Journal of Research in Applied and Basic Medical Sciences

ORIGINAL RESEARCH ARTICLE

Comparative Phytochemical, Safety, and Analgesic Evaluation of Selected African Medicinal Plants in Swiss Albino Mice

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Published: 15 November 2025

Abstract

Background Moringa oleifera, Anogeissus leiocarpus, and Commiphora africana are medicinal plants traditionally used for managing pain and inflammation. Despite documented pharmacological properties, the comparative evaluation of their analgesic activities using standardized peripheral and central pain models is limited.This study evaluated and compared the analgesic activities, phytochemical profiles, and acute toxicity of methanol extracts of M. oleifera seeds, A. leiocarpus leaves, and C. africana leaves in Swiss albino mice.

Methods Plant materials were extracted with methanol and subjected to qualitative phytochemical screening. Acute oral toxicity was evaluated using Lorke's method. Analgesic effects were evaluated using the acetic acid-induced writhing assay and the hot plate method at doses of 150, 300, and 600 mg/kg (i.p.). Piroxicam (10 mg/kg) and pentazocine (1 mg/kg) served as standard drugs.

Results All extracts contained alkaloids, flavonoids, saponins, and tannins, with terpenoids detected only in M. oleifera and steroids only in A. leiocarpus. LD_{50} values were > 5000 mg/kg for M. oleifera and A. leiocarpus, and 1264.9 mg/kg for C. africana. In the acetic acid-induced writhing assay, a significant, dose-dependent reduction in abdominal constrictions was observed in the extract-treated group. Similarly, in the hot plate test, all extracts significantly prolonged reaction latencies, with C. africana at 600 mg/kg approaching the activity of pentazocine.

Conclusion Methanol extracts of M. oleifera, A. leiocarpus, and C. africana possess significant peripheral and central analgesic activities, likely mediated by their bioactive phytoconstituents. The study supports their ethnomedicinal use in pain management and highlights C. africana's potent central analgesic activity but narrower safety margin. Further bioassay-guided and mechanistic studies are warranted.

Keywords Acute toxicity, Analgesic activity, Anogeissus leiocarpus, Commiphora africana, Moringa oleifera, Phytochemicals

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Received: 29 July 2025/ Revised: 18 October 2025/ Accepted: 2 November 2025

1 Introduction

Pain is a complex physiological response that serves as a protective mechanism against harmful stimuli but can significantly impair quality of life when persistent or inadequately managed.[1] Despite the availability of numerous synthetic analgesic agents, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics, their long-term use is often associated with adverse effects, including gastrointestinal irritation, renal dysfunction, tolerance, and dependence.[2] This has driven continuous interest in exploring plant-derived compounds as potential alternatives, given their historical use in traditional medicine and the growing body of scientific evidence supporting their therapeutic potential. Medicinal plants have long played a fundamental role in traditional African healthcare, where they are widely used for inflammatory conditions, fever, and painful disorders. Moringa oleifera (family: Moringaceae), Anogeissus leiocarpus (family: Combretaceae), and Commiphora africana (family: Burseraceae) are medicinal plants widely distributed in tropical and subtropical regions of Africa and Asia. Ethnobotanical reports have documented their use in managing various ailments, including inflammatory conditions, fever, and pain.[3–5] M. oleifera seeds are rich in bioactive compounds such as flavonoids, terpenoids, and phenolic acids, which are recognized for their potent antioxidant and anti-inflammatory effects.[6,7] A. leiocarpus leaves contain tannins, flavonoids, and triterpenes with reported antimicrobial and anti-inflammatory properties.[3] C. africana, a source of aromatic resins, contains phytochemicals like alkaloids, saponins, tannins, and flavonoids, which have demonstrated analgesic and anti-inflammatory potential.[5,8]

Despite increasing scientific interest in M. oleifera, A. leiocarpus, and C. africana, available studies have largely evaluated these species independently under heterogeneous experimental conditions. Differences in extraction methods, dose ranges, and nociceptive models limit meaningful comparison of their relative analgesic efficacy and safety profiles.[9] A standardized, side-by-side evaluation using uniform experimental parameters is therefore necessary to enable reliable assessment and identification of the most promising candidate for further development.

Phytochemicals identified in these plants, including flavonoids, alkaloids, tannins, saponins, terpenoids, and steroids, are known to modulate pain pathways through multiple mechanisms. Flavonoids and saponins have been reported to inhibit cyclooxygenase-mediated prostaglandin synthesis, thereby reducing peripheral nociceptor sensitization. Alkaloids and certain terpenoids may interact with central neurotransmitter systems, including opioidergic and monoaminergic pathways, influencing supraspinal pain processing. Additionally, antioxidant properties of phenolic compounds may attenuate oxidative stress–mediated inflammatory signaling.[10,11] These multimodal actions suggest potential involvement of both peripheral inflammatory suppression and central modulation of nociceptive transmission. Accordingly, the present study provides a direct evaluation of the phytochemical profiles, acute toxicity, and peripheral and central analgesic activities of methanol extracts of these three medicinal plants under identical experimental conditions in Swiss albino mice.

2 Methods

Animals

Swiss albino mice (18-24 g) of both sexes were obtained from the Animal House facility of the Department of Pharmacology, Bauchi State University, Gadau. The animals were maintained under standard laboratory conditions (12-hour light and 12-hour daily cycle) in accordance with protocols approved by the university ethical committee on the use and care of experimental animals, and fed with standard laboratory diet and water ad libitum.

Collection and Identification of Plant

The seeds of M. oleifera were collected from Jama'are Local Government Area, Bauchi State, Nigeria, while fresh leaves of C. africana were obtained from the University Garden, Sa'adu Zungur University, Bauchi State. Fresh leaves of A. leiocarpus were collected from Podo, Ibadan, Oyo State, Nigeria. All plant materials were authenticated at the Herbarium Unit, Department of Biological Sciences, Sa'adu Zungur University, Bauchi State, Nigeria, and voucher specimens were deposited for future reference under the following numbers: Moringa oleifera (SZU/HB/25/026), Anogeissus leiocarpus (SZU/HB/25/032), and Commiphora africana (SZU/HB/25/013).

Preparation and Extraction of Plant Materials

The collected plant materials were air-dried at room temperature and ground into fine powder using a mechanical grinder. For each plant, 200 g of the powdered material was macerated in 1 L of methanol for 72 hours with intermittent shaking to enhance solvent penetration and extraction of phytoconstituents. After maceration, the mixtures were filtered separately and dried in an oven at 45–50°C until a consistent weight was achieved.

Acute Toxicity Studies

The oral acute toxicity of the methanol extracts of M. oleifera seeds, A. leiocarpus leaves, and C. africana leaves was evaluated as described by Lorke in two

distinct phases.[12] In the first phase, nine healthy mice of either sex were randomly divided into three groups of three animals each. The groups received graded doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg of the respective extracts. Based on the outcomes of this phase, the second phase was carried out using three additional mice for each extract. These animals received higher doses of 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg to determine the median lethal dose (LD_{50}). All animals were observed closely for 24 hours for signs of toxicity, behavioral changes, or mortality.

The LD50 for the respective extracts was calculated as:

$$LD_{50} = \sqrt{\text{highest dose with no mortality} \times \text{lowest dose with mortality}}$$

Phytochemical Screening

The methanol extracts of the plants were subjected to qualitative phytochemical screening to identify the presence of various secondary metabolites using standard procedures.[13,14] The extracts were tested for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, phenols, and carbohydrates.

Acetic Acid-Induced Abdominal Writhing Test

The peripheral analgesic effect of the extracts was evaluated using the acetic acid-induced writhing model. The procedure was conducted according to the method described by Koster et al.[15] For each extract, 20 mice were randomly divided into five groups of four animals each as follows:

- Group I (Negative Control): Received normal saline (10 mL/kg, i.p.)
- Groups II, III, and IV (Test Groups): Received 150, 300, and 600 mg/kg body weight of the respective plant extract (i.p.)
- Group V (Positive Control): Received piroxicam injection (10 mg/kg, i.p.)

The doses of 150, 300, and 600 mg/kg were strategically selected based on the acute toxicity profiles (LD50 = 1264 mg/kg and > 5000 mg/kg) determined for the respective methanol extracts, ensuring all test doses remained well below 50% of the lower LD50 value.[16] Thirty minutes after treatment, all mice were injected intraperitoneally with 10 mL/kg of 0.6% acetic acid solution to induce abdominal writhing. Each animal was then placed in a transparent observation cage. Following a 5-minute lag period, the number of abdominal constrictions (writhes), characterized by contraction of the abdominal muscles and stretching of the hind limbs, was counted for each mouse over 10 minutes. Abdominal writhing typically begins within 5 minutes following intraperitoneal acetic acid administration and reaches peak frequency within the first 10–15 minutes. Writhing is quantified during the 5–15 minute period, which corresponds to the peak nociceptive phase reported in validated protocols.[17]

Analgesic activity was determined by comparing the mean number of writhes in treated groups with the control group. The percentage inhibition of writhing was calculated using the formula:

$$\%\ inhibition = \frac{Mean\ no.\ of\ writhes\ (control) - Mean\ no.\ of\ writhes\ (test)}{Mean\ no.\ of\ writhes\ (control)} \times 100$$

This protocol was independently applied to each of the three plant extracts.

Hot Plate Method

To assess central analgesic activity, the hot plate method was employed for each of the extracts following the method described by Lanhers et al.[18] The hot plate apparatus was maintained at a constant temperature of 50 ± 1°C. For each extract, 20 mice were randomly divided into five groups (n = 4 per group) as follows:

- Group I (Negative Control): Received normal saline (10 mL/kg, i.p.)
- Groups II, III, and IV (Test Groups): Received 150, 300, and 600 mg/kg body weight of the respective plant extract (i.p.)
- Group V (Positive Control): Received pentazocine (1 mg/kg, i.p.)

Thirty minutes post-treatment, each mouse was individually placed on the hot plate, and the latency to pain response (paw licking or jumping) was recorded in seconds. An increase in reaction time compared to the control group was considered indicative of central analgesic activity. The test was carried out separately for each extract, and the results were compared with the control. The standard drug doses, piroxicam (10 mg/kg, i.p.) for peripheral analgesia and pentazocine (1 mg/kg, i.p.) for central analgesia, were selected based on established pharmacological protocols in murine pain models.[19,20]

Statistical Analysis

Data obtained from the experiments were expressed as mean ± standard error of the mean (SEM). Statistical comparisons were conducted using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test, with the control group serving as the reference. The analysis was carried out using GraphPad Prism software version 8, and Differences were considered statistically significant at $p \leq 0.05$.

3 Results

Extraction

The extraction of plant materials yielded varying quantities of crude extracts. From 200 g of dried leaf powder of A. leiocarpus, 10 g of methanol extract was obtained, corresponding to a yield of 5%. Extraction of

200 g of crushed, powdered M. oleifera seeds using a methanol-water mixture produced 14.9 g of a yellowish, honey-like dry extract, representing a 7.45% yield. Similarly, methanol extraction of 200 g of C. africana leaf powder yielded 9 g of dry extract, corresponding to a 4.5% yield.

Phytochemical Analysis

Qualitative phytochemical analysis of the methanol extracts revealed the presence of various secondary metabolites known for their pharmacological potential. All three extracts tested positive for alkaloids, flavonoids, saponins, and tannins. Notably, terpenoids were detected only in the M. oleifera extract, while steroids were found exclusively in the A, leiocarpus extract. Carbohydrates were present only in the extract of C. africana. However, none of the extracts tested positive for glycosides or anthraquinones (Table 1).

Table 1 Phytochemical constituents of methanol extracts of the studied plants

Phytochemical constituents	M. oleifera	A. leiocarpus	C. africana
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	+	–	–
Steroids	–	+	–
Anthraquinones	–	–	–
Carbohydrates	–	–	+
Glycosides	–	–	–

Key: "+" = Present, "–" = Absent

Acute Toxicity Profile

Acute toxicity studies were conducted on the methanol extracts of Anogeissus leiocarpus, Commiphora africana, and Moringa oleifera using the method described by Lorke. The experiment was carried out in two phases with oral administration of increasing doses of each extract to mice. Animals were observed for signs of toxicity or mortality within the first 24 hours (Table 2).

No mortality or severe toxicity signs were observed up to 5000 mg/kg A. leiocarpus and M. oleifera treated mice; hence, the LD_{50} is estimated to be greater than 5000 mg/kg (Table 2). For C. Africana, the LD50 was calculated as:

$$LD_{50} = \sqrt{\text{highest dose with no mortality} \times \text{lowest dose with mortality}}$$

$$LD_{50} = \sqrt{1000\ \text{mg/kg} \times 1600\ \text{mg/kg}}$$

$$LD_{50} = 1264.9\ \text{mg/kg}$$

Table 2 LD_{50} determination of the studied plants

Phases	Dose (mg/kg)	No. of dead mice (24 hours)		
		A. leiocarpus	C. africana	M. oleifera
Phase I	10	0/3	0/3	0/3
Phase I	100	0/3	0/3	0/3
Phase I	1000	0/3	0/3	0/3
Phase II	1600	0/1	1/1	0/1
Phase II	2900	0/1	1/1	0/1
Phase II	5000	0/1	1/1	0/1

Acetic Acid-Induced Writhing Test

The methanol leaf extract of A. leiocarpus (MEAL) significantly reduced the number of abdominal writhing induced by acetic acid in mice in a dose-dependent manner. The highest dose (600 mg/kg) produced the greatest reduction in writhing and was comparable to the standard drug, piroxicam (PRM 10 mg/kg). All treatment groups showed significant reductions ($p < 0.05$) compared to the control (Figure 1). Similarly, the methanol extract of C. africana (MECA) at doses of 150, 300, and 600 mg/kg significantly decreased writhing responses in a dose-dependent manner compared to the negative control (NS 10 mg/kg, $p < 0.05$) (Figure 2). The highest inhibition was observed with piroxicam (PRM 10 mg/kg). The M. oleifera leaves also significantly reduced acetic acid-induced writhing at all tested doses (150, 300, and 600 mg/kg) in a dose-dependent fashion when compared to the negative control group (Figure 3).

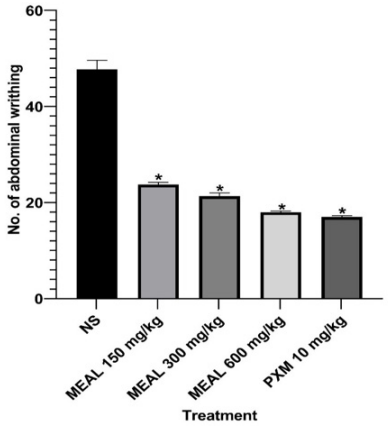

Figure 1 Effect of MEAL in acetic acid-induced writhing in mice. NS = Normal saline; MEAL = Methanol extract of A. leiocarpus; PXM = Piroxicam; * = $p \leq 0.05$ vs NS

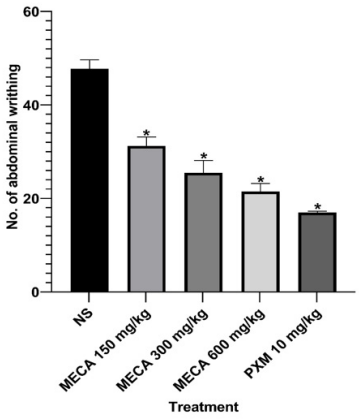

Figure 2 Effect of MECA in acetic acid-induced writhing in mice. NS = Normal saline; MECA = Methanol extract of C. africana; PXM = Piroxicam; * = $p \leq 0.05$ vs NS

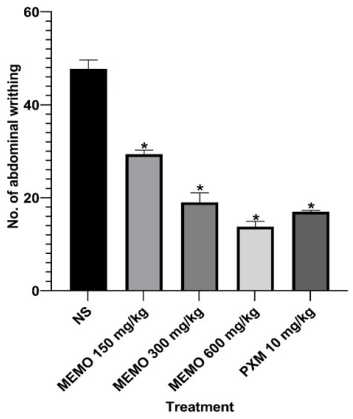

Figure 3 Effect of MEMO in acetic acid-induced writhing in mice. NS = Normal saline; MEMO = Methanol extract of M. oleifera; PXM = Piroxicam; * = $p \leq 0.05$ vs NS

When the three extracts were examined descriptively at equivalent dose levels, C. africana consistently demonstrated a higher percentage inhibition of writhing, particularly at 600 mg/kg, followed by A. leiocarpus and M. oleifera. However, as statistical testing was conducted relative to the negative control group only, these inter-extract differences should be interpreted as comparative trends rather than statistically confirmed differences.

Hot Plate-induced Pain

The MECA significantly increased the pain reaction time in mice in the hot plate test in a dose-dependent manner (Table 3). At 30, 60, and 90 minutes post-administration, all tested doses (150, 300, and 600 mg/kg) produced significant ($p < 0.05$) increases in latency compared to the negative control (N/S 10 ml/kg).

The methanol leaf extract of A. leiocarpus significantly increased the pain reaction time in mice during the hot plate test in a dose-dependent manner at 30, 60, and 90 minutes compared to the negative control (Table 4). MEAL at 600 mg/kg showed the most pronounced effect, with peak latency at 30 minutes and sustained effects up to 90 minutes. The 300 mg/kg dose also significantly increased latency at all time points, while the 150 mg/kg dose showed moderate but significant increases at 30 and 90 minutes.

The methanol extract of M. oleifera (MEMO) significantly increased pain reaction time in the hot plate test in a dose-dependent manner. At 600 mg/kg, MEMO produced the highest latency, at 60 minutes, with sustained effects up to 90 minutes. The 300 mg/kg dose also showed significant increases, especially at 60 and 90 minutes, while the 150 mg/kg dose had moderate effects (Table 5).

Descriptive examination of the plants' effects in the hot plate model revealed that C. africana showed greater increases in reaction latency at higher doses compared with the other extracts. A. leiocarpus and M. oleifera produced moderate, dose-dependent effects. Since formal pairwise statistical comparisons between the extracts were not performed, these observations represent relative trends and do not imply statistically significant superiority between plant extracts.

4 Discussion

The findings of this study demonstrate that the methanol extracts of M. oleifera seeds, A. leiocarpus leaves, and C. africana leaves possess significant analgesic activities in both peripheral and central pain models. Phytochemical screening revealed that all three extracts contained alkaloids, flavonoids, saponins, and tannins, which are known to contribute to analgesic and anti-inflammatory effects through multiple mechanisms, including inhibition of prostaglandin synthesis, modulation of nociceptor activity, and antioxidant actions.[21] The presence of terpenoids in M. oleifera and steroids in A. leiocarpus may further enhance these effects through synergistic interactions. The acute toxicity studies revealed that M. oleifera and A. leiocarpus were safe up to 5000 mg/kg. Previous study on the root extract of A. leiocarpus reported an LD_{50} of above 10,000 mg/kg.[22] C. africana had a lower LD_{50} of 1264.9 mg/kg. highlighting the importance of dose optimization for therapeutic applications.

In the acetic acid-induced writhing test, all extracts produced a significant and dose-dependent reduction in the number of abdominal constrictions, suggesting effective inhibition of peripheral nociception. This effect is likely mediated by suppression of inflammatory mediators such as prostaglandins (particularly PGE_2) and bradykinin, which are implicated in the sensitization of peripheral nociceptors.[23,24] The magnitude of inhibition observed at higher doses was similar to that of the standard NSAID, piroxicam, indicating substantial peripheral

Table 3 Effect of MECA on hot plate test in mice

Treatment (mg/kg)	0 min	30 min	60 min	90 min
N/S (10 ml/kg)	5.23 ± 0.11	4.00 ± 0.18	5.25 ± 0.10	2.25 ± 0.14
MECA 150	4.00 ± 0.87	8.00 ± 2.75*	9.63 ± 1.25*	8.50 ± 2.90*
MECA 300	5.25 ± 0.25	9.75 ± 2.86*	10.75 ± 1.11*	11.25 ± 1.65*
MECA 600	5.25 ± 0.46	15.00 ± 0.91*	10.50 ± 1.32*	17.50 ± 1.85*
PENT 10	6.50 ± 0.00*	16.00 ± 0.47*	11.25 ± 0.10*	8.25 ± 0.14*

Results are expressed as mean ± SEM, with n = 4 per group. * indicates differences with $p \leq 0.05$ considered statistically significant.

Table 4 Effect of MEAL in the hot plate test

Treatment (mg/kg)	0 min	30 min	60 min	90 min
NS (10 ml/kg)	5.23 ± 0.11	4.00 ± 0.18	5.25 ± 0.10	2.25 ± 0.14
MEAL 150	5.25 ± 0.32	7.00 ± 0.18*	5.50 ± 0.31	4.50 ± 0.54*
MEAL 300	5.75 ± 0.32*	10.00 ± 0.25*	7.25 ± 0.38*	7.25 ± 0.52*
MEAL 600	6.30 ± 0.12*	13.25 ± 0.27*	10.43 ± 0.17*	10.50 ± 0.20*
PENT 10	6.50 ± 0.00*	16.00 ± 0.47*	11.25 ± 0.10*	8.25 ± 0.14*

Results are expressed as mean ± SEM, with n = 4 per group. * indicates differences with $p \leq 0.05$ considered statistically significant.

Table 5 Effects of MEMO in hot plate test

Treatment (mg/kg)	0 min	30 min	60 min	90 min
NS 10 ml/kg	5.23 ± 0.11	4.00 ± 0.18	5.25 ± 0.10	2.25 ± 0.14
MEMO 150	4.25 ± 1.70	7.00 ± 0.53*	9.25 ± 0.97	5.75 ±1.09*
MEMO 300	5.00 ± 2.55	7.50 ± 0.77*	12.00 ± 2.07*	8.00 ±1.87*
MEMO 600	5.50 ± 3.41	13.25 ± 0.36*	14.75 ± 0.60*	9.25 ±1.92*
PENT 10	6.50 ± 0.00*	16.00 ± 0.47*	11.25 ± 0.10*	8.25 ± 0.14*

Results are expressed as mean ± SEM, with n = 4 per group. * indicates differences with $p \leq 0.05$ considered statistically significant.

analgesic potential. The hot plate test results further support the central analgesic activity of the extracts, as evidenced by the significant prolongation of reaction latency times in treated mice. This suggests possible involvement of supraspinal and spinal pain pathways, potentially through interaction with opioid receptors or modulation of endogenous pain inhibitory systems. Notably, C. africana exhibited pronounced effects at 600 mg/kg, approaching those of pentazocine, a centrally acting opioid analgesic, indicating strong central pain-modulating activity. These findings are consistent with previous reports on the analgesic and anti-inflammatory potentials of these plants and provide additional evidence supporting their traditional use in pain management.[5,7,25] Mechanistically, the pattern of activity across tests supports a mixed mode of action. The inhibition of acetic acid-induced writhing by all three extracts indicates suppression of peripheral mediators (e.g., prostaglandins, bradykinin) as reported for other plant extracts with similar phytochemical composition, while the increased hot-plate latencies implicate central modulation (opioidergic or descending inhibitory pathways). These dual actions are well documented for plant extracts rich in flavonoids, alkaloids, and terpenoids, and align with mechanistic proposals in earlier reports.[26–28]

While the extracts demonstrated significant peripheral and central analgesic effects, it is important to note that the mechanisms proposed in this study remain speculative. No mechanistic assays, such as naloxone antagonism, COX-1/COX-2 inhibition, or cytokine/prostaglandin quantification, were performed. Therefore, the suggestions regarding possible inhibition of inflammatory mediators or interaction with opioid pathways are based solely on the known pharmacological activities of the phytochemicals identified and on previously published literature. The present findings indicate analgesic activity but do not confirm the specific pathways involved. Future studies incorporating receptor-blocking experiments, enzyme inhibition assays, and inflammatory biomarker analysis will be required to elucidate the precise mechanisms of action.

Compared with prior studies, the novelty of the present work lies in the direct comparison of M. oleifera, A. leiocarpus, and C. africana using the same extraction solvent, dose ranges, and validated nociceptive models. While earlier papers established analgesic activity for individual species, our results enable relative ranking under uniform conditions and highlight C. africana's strong central effect at higher doses and its narrower safety margin; observations that have practical implications for

future fractionation and lead-isolation studies.

Despite demonstrating significant antinociceptive activity in peripheral and central models, several limitations should be acknowledged. The study utilized crude extracts without bioassay-guided fractionation or phytochemical standardization; therefore, the specific active constituents and their potential synergistic interactions remain unidentified. Although established nociceptive assays were employed, molecular mechanistic investigations were not conducted, rendering the pharmacodynamic basis inferential. Furthermore, only acute pain models were assessed, and sub-chronic toxicity, organ-specific safety, and pharmacokinetic evaluations were not performed, precluding conclusions regarding long-term safety or therapeutic index.

5 Conclusion

The present study confirms that methanol extracts of M. oleifera seeds, A. leiocarpus leaves, and C. africana leaves possess significant peripheral and central analgesic activities in Swiss albino mice. Phytochemical analysis revealed flavonoids, alkaloids, tannins, and saponins in all three plants, with terpenoids present in M. oleifera and steroids in A. leiocarpus, suggesting that these secondary metabolites contribute synergistically to the observed analgesic effects. Acute toxicity testing showed high safety margins for M. oleifera and A. leiocarpus (LD_{50} > 5000 mg/kg orally) but a lower LD_{50} for C. africana, indicating the need for careful dose selection. By evaluating all three plants under identical conditions, this study offers a clearer understanding of their relative efficacy and safety profiles, reinforcing their ethnomedicinal use in pain management. Further investigations, including bioassay-guided isolation of active compounds, mechanistic studies, and long-term toxicity evaluations, are warranted to advance their development as safe and effective plant-derived analgesics.

Declarations

Acknowledgments
The authors express their gratitude to the staff of the General Pharmacology Laboratory, Department of Pharmacology, Sa'adu Zungur University, Bauchi State.

Artificial Intelligence Disclosure
No artificial intelligence tools were used in study design, data collection, data analysis, data interpretation, or the generation of scientific conclusions. The authors take full responsibility for the content, accuracy, and integrity of the work.

Authors' Contributions
Albashir Tahir and Khaleel Ibrahim Muazu participated in the study design, interpretation of the study results, data analysis, and writing the initial draft of the manuscript. Sanusi Ahmad, Saddam Ahmad and Muhammad Muhammad Lawal conducted the experiments and contributed to the data analyses. Musab Abba Usman and Muslim Ahmad Muhammad supervise and review the final draft of the manuscript. All authors approved the final article before submission.

Availability of Data and Materials
All data supporting the findings of this study are available within the manuscript.

Conflict of Interest
The authors declare no conflicting interests regarding the publication of this manuscript.

Consent for Publication
Not applicable.

Ethical Considerations
All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals. Ethical approval for this study was obtained from the Faculty of Basic Medical Sciences Research and Ethics Committee (FBMSRC) of Bauchi State University, Gadau uder the Code of Ethics BASUG/FBMS/REC/VOL.08/01048.

Funding
This research received no external funding.

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