/kg extract once daily by oral gavage for 21 days. Body weights were recorded on Days 1, 7, 14 and 21. At the end of exposure, animals were anaesthetised and blood was collected by cardiac puncture for haematological and serum biochemical analyses. The heart, lungs, kidneys, liver, spleen and testes were removed and weighed. The original tables reported absolute organ weights rather than organ-to-body-weight ratios. Consequently, the findings are described as organ-weight observations and not as relative-organ-weight effects. Histopathological examination was not included in the available dataset, which limits attribution of numerical organ-weight differences to tissue injury.

3.7 Haematological, hepatic, renal and lipid measurements. Packed cell volume, total white blood cell count, neutrophils, lymphocytes, monocytes and eosinophils were measured using an automated haematology analyser. Serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were measured as hepatic indices. Urea, creatinine, sodium, potassium, bicarbonate and chloride were measured as renal and electrolyte indices. Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol and triglycerides were also determined. Units in the original records were harmonised to conventional reporting units where the numerical ranges supported correction: enzymes are reported as U/L, electrolytes and lipids as mmol/L, and creatinine as µmol/L. These unit corrections should be verified against the analyser printouts before submission.

3.8 Acetic acid-induced writhing. Twenty-five mice weighing approximately 25-30 g were allocated to five groups (n = 5). The vehicle group received normal saline, the reference group received indomethacin 10 mg/kg, and the treatment groups received 50, 100 or 200 mg/kg extract orally. Thirty minutes later, 0.7% acetic acid was administered intraperitoneally to induce abdominal constrictions. Writhes were counted during the observation period. Percentage inhibition was recalculated from the group means using: inhibition (%) = [(Wc - Wt)/Wc] × 100, where Wc is the mean number of writhes in the vehicle group and Wt is the mean in the treated group. Recalculation was necessary because several percentages in the original table did not correspond exactly with the reported group means.

3.9 Hot-plate test. Twenty-five rodents were allocated to five groups (n = 5) after pre-screening on a hot plate maintained at 55 ± 0.1 °C. Animals with a pre-treatment latency above 15 seconds were excluded. The groups received normal saline, indomethacin 10 mg/kg, or E. speciosa extract at 50, 100 or 200 mg/kg orally. Thirty minutes after administration, each animal was placed on the heated surface and the latency to hind-paw licking, withdrawal, flicking or jumping was recorded. A 30-second cut-off was applied to minimise tissue injury. Latencies were recorded at baseline and at 30, 60, 90 and 120 minutes. Because only group means and SEMs were available, the repeated-measures covariance structure could not be reconstructed; interpretation therefore focuses on the time-response pattern and the inferential annotations retained in the original laboratory output.

3.10 Statistical analysis and data integrity. Results are presented as mean ± standard error of the mean (SEM). The original analysis used one-way analysis of variance in SPSS, with statistical significance defined at $p < .05$. The available records did not identify the post hoc test for the first manuscript or provide exact p values, confidence intervals, effect sizes or raw observations. No additional inferential analysis was performed from

summary statistics alone. Instead, descriptive percentage changes and recalculated writhing inhibition were used to strengthen interpretation. This approach prevents false precision but also means that several numerical trends cannot be classified confidently as treatment effects. A future confirmatory study should pre-specify a primary outcome, justify sample size, use randomisation and blinding, retain animal-level data and apply a mixed-effects model for repeated hot-plate measurements.

Table 1. Experimental design, exposure and primary endpoints

Component	Species/sample	Groups and doses	Duration/timing	Primary endpoints
Acute oral toxicity	13 mice	10, 100, 1,000 mg/kg; then 1,600, 2,900 and 5,000 mg/kg; vehicle	Observation to 72 h and delayed follow-up	Clinical signs and mortality; LD50 boundary
Repeated-dose toxicity	20 male Wistar rats (4 groups; n = 5)	Saline; extract 50, 100 or 200 mg/kg orally	Once daily for 21 days	Body and organ weights; haematology; liver, kidney and lipid indices
Acetic acid writhing	25 mice (5 groups; n = 5)	Saline; indomethacin 10 mg/kg; extract 50, 100 or 200 mg/kg	Acetic acid 30 min after treatment	Number of writhes and inhibition (%)
Hot-plate test	25 rodents (5 groups; n = 5)	Saline; indomethacin 10 mg/kg; extract 50, 100 or 200 mg/kg	Latency at 0, 30, 60, 90 and 120 min	Thermal response latency (30-s cut-off)

Note. *Doses and sample sizes are reported from the analyzable numerical records. Narrative inconsistencies should be checked against original laboratory notebooks.*

Table 2. Qualitative phytochemical profile and acute oral toxicity findings

Domain	Test/constituent	Finding	Analytical interpretation
Phytochemistry	Alkaloids	Present	Potential receptor, ion-channel or enzyme activity; requires compound-level confirmation
Phytochemistry	Tannins	Present	Possible antioxidant, mediator-modulating and protein-binding effects
Phytochemistry	Saponins	Present	Possible membrane and inflammatory-pathway effects
Phytochemistry	Terpenoids	Present	Potential anti-inflammatory and neuromodulatory activity
Phytochemistry	Steroids	Present	Broad structural class; biological relevance requires identification
Phytochemistry	Glycosides	Present	Possible systemic activity; aglycone and sugar moieties not identified
Phytochemistry	Flavonoids	Not detected	Qualitative result; chromatographic confirmation required
Phytochemistry	Reducing sugars	Not detected	Qualitative result
Acute toxicity	Clinical signs/mortality up to 5,000 mg/kg	None observed	Oral LD50 > 5,000 mg/kg under test conditions; does not establish long-term safety

Table 3. Body-weight trajectory and absolute organ weights after 21 days

Panel A: Body weight (g), mean ± SEM				
Treatment	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Normal saline (20 mL/kg)	143.43 ± 3.08	144.96 ± 20.47	140.28 ± 13.18	144.35 ± 14.30
E. speciosa 50 mg/kg	184.75 ± 13.15	186.43 ± 16.42	180.40 ± 14.20	174.83 ± 7.93
E. speciosa 100 mg/kg	175.50 ± 17.70	172.67 ± 11.67	171.43 ± 10.23	184.23 ± 9.03
E. speciosa 200 mg/kg	164.48 ± 46.40	163.03 ± 50.98	174.73 ± 48.70	162.33 ± 27.30
Panel B: Absolute organ weight (g), mean ± SEM				
Treatment	**Heart**	**Lungs**	**Kidneys**	**Liver**
Normal saline	0.23 ± 0.11	0.50 ± 0.13	0.48 ± 0.06	4.40 ± 0.20
E. speciosa 50 mg/kg	0.30 ± 0.22	0.65 ± 0.15	0.45 ± 0.03	3.80 ± 0.16
E. speciosa 100 mg/kg	0.34 ± 0.02	0.60 ± 0.08	0.44 ± 0.07	3.50 ± 0.24

E. speciosa 200 mg/kg	0.33 ± 0.06	0.63 ± 0.18	0.45 ± 0.06	3.55 ± 0.20
Panel C: Additional absolute organ weights (g), mean ± SEM				
Treatment	**Spleen**	**Testes**		
Normal saline	0.42 ± 0.07	1.36 ± 0.30		
E. speciosa 50 mg/kg	0.48 ± 0.04	1.30 ± 0.10		
E. speciosa 100 mg/kg	0.45 ± 0.03	1.40 ± 0.06		
E. speciosa 200 mg/kg	0.42 ± 0.03	1.35 ± 0.05		

Note. *Absolute organ weights are reported because terminal body weights required for relative-organ-weight calculations were unavailable.*

Table 4. Haematological, hepatic, renal and lipid indices after 21 days

Parameter	Normal saline	50 mg/kg	100 mg/kg	200 mg/kg
PCV (%)	55.20 ± 2.10	42.36 ± 12.20	45.28 ± 6.10	45.56 ± 2.60
WBC (×10⁹/L)	7.80 ± 1.90	5.00 ± 4.90	6.10 ± 3.80	3.50 ± 1.80
Neutrophils (%)	35.10 ± 8.70	33.45 ± 11.20	33.45 ± 11.20	32.48 ± 16.00
Lymphocytes (%)	60.11 ± 13.60	63.51 ± 14.50	68.10 ± 19.00*	65.70 ± 6.30
Monocytes (%)	2.45 ± 0.50	1.67 ± 0.02	1.28 ± 0.01	1.22 ± 0.05
Eosinophils (%)	3.50 ± 1.00	3.54 ± 0.50	1.67 ± 0.50	2.10 ± 0.20
AST (U/L)	33.20 ± 2.38	32.15 ± 1.50	33.11 ± 2.35	30.14 ± 2.33
ALT (U/L)	20.40 ± 1.35	18.42 ± 2.10	22.40 ± 2.40	23.28 ± 2.62
ALP (U/L)	45.18 ± 2.55	50.20 ± 1.61	52.09 ± 2.32	51.13 ± 1.56
Urea (mmol/L)	6.20 ± 3.00	6.40 ± 1.20	6.00 ± 3.20	6.40 ± 1.70
Sodium (mmol/L)	136 ± 8.50	136 ± 4.70	134 ± 1.80	136 ± 12.50
Potassium (mmol/L)	3.40 ± 0.50	3.50 ± 2.10	3.30 ± 0.20	3.40 ± 0.70
Bicarbonate (mmol/L)	22.30 ± 0.91	21.32 ± 8.10	22.25 ± 1.70	21.35 ± 1.90
Chloride (mmol/L)	102 ± 11.80	100 ± 7.50	98 ± 9.30	96 ± 11.60
Creatinine (µmol/L)	132 ± 7.80	136 ± 11.30	135 ± 11.45	132 ± 12.90
Total cholesterol (mmol/L)	1.70 ± 1.00	1.60 ± 0.15	2.00 ± 0.02	1.50 ± 0.03
HDL (mmol/L)	0.40 ± 0.02	0.40 ± 0.03	0.60 ± 0.02	0.40 ± 0.01
LDL (mmol/L)	1.10 ± 0.04	1.00 ± 0.05	1.10 ± 0.03	0.90 ± 0.08
VLDL (mmol/L)	0.20 ± 0.01	0.20 ± 0.02	0.30 ± 0.02	0.20 ± 0.02
Triglyceride (mmol/L)	0.50 ± 0.71	0.40 ± 0.02	0.60 ± 0.01	0.50 ± 0.03

Note. *Values are mean ± SEM (n = 5). *Marked significant in the original table at p < .05. Conventional units were harmonised from the source record and should be verified against analyser printouts.*

Table 5. Antinociceptive outcomes in the acetic acid-induced writhing and hot-plate tests

Panel A: Acetic acid-induced writhing						
Treatment	**Dose**	**Mean writhes ± SEM**	**Inhibition reported (%)**	**Inhibition recalculated (%)**		
Normal saline	20 mL/kg	96.67 ± 3.87	–	–		
Indomethacin	10 mg/kg	3.40 ± 1.91	91	96.5		
E. speciosa	50 mg/kg	28.63 ± 2.71	75	70.4		
E. speciosa	100 mg/kg	12.32 ± 1.82	85	87.3		
E. speciosa	200 mg/kg	5.96 ± 3.80	90	93.8		
Panel B: Hot-plate latency (s), mean ± SEM						
Treatment	**Dose**	**0 min**	**30 min**	**60 min**	**90 min**	**120 min**
Normal saline	20 mL/kg	10.17 ± 0.45	10.00 ± 0.58	10.67 ± 0.33	10.55 ± 0.22	10.50 ± 0.20
Indomethacin	10 mg/kg	7.50 ± 0.62	17.50 ± 0.72	19.17 ± 0.60	21.17 ± 0.87	23.33 ± 0.95
E. speciosa	50 mg/kg	6.50 ± 0.43	8.50 ± 0.50	10.83 ± 0.83	11.67 ± 0.71	13.17 ± 0.60
E. speciosa	100 mg/kg	7.50 ± 0.76	9.33 ± 0.76	11.83 ± 0.70	13.50 ± 0.56	16.80 ± 0.68
E. speciosa	200 mg/kg	8.00 ± 0.52	10.17 ± 0.79	12.50 ± 0.88	14.67 ± 0.56	17.50 ± 0.34

Note. *Recalculated inhibition = [(vehicle mean − treated mean)/vehicle mean] × 100. The original table marked treatment effects as significant at p < .05 but did not provide exact p values.*

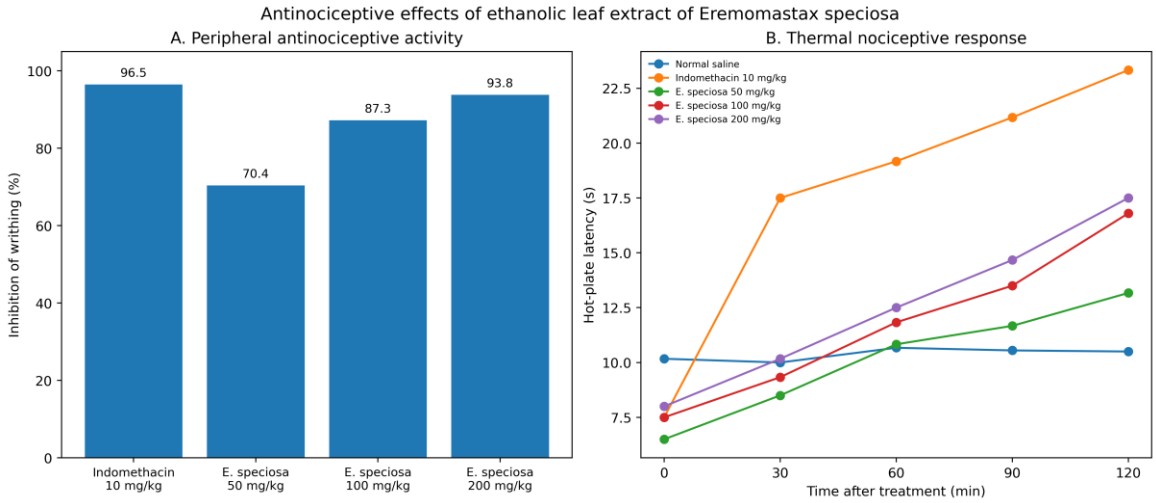

Figure 1. Antinociceptive activity of the ethanolic leaf extract of Eremomastax speciosa. Panel A shows writhing inhibition recalculated from reported group means. Panel B shows mean hot-plate latency over time. Values are derived from the original group summaries; error bars for Panel A were not recalculated because raw data were unavailable.

Results and Discussion

4.1 Phytochemical profile and acute toxicity. The extract tested positive for alkaloids, tannins, terpenoids, saponins, steroids and glycosides, while flavonoids and reducing sugars were not detected. This profile provides several plausible routes for biological activity. Alkaloids and terpenoids can interact with neuronal receptors and inflammatory signalling pathways, while tannins and saponins may alter mediator release, membrane processes and oxidative responses. The findings are consistent with the wider observation that African medicinal plants frequently contain multiple classes of secondary metabolites with overlapping pharmacological actions (Oguntibeju, 2018). They also reinforce the need for chemical standardisation: two extracts labelled with the same plant name may differ if they are collected from different environments or processed differently. The qualitative tests cannot identify the active constituent, and absence in a colour reaction should not be interpreted as chemical absence without chromatographic confirmation.

No mortality or overt clinical signs were recorded up to 5,000 mg/kg during acute oral testing. The defensible conclusion is therefore that the oral LD50 was greater than 5,000 mg/kg under the conditions of this experiment. It would be inappropriate to describe the extract as universally safe on this basis. Acute mortality is a coarse endpoint, the phase-two groups contained one animal per dose, and delayed, reproductive, genotoxic or subtle organ effects were not assessed. Nevertheless, the result indicates a relatively wide margin between the doses used in the repeated-dose and antinociceptive experiments and the highest non-lethal acute dose. Similar high acute thresholds have been reported for several plant extracts, but subsequent repeated-dose studies have sometimes identified target-organ or haematological changes (Mukinda & Syce, 2007). The present study therefore appropriately continued beyond acute testing.

4.2 Body weight and organ weights. Body weight did not show a consistent dose-related deterioration over 21 days. The saline group changed from 143.43 g on Day 1 to 144.35 g on Day 21. The 50 mg/kg group declined from 184.75 to 174.83 g, the 100 mg/kg group increased from 175.50 to 184.23 g, and the 200 mg/kg group remained broadly stable, changing from 164.48 to 162.33 g. Baseline weights differed considerably between groups, and the very large SEMs in the 200 mg/kg group indicate substantial heterogeneity. For this reason, direct comparison of final weights is less informative than within-group change. The absence of a monotonic pattern argues against a clear dose-related effect on growth or food utilisation, but the design would have been stronger with individual weight trajectories, food intake and analysis adjusted for baseline weight.

Absolute organ weights were numerically similar across groups for the heart, lungs, kidneys, spleen and testes. Liver weight was lower in the extract groups than in the saline group, with means of 3.80, 3.50 and 3.55 g at 50, 100 and 200 mg/kg compared with 4.40 g in controls. The original record did not mark these differences as statistically significant and did not provide terminal body weights for calculation of relative organ weight. It is therefore not justified to label the pattern hepatotoxicity or hepatic atrophy. Organ weight should be interpreted jointly with serum biomarkers and histology, neither of which demonstrated a coherent injury pattern here. The observation is best treated as a signal requiring confirmation. A repeat study should report organ-to-body-weight ratios, gross pathology and blinded histological scoring, because absolute weights are sensitive to baseline body size (Lazic et al., 2020).

4.3 Haematological findings. Packed cell volume was lower in all extract groups than in controls: 42.36%, 45.28% and 45.56% at 50, 100 and 200 mg/kg, respectively, compared with 55.20% in the saline group. Total white-cell counts were also lower, particularly at 200 mg/kg, where the mean was 3.50 compared with 7.80 in controls. Neutrophil proportions were similar, while lymphocyte proportions were numerically higher in the 100 and 200 mg/kg groups. Monocyte and eosinophil values tended to be lower at the two higher doses. The original results marked only the lymphocyte value at 100 mg/kg as significant, and the discussion record stated that overall haematological variation was not statistically significant. Thus, the lower PCV and white-cell means should be described as possible safety signals rather than established anaemia or immunosuppression.

The pattern is nevertheless biologically important enough to justify follow-up. A fall in PCV may reflect reduced erythrocyte production, haemodilution or increased destruction, while a lower white-cell count may reflect altered production, redistribution or immune effects. The present dataset cannot distinguish these mechanisms because haemoglobin, red-cell count, indices, platelets, reticulocytes and bone-marrow findings were not measured. Baseline haematological values were also unavailable. In environmental-health terms, this uncertainty matters because herbal preparations may be used by people with malaria, nutritional anaemia, chronic infection or other conditions that already affect blood indices. A standardisation programme for E. speciosa should therefore include a more complete haematological panel and longer exposure before community-level safety claims are made.

4.4 Hepatic, renal and lipid indices. Serum AST ranged from 30.14 to 33.20 U/L, ALT from 18.42 to 23.28 U/L and ALP from 45.18 to 52.09 U/L. These narrow ranges did not show a consistent dose-response. The modest decline in AST at 200 mg/kg occurred alongside a modest increase in ALT and ALP, but the changes were not supported by bilirubin, albumin, total protein or histological evidence. The results therefore do not demonstrate hepatocellular injury. This interpretation differs from the stronger hepatotoxicity language used in the original draft and reflects a more conservative reading of the numerical data. Clinical chemistry should be interpreted through coherent patterns, not isolated direction changes, especially in small groups.

Renal and electrolyte indices were similarly stable. Urea remained between 6.0 and 6.4 mmol/L, sodium between 134 and 136 mmol/L, potassium between 3.3 and 3.5 mmol/L, bicarbonate between 21.32 and 22.30 mmol/L and creatinine between 132 and 136 µmol/L. Chloride decreased numerically from 102 mmol/L in controls to 96 mmol/L at 200 mg/kg, but the large SEMs and absence of inferential significance limit interpretation. Taken together, the data do not indicate a clear nephrotoxic effect after 21 days. This is consistent with the principle that renal injury should be supported by convergent changes in filtration markers, electrolytes, urine findings and tissue morphology. The results are also relevant to previous work by Akpan et al. (2024), which used renal biomarkers to evaluate a different medicinal-plant extract and illustrates the value of maintaining comparable safety endpoints across a research programme.

The lipid profile showed no coherent adverse pattern. Total cholesterol was 1.70 mmol/L in controls and 1.60, 2.00 and 1.50 mmol/L in the 50, 100 and 200 mg/kg groups. HDL was higher at 100 mg/kg, while LDL and VLDL varied only slightly. Triglyceride means ranged from 0.40 to 0.60 mmol/L. These data do not support the claim that a small reduction in cholesterol is evidence of liver damage; lipid values are influenced

by diet, fasting, metabolic state and hepatic synthesis, and the direction observed was neither monotonic nor accompanied by enzyme evidence of injury. The broader body of local pharmacological research, including work on honey and plant extracts, also shows that metabolic indices can move independently and should be interpreted as a panel rather than as single markers (Erejuwa et al., 2018; Eke et al., 2025).

4.5 Acetic acid-induced writhing. The extract produced a strong and dose-related reduction in abdominal constrictions. The saline group recorded 96.67 ± 3.87 writhes, compared with 28.63 ± 2.71 at 50 mg/kg, 12.32 ± 1.82 at 100 mg/kg and 5.96 ± 3.80 at 200 mg/kg. When inhibition was recalculated directly from these means, the corresponding values were 70.4%, 87.3% and 93.8%. Indomethacin produced 3.40 ± 1.91 writhes, equivalent to 96.5% inhibition. The original table indicated significant activity for all treatment groups. The close approach of the 200 mg/kg response to the reference drug is notable, although the small sample and lack of confidence intervals make it difficult to determine whether the two effects were statistically equivalent.

Acetic acid evokes a complex visceral response involving peripheral mediators, including prostaglandin-related pathways, and is highly sensitive to anti-inflammatory and antinociceptive agents. The dose-response observed here is therefore compatible with peripheral inhibition of mediator generation or action. The phytochemical profile offers plausible candidates, particularly tannins, terpenoids, saponins and alkaloids, but mechanism cannot be assigned without fractionation, receptor or enzyme studies. Reduced writhing could also arise from sedation or impaired movement; a locomotor control was not included. These limitations do not negate the effect, but they define the next experimental steps. Future work should include open-field activity, inflammatory mediator assays and chemically standardised fractions.

4.6 Hot-plate response. Hot-plate latency increased over time in the reference and extract groups, but the magnitude was lower for the extract than for indomethacin. At 120 minutes, latency was 10.50 ± 0.20 seconds in the saline group, 23.33 ± 0.95 seconds with indomethacin, and 13.17 ± 0.60, 16.80 ± 0.68 and 17.50 ± 0.34 seconds with 50, 100 and 200 mg/kg extract. The response was dose ordered at later time points, and the 200 mg/kg group increased progressively from a baseline of 8.00 seconds to 17.50 seconds at 120 minutes. The original narrative stated that early effects reached significance, while later comparisons varied. The plotted time course nevertheless suggests a sustained, dose-related thermal antinociceptive signal, with a smaller maximal effect than the reference treatment.

The divergence between the two models is informative. E. speciosa nearly matched the reference drug in the writhing assay at 200 mg/kg but produced a more moderate increase in hot-plate latency. This pattern may indicate stronger peripheral than centrally integrated activity, different assay sensitivity, slower absorption or a mechanism that more effectively suppresses inflammatory mediator-driven nociception than thermal response. It also cautions against a simple statement that the extract is a potent central analgesic. A more precise conclusion is that the extract showed robust peripheral antinociception and moderate, dose-related activity in a thermal model. Pharmacokinetic studies and antagonist experiments would be required to determine whether opioid, adrenergic, serotonergic or other central pathways are involved.

4.7 Integrated interpretation, strengths and limitations. The strongest evidence in this study is the dose-related reduction in writhing, supported by a progressive hot-plate response at higher doses. The toxicological findings are more reassuring than the original draft suggested: acute mortality was absent at 5,000 mg/kg, and repeated dosing did not produce a coherent biochemical or organ-weight pattern of liver or kidney injury. At the same time, lower PCV and white-cell means should not be dismissed. The appropriate overall interpretation is therefore a favourable short-term efficacy signal with unresolved haematological questions. This balanced position is more useful for decision-making than either an unqualified safety claim or an unsupported conclusion of hepatotoxicity.

The study's strengths include the use of complementary nociceptive models and a multi-system repeated-dose panel. Its limitations include small group sizes, incomplete reporting of animal sex and environmental conditions, lack of voucher number and extraction yield, absence of raw data and exact p values,

no histopathology, no locomotor control, and inconsistent doses between some narrative records and numerical tables. The manuscript resolves internal inconsistencies by reporting the doses and values contained in the tables, but author verification against the laboratory notebooks remains essential. These limitations should inform, rather than prevent, further research. A confirmatory study with ARRIVE-compliant reporting, pre-specified endpoints, individual-level data and chemical fingerprinting could determine whether the activity is reproducible and whether the haematological signals are real.

Policy and Practice Implications

The findings have implications for environmental health, traditional-medicine governance and sustainable medicinal-plant development. First, regulatory and public-health communication should avoid presenting a high acute LD50 as proof of safety. Any community or commercial product derived from E. speciosa should be supported by authenticated plant material, defined extraction procedures, contaminant testing, batch-level chemical fingerprints and repeated-dose safety data. Particular attention should be given to haematological monitoring because the present PCV and white-cell patterns, although not conclusive, could be important in populations with prevalent anaemia or infection. Pharmacovigilance systems for herbal products should include simple reporting routes for adverse reactions and should document concurrent use with conventional medicines.

Second, promising pharmacological activity can increase harvesting pressure. Environmental agencies, forestry authorities, researchers and local communities should therefore develop cultivation and resource-monitoring plans before promoting large-scale use. Collection should be traceable to location and season, and harvesting methods should protect plant regeneration. Community knowledge holders should be acknowledged and included in benefit-sharing arrangements. The broader environmental literature shows that conservation rules are more likely to be socially sustainable when they recognise livelihood dependence and provide viable alternatives (Eneyo et al., 2026). Cultivation trials could reduce pressure on wild populations while also allowing investigation of how soil, shade, water and other environmental variables influence phytochemical composition.

Third, the data justify further research but not immediate clinical recommendation. The next stage should prioritise taxonomic confirmation with a deposited voucher, quantitative phytochemistry, chromatographic standardisation, 28- or 90-day toxicity, full blood counts, histopathology, locomotor testing and mechanism-focused antinociceptive experiments. If these stages confirm efficacy and safety, formulation, dose standardisation and controlled clinical investigation may be considered. This staged approach protects participants and ecosystems while creating a transparent evidence pathway from indigenous knowledge to sustainable innovation.

Conclusion

The ethanolic leaf extract of Eremomastax speciosa contained alkaloids, tannins, saponins, terpenoids, steroids and glycosides and produced marked, dose-related antinociceptive activity in the acetic acid writhing model, with moderate dose-related activity in the hot-plate test. No mortality occurred at 5,000 mg/kg, and 21-day administration did not produce a coherent pattern of adverse change in body weight, organ weight, hepatic enzymes, renal indices or lipid profile. Lower packed cell volume and white-cell means constitute safety signals that require confirmation with larger, better characterised studies. Overall, the evidence supports E. speciosa as a candidate for further pharmacological and chemical investigation, not as a clinically established analgesic or a proven safe herbal medicine. Future development should integrate rigorous toxicology with botanical authentication, standardisation, sustainable cultivation and environmental-health oversight.

Declarations

Funding: No external funding was reported in the source manuscript.

Ethics approval: The study involved laboratory animals and was reported to have followed applicable institutional and international guidelines for animal care and use.

Conflict of interest: The authors declare that they have no conflict of interest.

Data availability: The group-level data underlying the reported tables and figures are contained in this article.

Author contributions: All authors contributed to the conception and design of the study, acquisition, analysis and interpretation of data, drafting or critical revision of the manuscript, and approval of the final version. All authors agree to be accountable for the work.

Author declaration: The authors affirm that the manuscript is original, has not been published in the present form,

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