

Original Research Article

Analgesic activity and phytochemical evaluation of ethanol stem bark extract of *Dennettia tripetala* in experimental pain models

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Abstract

Purpose: To evaluate the analgesic potential of ethanol stem bark extract of *Dennettia tripetala*.

Methods: Ethanol stem bark extract of *Dennettia tripetala* was prepared and screened phytochemically using standard procedures. Analgesic activity was assessed in mice ($n = 6$) using acetic acid-induced writhing test for peripheral nociception and hot plate and radiant heat-tail beat method models for central analgesic activity. Animals received a once-daily dose of 250, 500 and 1000 mg/kg of extract, while diclofenac (50 mg/kg) and morphine (2 mg/kg) served as standard reference drugs. Pain responses were measured as reaction latency and observed number of writhes.

Results: The extract produced a significant, dose-dependent reduction in writhing response (23.7 ± 0.22 , 17.4 ± 0.54 , and 13.3 ± 0.44) compared with control animals ($p < 0.05$), indicating peripheral analgesic activity. In thermal nociceptive models, extract-treated groups showed significant prolongation of reaction latency times relative to controls ($p < 0.05$), with the highest dose of 1000 mg/kg producing responses comparable to standard drugs. Phytochemical analysis confirmed the presence of bioactive metabolites associated with analgesic effects.

Conclusion: Ethanol stem bark extract of *Dennettia tripetala* possesses significant central and peripheral analgesic activities, supporting its traditional use in pain management and suggesting potential for development of plant-derived analgesic agents.

Keywords: Analgesic activity, *Dennettia tripetala*, Experimental mice, Pain models, Phytochemicals

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INTRODUCTION

Pain is a complex physiological and pathological phenomenon that serves as an important protective mechanism but becomes debilitating when persistent [1]. Acute and chronic pain conditions significantly affect quality of life and remain leading causes of medical consultations worldwide. Although non-steroidal anti-

inflammatory drugs (NSAIDs) such as piroxicam and diclofenac and opioid analgesics morphine and pentazocine are effective in managing pain, their long-term use is associated with adverse effects, including gastrointestinal irritation, renal toxicity for NSAIDs and tolerance and dependence for opioids. Consequently, there is increasing interest in medicinal plants as alternative sources of safer analgesic

compounds [2]. Traditional medicine remains the primary healthcare option for many populations in Africa. Numerous indigenous plants are employed for pain management, often based on empirical knowledge accumulated over generations [3]. *Dennettia tripetala* G. Baker, belonging to the family Annonaceae, is commonly known as pepper fruit in southeastern region of Nigeria and widely distributed across West Africa. The stem bark and leaves of the plant are extensively used in traditional medicine for the management of fever, cough, stomach discomfort, toothache, and painful inflammatory conditions [4-7].

Previous scientific investigations have demonstrated antimicrobial, antioxidant, anti-inflammatory, and gastroprotective activities of stem bark extract of *Dennettia tripetala* (*D. tripetala*), properties that could contribute to its analgesic effects. Phytochemical constituents such as flavonoids, alkaloids, and phenolic compounds commonly associated with analgesic mechanisms have also been reported in the plant [8-10].

Despite its broad traditional applications and comprehensive studies, evaluating analgesic effects of stem bark extracts of the plant remains limited. Scientific validation of these claims is necessary to establish pharmacological relevance and support development of plant-based analgesics. The present study, therefore, aimed to evaluate the central and peripheral antinociceptive effects of ethanol stem bark extract of *Dennettia tripetala* using standard mice pain models.

EXPERIMENTAL

Plant collection and authentication

Stem bark of *Dennettia tripetala* was collected on 12 August 2024 from a local botanical garden in Tanzania (3°21'00" S, 37°20'00" E) and authenticated by a qualified taxonomist. A voucher specimen was deposited at the Department of Botany, Kampala International University, with the number 112765 for reference. Collected stem bark materials were washed with clean water, air-dried at ambient temperature to preserve thermolabile compounds, and pulverised into coarse powder using a mechanical grinder.

Preparation of ethanol extract

Pulverised 7.2 kg of the plant material was subjected to Soxhlet extraction using analytical-grade 75% ethanol as solvent. Extraction was

continued until complete exhaustion of plant constituents. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at controlled temperature to prevent compound degradation. The crude extract obtained was weighed to determine percentage yield and stored at 4 °C until use.

Animals

Healthy adult Swiss albino mice weighing between 20 and 30 g were obtained from a 'Dar es Salaam' laboratory animal facility in Tanzania. Animals were maintained under standard laboratory conditions with controlled temperature, humidity, and light cycles. Standard feed and water were provided *ad libitum*, and animals were acclimatised for seven days prior. The Animal Ethics Committee of the College of Health Sciences, Kampala International University, approved the study (KIU/2025/11). Mice (n = 6) were randomly assigned to different groups. All procedures complied with international animal care guidelines [11].

LD₅₀ determination of stem bark of *Dennettia tripetala*

An acute oral toxicity assessment of the *stem bark of Dennettia tripetala* was performed in mice and male albino rats (rat between 150 – 210 g body weight were obtained from an Durasallam laboratory animal facility in Tanzania) employing the Up-and-Down Procedure at single dose levels of 250, 500, 2500 and 5000 mg/kg, following the Organisation for Economic Co-operation and Development (OECD) Guideline 425 [12]. For each dose group, each animal species was utilised. Animals were individually selected, weighed, and administered an appropriate volume of the extract prepared in distilled water. Oral dosing was conducted using a gastric gavage tube.

Immediately after administration, each animal was monitored for the first five minutes to detect possible regurgitation and subsequently housed in metallic cages for continued observation. Clinical signs were recorded at 15-minute intervals during the initial four hours, followed by observations every 30 minutes up to six hours post-treatment, and thereafter daily for 48 hours. Behavioural and physiological parameters assessed included alterations in skin, fur, ocular and mucosal appearance, respiratory and circulatory responses, autonomic and central nervous system activity, motor coordination, convulsions, tremors, salivation, diarrhoea, lethargy, and sleep patterns, consistent with OECD recommendations. Animals were further

observed over a 14-day period to identify any delayed mortality or persistent toxic effects. Body weight measurements were obtained on days 1, 7, and 14 to evaluate treatment-related changes over time.

Phytochemical Screening

Qualitative phytochemical analysis was conducted using standard procedures to detect major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, steroids, carbohydrates, proteins, and fats [13].

Hot plate test in mice

The method described by Eddy and Leimbach [14] was adopted. Mice with body weight of 26-29 g were selected and divided into five groups, with six animals in each group. Group 1 was administered distilled water orally (10 ml/kg), group 2 received morphine (2 mg/kg) intraperitoneally, while groups 3, 4, and 5 received 250, 500, and 1000 mg/kg oral doses of the extract, respectively. The dose administered was determined from available literature [14] and by calculating one-fifth of the lethal dose. Mice were placed individually on a hot plate. Hot plates were maintained at $(45 \pm 1^\circ\text{C})$ prior to drug treatment so that each animal received its own application; this served as the baseline latency time. The time it takes for the animal to lick its paw, flap a few paws, or jump is considered the reaction time and is turned off. A cut-off time of 20s was used to prevent paw tissue damage. The incubation time was observed and recorded after 0, 30, 60, 90 and 150 min; please align the time points.-min following treatment.

Central antinociceptive activity study

The central antinociceptive activity of the stem bark of *Dennettia tripetala* was determined using the radiant heat-tail beat method [15]. Group 1 mice received distilled water; test groups received 250, 500 and 1000 mg/kg of extract, while group 2 received morphine (2 mg/kg), which was injected subcutaneously. A 30 min control period was used to ensure the effective absorption of the product. Each mouse was placed in a cage with nearly one-third of its tail exposed to a thin wire handle. Tail movement time was measured after 30 minutes of administration of drug and extract using an anaesthetic testing device (Medicraft, Cochin, India). A low current (3 Amps) was allowed to flow through the wire and heat it. Animals either wag their tails to the side or try to run. The percentage elongation (E; %) of reaction time

due to the action of the stem bark of *Dennettia tripetala* was calculated using Eq 1.

$$E (\%) = \{(T_t - T_c) / T_t\} 100 \dots\dots\dots (1)$$

Where T_t is the average reaction time of test group and T_c is average reaction time of control group.

Peripheral antinociceptive activity

The peripheral antinociceptive activity of the stem bark of *Dennettia tripetala* was determined using the acetic acid-induced writhing inhibition method [16]. Thirty mice were divided into five groups, with five animals in each group. Forty minutes after treatment (group 1 received distilled water orally, groups 3 - 5 received 250, 500, and 1000 mg/kg oral doses of the extract, while group 2 received 50 mg/kg dose of diclofenac intraperitoneally), each mouse was intraperitoneally injected with acetic acid (0.6 % v/v saline, 10 mL/kg) to induce pain. Five minutes after the application of acetic acid, the number of contractions in 15 min was counted, which is an index of the pain response to chemical stimulation, characterised by contraction of the abdominal muscles with rotation of the body and extension of the limbs. The response of the extract and 50 mg/kg dose of diclofenac sodium was compared to that of the control animals [16]. The percentage of writhing inhibition (I) compared with the control group was used as the analgesic index and was calculated using Eq 2.

$$I (\%) = \{(W_c - W_t) / W_c\} 100 \dots\dots\dots (2)$$

Where W_c is the average number of writhing reflexes in the control group and W_t is the average number of writhing reflexes in the test group.

Statistical analysis

The data obtained from the study were analysed using the Statistical Package for Social Sciences (SPSS-25). Results are presented as the mean $\pm$ standard error of the mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Acute toxicity test (LD_{50})

Oral administration of the stem bark of *Dennettia tripetala* at dose levels of 200, 500, 2500, and 5000 mg/kg, evaluated using the Up-and-Down

acute toxicity testing approach, resulted in no mortality among both mice and rats. No fatal responses were observed during either the immediate post-administration period or the prolonged observation phase. Furthermore, animals did not display any discernible behavioural or physiological signs indicative of toxicity throughout the 14-day monitoring period. These results indicate that the stem bark extract is well tolerated at the investigated doses, with the median lethal dose (LD_{50}) greater than 4000 mg/kg following oral exposure in both species.

Phytochemical screening

Results of qualitative phytochemical screening of ethanol stem bark extract of *Dennettia tripetala*, as presented in Table 1, demonstrated the presence of several bioactive secondary metabolites, including flavonoids, alkaloids, tannins, saponins, and glycosides. These compounds were consistently detected across replicate assays, indicating that the extract contains pharmacologically relevant phytochemical constituents (Table 1).

Effect of hot plate test (Central analgesic activity)

Baseline reaction latency times were comparable among groups prior to treatment administration, indicating uniform nociceptive response across animals, as seen in Table 2. Following treatment, extract-treated groups demonstrated increased reaction latency compared with control animals, indicating reduced sensitivity to thermal pain stimulus.

A dose-dependent increase and time-dependent latency period were observed, with higher extract doses producing greater prolongation of response time. Statistical comparison showed significant increases in reaction latency in treated groups relative to control values at successive observation periods ($p < 0.05$ to $p < 0.001$). The highest extract dose produced latency values approaching those observed in diclofenac-treated animals, indicating strong analgesic effect (Table 2).

Effect of acetic acid-induced writhing test (Peripheral analgesic activity)

Administration of acetic acid produced characteristic abdominal constrictions in control animals, representing peripheral nociceptive response. Animals treated with the extract showed significant ($p < 0.05$) reduction in

number of writhes compared with control group (Table 3).

The reduction in writhing frequency was dose-dependent, with higher extract doses producing greater inhibition of nociceptive responses. Statistical evaluation revealed significant reductions in writhing counts in all treated groups relative to control animals ($p < 0.001$). The highest extract dose demonstrated inhibition comparable to that observed in diclofenac-treated animals, confirming strong peripheral analgesic activity (Table 3).

Effect of radiant heat-tail heat (Central analgesic activity)

Baseline tail withdrawal latency times were similar across groups prior to treatment. Following administration of the extract, animals exhibited prolonged tail withdrawal latency compared with control animals, indicating increased pain threshold. This is presented in Table 4.

Latency increases were dose-dependent, with statistically significant differences observed in treated groups compared with controls ($p < 0.05$). The highest extract dose elicited latency responses comparable to those in morphine-treated animals, indicating substantial central analgesic activity (Table 4).

Table 1: Results of Phytochemical Analysis of ethanol stem bark extract of *D. tripetala*

Phytochemical	Crude extract
Saponins	++ +
Tannins	+++
Carbohydrates	+
Reducing sugars	+
Flavonoids	+++
Alkaloids	+++
Glycosides	+++
Steroids	++
Fats and oils	++
Proteins	++
Acidic compounds	-

- Not present, + Present in small concentrations, ++ Present in moderately high concentration, +++ Present in high concentration

DISCUSSION

The present investigation provides comprehensive evidence demonstrating that the ethanol stem bark extract of *Dennettia tripetala* possesses significant analgesic activity in both peripheral and central nociceptive models.

Table 2: Effect of ethanol stem bark extract of *D. tripetala* on the hot plate test in mice

Group	0 min	30 min	60 min	90 min	150 min
Distilled water (10 ml/kg)	2.45±0.87	1.38±0.13	1.76±1.43	1.30±1.42	1.56±0.76
Morphine (2 mg/kg)	2.32±0.21	9.43±0.71*	12.65±2.00*	16.00±2.44*	16.20±1.32
ETEDT (250 mg/kg)	1.30±3.43	3.72±2.31*	3.80±0.18	4.84±1.66	5.11±2.54
ETEDT (500 mg/kg)	1.41±2.00	5.52±1.20*	6.74±0.59	7.67±0.87*	9.77±3.54
ETEDT (1000 mg/kg)	2.40±0.14	10.00±1.75*	12.38±1.00*	17.10±1.78*	17.75±1.78*

Values presented as Mean ± SEM, * $p < 0.05$ vs control. ETEDT =Ethanol extract of *D. tripetala*, n = 6

Table 3: Peripheral antinociceptive activity of ethanol stem bark extract of *D. tripetala*

Group	Dose (mg/kg)	Number of writhings	Inhibition of writhing (%)
Distilled water		32.1±0.55	
Diclofenac sodium	50	13.8±1.21	58.86***
ETEDT	250	23.7±0.22	37.12*
ETEDT	500	17.4±0.54	53.23**
ETEDT	1000	13.3±0.44	57.22***

Values presented as Mean ± SEM, * $p < 0.05$ vs control. ETEDT = Ethanol extract of *D. tripetala*, n = 6

Table 4: Central antinociceptive activity of ethanol stem bark extract of *D. tripetala* in mice model

Group	Dose (mg/kg)	Reaction time			
		30 min (% elongation)	60 min (% elongation)	90 min (% elongation)	120 min (% elongation)
Control (Distilled water)		6.10±0.47	5.33±0.21	5.12±0.78	5.13±0.96
Morphine	2	15.1±0.21	12.17±0.13	10.21±0.23	9.07±0.11*
ETEDT	250	10.26±0.22	9.43±0.10	8.43±0.379	8.12±0.37*
ETEDT	500	12.01±0.20	10.22±0.18	10.21±0.26	9.19±0.12*
ETEDT	1000	14.6±0.57	12.29±0.29	11.16±0.88	10.±0.58*

Values presented as Mean ± SEM, * $p < 0.05$ vs control. ETEDT =Ethanol extract of *D. tripetala*, n = 6

The results obtained from acetic acid-induced writhing, hot plate, and radiant heat-tail beat tests collectively suggest that the extract modulates pain perception through multiple mechanisms involving both peripheral inflammatory pathways and central nociceptive processing [17].

Pain sensation arises from activation of peripheral nociceptors followed by transmission and processing of signals within the spinal cord and higher brain centres [5]. Peripheral inflammatory mediators such as prostaglandins, bradykinin, histamine, and cytokines play major roles in sensitizing nociceptors, while central mechanisms regulate pain perception and modulation via neurotransmitters and endogenous opioid systems [8]. Therefore, evaluation using multiple models enables determination of whether analgesic effects are peripheral, central, or combined in nature.

The acetic acid-induced writhing model is widely accepted for evaluating peripheral analgesic activity because it mimics visceral inflammatory pain through chemical irritation of the peritoneal cavity. Intraperitoneal injection of acetic acid leads to release of inflammatory mediators, including prostaglandins (particularly PGE2 and PGF2 α), bradykinin, and substance P, which

stimulate nociceptive neurons and produce abdominal constrictions or writhing behaviour. Drugs that inhibit prostaglandin synthesis, such as non-steroidal anti-inflammatory drugs, typically reduce writhing responses in this model [7].

In the present study, administration of ethanol stem bark extract produced significant and dose-dependent reductions in writhing frequency compared with control animals. This observation indicates that the extract interferes with peripheral pain mechanisms, possibly by inhibiting synthesis or release of inflammatory mediators or by stabilizing peripheral nociceptor membranes. Comparable inhibition observed between the highest extract dose (1000 mg/kg) and diclofenac treatment further supports peripheral analgesic activity. Since diclofenac acts primarily by inhibiting cyclooxygenase-mediated prostaglandin synthesis, it is plausible that similar mechanisms may contribute to the extract's effect, although this requires further biochemical confirmation.

The hot plate test evaluates centrally mediated nociceptive responses involving supraspinal integration of pain stimuli [12]. Reaction behaviours such as paw licking and jumping

reflect central processing of thermal pain, and drugs that prolong reaction latency are generally considered to possess central analgesic activity. Opioid analgesics and certain centrally acting drugs typically show effectiveness in this model.

Extract-treated animals exhibited significant prolongation of reaction latency in the hot plate test, with responses increasing progressively with dose. These findings indicate that the extract influences central nociceptive processing, possibly through modulation of neurotransmitter systems or activation of endogenous analgesic pathways. Although the extract did not completely replicate the effect of a potent opioid analgesic like morphine, the latency increases observed suggest meaningful central analgesic potential. Similarly, the radiant heat-tail beat model assesses spinal reflex responses to thermal nociception [12]. Increased tail withdrawal latency following treatment indicates elevated pain threshold at spinal or supraspinal levels. Extract administration resulted in prolonged withdrawal times comparable to standard analgesic treatment, confirming that central analgesic mechanisms contribute to observed effects.

The demonstration of analgesic activity in both peripheral and central models indicates that the extract likely contains multiple bioactive compounds acting synergistically on diverse pain pathways. Plant extracts often contain complex mixtures of secondary metabolites that produce combined pharmacological effects rather than single-target actions typical of synthetic drugs.

Qualitative phytochemical screening conducted in the present study revealed the presence of flavonoids, alkaloids, tannins, saponins, and glycosides, classes of compounds widely reported to exhibit analgesic and anti-inflammatory properties. Flavonoids are known to inhibit enzymes involved in inflammatory mediator production, including cyclooxygenase and lipoxygenase pathways, thereby reducing prostaglandin synthesis [18]. They also possess antioxidant properties that mitigate oxidative stress associated with inflammation and tissue injury [18].

Alkaloids represent another group of compounds known for their interaction with central nervous system receptors, including opioid receptors in certain cases. Such interactions may contribute to central analgesic effects observed in thermal nociception models. Additionally, alkaloids may modulate neurotransmitter release and neuronal excitability, influencing pain perception [18].

Tannins exhibit astringent and anti-inflammatory properties and are capable of forming protective layers over mucosal surfaces, thereby reducing tissue irritation and inflammatory responses [7]. Saponins have also been reported to exhibit anti-inflammatory and membrane-stabilizing properties, which could contribute to analgesic effects by reducing inflammatory mediator release [10].

The presence of multiple analgesic-related phytoconstituents suggests that synergistic interactions among these compounds may enhance therapeutic efficacy. Such synergy may also reduce the likelihood of adverse effects compared with single-compound drugs. Dose-dependent increases in analgesic response observed across all models further support pharmacological relevance of the extract. Gradual improvement in response to increasing dose suggests that sufficient concentrations of active constituents are required to achieve therapeutic effects. However, beyond certain levels, additional increases may not proportionally enhance activity, indicating potential plateau effects or pharmacokinetic limitations [11].

Traditional use of *Dennettia tripetala* stem bark for management of painful conditions gains scientific credibility through these findings. In many West African communities, the plant is consumed both as food spice and medicinal remedy, suggesting relative safety at commonly used doses. Nonetheless, systematic toxicity and chronic exposure studies are necessary before clinical application can be recommended [18].

Pain management remains a significant global health concern, particularly in regions with limited access to modern healthcare services. Plant-derived analgesics offer potential advantages including affordability, accessibility, and cultural acceptability. Identification and validation of medicinal plants with analgesic potential may contribute to development of novel therapeutic agents or complementary treatment strategies [1].

Limitations of this study

Despite promising results, certain limitations of the present study should be acknowledged. The investigation employed crude extract rather than isolated compounds, making it difficult to determine specific active constituents responsible for analgesic effects. Furthermore, mechanisms underlying analgesic activity were inferred from pharmacological models rather than directly measured through biochemical or

receptor studies. Future investigations involving isolation of bioactive compounds, receptor-binding studies, and molecular pathway analysis would provide deeper understanding of analgesic mechanisms.

Additionally, evaluation of anti-inflammatory biomarkers, oxidative stress parameters, and neurotransmitter interactions could further elucidate mechanisms underlying analgesic effects. Long-term toxicity, pharmacokinetic profiling, and interaction studies with conventional drugs are also essential before therapeutic development.

Overall, the findings demonstrate that ethanol stem bark extract of *Dennettia tripetala* exhibits substantial analgesic activity mediated through both peripheral and central mechanisms. Combined pharmacological effects likely arise from interactions among multiple phytochemicals present within the extract. Scientific validation of traditional medicinal use supports continued exploration of the plant as a potential source of novel analgesic compounds.

CONCLUSION

Ethanol stem bark extract of *Dennettia tripetala* exhibits significant central and peripheral analgesic effects in various models. The activity appears dose-dependent and comparable to standard analgesic drugs. The presence of multiple bioactive phytochemicals likely contributes to the observed effects. These findings support traditional use of the plant for pain management and highlight its potential as a source of novel analgesic agents.

DECLARATIONS

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Ethics approval

The Animal Ethics Committee of the College of Health Sciences, Kampala International University, Tanzania, approved the use of animals in this study (KIU/2025/11)

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and

clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. JOS, AAT and JOT conducted the actual study, carried out the statistical analysis, and wrote the manuscript. JOS was involved in developing the idea, designing the study, and writing the manuscript. AAT and JOT were involved in developing the idea and designing the study. All authors approved the submitted final version of the manuscript.

REFERENCES

1. Brunton LL, Hilal-Dandan R, Knollmann BC. Goodman & Gilman's *The Pharmacological Basis of Therapeutics*. 13th ed. McGraw-Hill; 2018.
2. Calixto JB. Twenty-five years of research on medicinal plants in pain control. *Pharmacol Res* 2009; 60: 1–8.
3. Oyepata JS, Opeyemi JT. Evaluation of proliferative potential of *Musa paradisiaca* stem juice on the pancreatic cells of Wistar rats. *Trop J Nat Prod Res* 2024; 8(1): 6035–6038. <http://www.doi.org/10.26538/tjnpr/v8i1.46>
4. Oyemitan IA, et al. Antinociceptive and anti-inflammatory effects of essential oil of *Dennettia tripetala*. *J Ethnopharmacol* 2010; 130: 191–197.
5. Burkill HM. *The Useful Plants of West Tropical Africa*. Vol.1. Royal Botanic Gardens, Kew; 1985.
6. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books; 1993.
7. Gill LS. *Ethnomedicinal Uses of Plants in Nigeria*. University of Benin Press; 1992.
8. Joseph OS, Joseph OT. Hematological, biochemical and histological evaluation of sub-chronic administration of ethanol. *JOPAT* 2025; 23(1): 1361–1375. <https://dx.doi.org/10.4314/jopat.v23i1.11>

9. Okolie NP, et al. *Phytochemical and biological evaluation of Dennettia tripetala*. *BMC Complement Altern Med* 2016; 16: 1–10.
10. Harborne JB. *Phytochemical Methods*. 3rd ed. Chapman & Hall; 1998.
11. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC): National Academies Press (US); 2011. PMID: 21595115
12. Organisation for Economic Co-operation and Development. *OECD Guideline for Testing of Chemicals 425: Acute Oral Toxicity—Up-and-Down Procedure*. Paris; 2008.
13. Oyepata JS, Opeyemi JT, Adekunle AT, Moses OA. *Effect of Pterocarpus santalinus ethanol leaf extract on haematological, histopathological and lipid profile indices in Wistar rats*. *Trop J Nat Prod Res* 2024; 8(3): 6681-6685. <https://doi.org/10.26538/tjnpr/v8i3.30>
14. Joseph OS, Joseph OT, El-Gazzar AM, Mahmoud MH, Omoirri MA, Gaber ESB. *Toxicological evaluation of ethanol leaf extract of Pterocarpus santalinus on lungs, stomach, brain and hematological parameters of Wistar rats*. *Cogent Food Agric* 2024; 10(1): 2303828. <https://www.tandfonline.com/doi/full/10.1080/23311932.2024.2303828>
15. D'Amour FE, Smith DL. *A method for determining loss of pain sensation*. *J Pharmacol Exp Ther* 1941; 72: 74–79.
16. Koster R, Anderson M, De Beer EJ. *Acetic acid for analgesic screening*. *Fed Proc* 1959; 18: 412.
17. Rang HP, Ritter JM, Flower RJ, Henderson G. *Rang and Dale's Pharmacology*. Elsevier; 2020.
18. Simeon JO, Tosin JO. *Toxicological evaluation of sub-chronic administration of ethanol leaf extract of Ocimum canum on lipid parameters, heart histology and feed consumption of Wistar rats*. *Caliphate J Sci Technol* 2024; 6(1): 70-76.