

Anxiolytic and Peripheral Analgesic Effects of *Myrianthus Arboreus* Stem Bark Extract and its Fractions in Mice.

Adebayo M.A¹, Akinpelu L.A^{2*}, Sanusi A³, Aliyu A⁴, David S.M⁴, Shittu A.O⁵,
Anifowoshe K.V¹, and Mukaila F.S.¹

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¹Department of Pharmacognosy, College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria.

²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Kwara State, Nigeria.

³Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Ilorin, Kwara State, Nigeria.

⁴Department of Pharmaceutical Microbiology & Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.

⁵Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, Kwara State, Nigeria.

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ABSTRACT

Myrianthus arboreus stem bark is used as an anti-anxiety and pain reliever in traditional medicines. However, scanty scientific reports are available to corroborate these ethnomedicinal claims. Hence, this study investigated the anxiolytic and peripheral analgesic potentials of methanol stem bark extract and fractions of *Myrianthus arboreus* in mice. The anxiolytic potential was evaluated using elevated plus maze while the peripheral analgesic effect was investigated using mouse model of acetic acid induced writhing test. The Fourier-transform infrared analysis of the dichloromethane fraction was also carried out. The *Myrianthus arboreus* extract (125-500 mg/kg), dichloromethane and aqueous fractions at 250 mg/kg significantly ($p < 0.05$) reduced the anxiety indices of mice compared to control, consistent with an anxiolytic effect. The crude extract and all the investigated fractions significantly ($p < 0.05$) reduced the number of abdominal constrictions elicited by acetic acid compared to control, consistent with peripheral analgesic potentials. The Fourier-transform infrared analysis showed several functional groups in the dichloromethane fraction. The hydroxyl functionality in this fraction may be an integral part of some phenolic compounds such as flavonoid and tannins. This study concluded that *Myrianthus arboreus* may possess secondary plant metabolites responsible for its anxiolytic and peripheral analgesic potentials.

Keywords: *Myrianthus arboreus*, dichloromethane fraction, Fourier-transform infrared analysis, anxiolytic, analgesic

Corresponding Author: Akinpelu, L.A.

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences,
University of Ilorin, Kwara State, Nigeria

Email: akinpelu.la@unilorin.edu.ng, Tel: +2348038590621



Introduction

The concomitant occurrence of chronic pain with anxiety is a major public health issue culminating in routine screening in clinical settings, specialty care, and development of innovative treatment (De La Rosa *et al.*, 2024).

Treatment of anxiety and pain with the currently available synthetic agents are associated with numerous side effects (Chen *et al.*, 2017; Akinpelu *et al.*, 2019). For instance, Phootha *et al.* (2022) reported drug dependence, abuse liability, amnesia, sleep disturbances and sexual dysfunction, among others, as untoward effects associated with anxiolytics. Most known and currently used pain relievers are also known to produce undesirable effects such as renal damage, gastrointestinal disturbances, and respiratory depression among others (Woode and Abotsi, 2011).

Due to the several side effects associated with modern synthetic drugs, herbal drugs have gained acceptance from the scientific world in recent years as a dependable source of alternative drugs with minimal or no undesirable effects (Sobhani *et al.*, 2017; Mohammadi *et al.*, 2018).

Medicinal plants such as *Lavandula angustifolia* and *Chamomilla recutita* have been scientifically validated for their anxiolytic and antinociceptive potentials based on their long history of use in ethnomedicine (Chaves *et al.*, 2020; Lejeune *et al.*, 2023). Numerous plants used in folkloric medicine have been evaluated for their anxiolytic and analgesic potentials (Oyemitan *et al.*, 2014; Adebayo *et al.*, 2020; Ayanaw *et al.*, 2023; Akinpelu *et al.*, 2024). However, many have been evaluated but thousands are yet to be validated for the pharmacological basis of their action (Alonso-Castro *et al.*, 2018). One of such plants with the ethnomedicinal claims as pain reliever and in management of the panic attack is *Myrianthus arboreus* (*M. arboreus*) (Bobuya *et al.*, 2023).

Myrianthus arboreus P. Beauv otherwise called giant yellow mullberry or monkey fruits is a shrub or tree plant which belongs to the Cecropiaceae or Urticaceae family (Bemmo *et al.*, 2023). It is widely spread in Tropical Africa where it grows up to 20 m in length (Bemmo *et al.*, 2023). The young leaves are eaten in vegetable soups, the fruit is eaten raw and the sweet pulp around the seed is edible (Olonode *et al.*, 2015).

Myrianthus arboreus was reported to be rich in therapeutic plant secondary metabolites such as total phenols, alkaloids, saponins, flavonoids, tannins and glycosides among other bioactive molecules (Awodi *et al.* 2017; Harley *et al.*, 2020; García-Pérez *et al.*, 2023). The different parts of the plant such as the stem bark, leaves and root are employed as traditional remedies against back pain, fever, panic attack, dysentery, epilepsy, diarrhea, eye aches, malaria, bronchitis, hernia and heart diseases (Olonode *et al.*, 2015; Bemmo *et al.*, 2023; Bobuya *et al.*, 2023).

Pharmacologically, the bark, leaves and roots of *M. arboreus* have demonstrated analgesic (Olonode *et al.*, 2015), wound healing and anti-infective (Agyvare *et al.*, 2014), antioxidant (Awodi *et al.*, 2017), gastroprotective (Agu *et al.*, 2023) antidiabetic (Tijani *et al.*, 2022) and used to treat female infertility (Mvondo *et al.*, 2020).

This study was designed to examine the health-beneficial effects of methanol stem bark extract and fractions of *M. arboreus* on anxiety and pain models in mice. Methanol was used as extractive solvent because of its high extractive index in comparison to other solvents (Chatepa *et al.*, 2024). Upon the establishment of anxiolytic and analgesic effects of the extract, the extract was partitioned with solvents of wide polarities to determine the most active fraction. Fourier-transform infrared analysis of most active fraction was carried out. Put together, the study aimed at providing scientific basis for the plant's long history of use against pain and panic attack (anxiety) in traditional medicines (Bobuya *et al.*, 2023).

Materials and Methods

Plant identification, collection and authentication

Myrianthus arboreus stem bark was collected within Igbinedion University Okada campus. The identification and authentication was done by Mr. Adebayo Muritala Ayofe of Department of Pharmacognosy, Prof. Dora Akunyili College of Pharmacy, Igbinedion University Okada. A voucher sample (*Myrianthus arboreus* IUO/18/189) was deposited.

Preparation of *Myrianthus arboreus* extract and fractions

Myrianthus arboreus stem bark was cut into pieces to facilitate drying and grinding. The chopped stem barks were air dried in the laboratory, pulverized and 300 g of the ground powder was extracted with 500 ml of methanol for 4 hours using the Soxhlet extractor and concentrated *in vacuo* and subsequently freeze-dried to yield 6 g of crude extract coded MSMA. A weighed 5.2 g of the methanol extract of *Myrianthus arboreus* was successively partitioned with n-hexane, dichloromethane, n-butanol and water to yield n-hexane (HFMA 0.2 g), dichloromethane (DCMA 0.45 g), n-butanol (BFMA 0.57 g) and aqueous (AFMA 2.71 g) fractions of *Myrianthus arboreus*. The various fractions of the extract were allowed to dry in a desiccator where it was stored before use.

Spectroscopic analyses of dichloromethane fraction of *Myrianthus arboreus*

The FT-IR spectrum of DCMA was obtained after mixing the fraction with the spectra-grade potassium bromide (KBr) in the ratio of 1:100 and introduced into the sample holder of Perkin Elmer Spectrophotometer. The spectrophotometer was operated in the range 4000 – 300 cm⁻¹ to detect the spectra data of the different functional groups inherent in the extract (Adebayo *et al.*, 2020).

Animals

Male adult mice (18-20 g) were purchased from the Central Animal House of the Igbinedion University, Okada, Edo State, Nigeria. They were housed in well-ventilated and lit room maintained under natural 12-hour light/12-hour dark condition. The mice had unrestricted access to clean drinkable water and standard commercial diet (Guinea feeds brand, Bendel Feeds, Benin City, Edo State, Nigeria). The experimental protocols followed in this study were in strict adherence to the set guidelines by the Igbinedion University Animal Ethical Committee which is in strict compliance to the United State National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

Drugs and chemicals

Diazepam (Roche, Basel, Switzerland), Dimethylsulfoxide (DMSO) (Sigma Aldrich, St. Louis, MO, USA), diclofenac (Supreme Pharm Nig Ltd Lagos, Nigeria), acetic acid (BDH Chemicals Ltd, Poole, England) and normal saline (Unique Pharmaceutical Limited, Lagos, Nigeria) were employed in this study. Plant extract and fractions were dissolved with 1% DMSO and made up to the required volume with normal saline.

Evaluation of anxiolytic effect of extract on elevated plus mazes (EPM)

Eighty-five male adult mice were used for the anxiolytic behavioural assays of *M. arboreus* extract and fractions using elevated plus maze as previously described (Adebayo *et al.*, 2020; Akinpelu *et al.*, 2024). Briefly, mice were randomized into 17 groups/n = 5. Group 1 mice (control group) received physiological saline (10 mL/kg, p.o.) only. Test groups 2-4 received graded doses of *M. arboreus* stem bark extract (125, 250 and 500 mg/kg) respectively, groups 5- 7 mice received HFMA as indicated above for the extract, groups 8-10 received DCMA at the same doses as

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used for the crude extract above, groups 11-13 mice received BFMA (125, 250 and 500 mg/kg), groups 14-16 received AFMA (125, 250 and 500 mg/kg) respectively, while group 17 received diazepam (1 mg/kg, i.p.) and served as positive control drug. One hour after oral dosing as above in groups 1 to 16 and 30 minutes following intraperitoneal injection of diazepam in group 17, each mouse was individually placed at the middle of the EPM, with the head facing one of the open arm of the EPM. The number of entries into and duration on the open arm of EPM was noted for 5 minutes. The percentage numbers of open arm entries and the percentage duration spent exploring the open arm of the EPM were calculated as earlier done using the formula below:

Percentage open arm entry (% OE) = [(number of open arm entries/ Total number of entries)] × 100

Percentage open arm time (% OT) = [(Time spent in open arm / Total time spent on EPM)] × 100

The open arm avoidance index (OAAI) interpreted as anxiety index was calculated as earlier done using the two parameters calculated above as below (Aiyelero *et al.*, 2023).

Open arm avoidance index (OAAI) = 100 – [(% OE + % OT)] / 2]

Evaluation of analgesic effect of *Myrianthus arboreus* extract and fractions in mice

Another fresh eighty-five male adult mice were used for the analgesic behavioural assays of *M. arboreus* extract and fractions using acetic acid for pain induction in mice as previously reported (Oyemitan *et al.*, 2014). The groupings and dosing of mice were carried out as above for anxiolytic behavioural assays. One hour after oral dosing as above in groups 1 to 16 and 30 minutes following intraperitoneal injection of diclofenac (100 mg/kg, i.p.) in group 17 which served as positive control group. Each mouse was intraperitoneally injected with 1% acetic acid (10 mL/kg). The number of abdominal constrictions elicited by each mouse

was counted with a manual counter and recorded for a time course of 20 minutes beginning from 5 minutes post acetic acid injection.

Statistical analysis

Experimental data were depicted as means ± SEM. The data were analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test. The significance level was set at $p < 0.05$.

Results

Effect of *Myrianthus arboreus* extract and fractions of *Myrianthus arboreus* on percentage number of open arm entry in mice

The crude extract, dichloromethane and aqueous fractions at all the evaluated doses significantly ($p < 0.05$) increased the percentage number of entry into open arm of the EPM when comparison was made to control. The n-hexane fraction at 125 mg/kg and n-butanol fraction at 250 and 500 mg/kg significantly ($p < 0.05$) increased the percentage number of entry into open arm of the EPM compared to control (Figure 1).

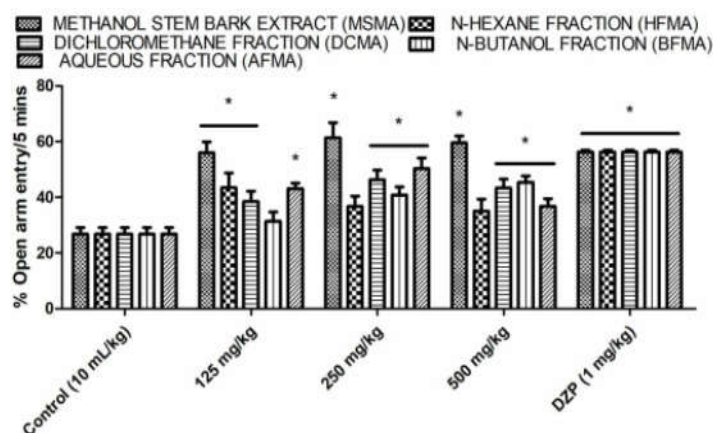


Figure 1: Effect of *Myrianthus arboreus* methanol extract and fractions on percentage open arm entry in mice.

Each bar represents Mean $\pm$ SEM, $n=5$, one-way ANOVA; Dunnett's post hoc test, $*p<0.05$ compared to control.

Effect of methanol extract and fractions of *Myrianthus arboreus* on percentage number of open arm duration in mice.

The crude extract at all the evaluated doses, dichloromethane and aqueous fraction at 250 mg/kg significantly ($p<0.05$) increased the percentage duration spent on the open arm of the EPM compared to control (Figure 2).

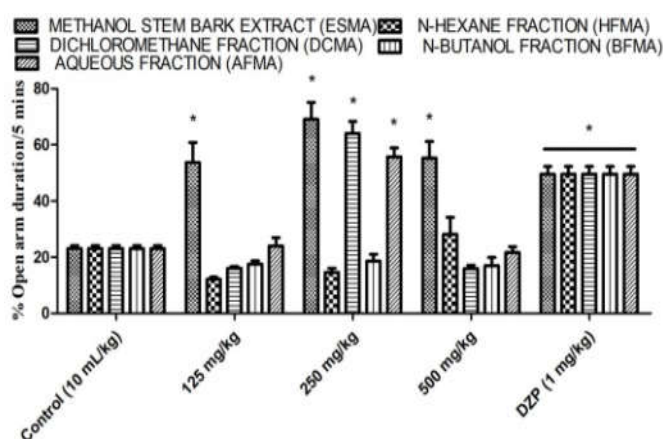


Figure 2: Effect of *Myrianthus arboreus* methanol extract and fractions on percentage open arm duration in mice.

Each bar represents Mean $\pm$ SEM, $n=5$, one-way ANOVA; Dunnett's post hoc test, $*p<0.05$ compared to control.

Effect of methanol extract and fractions of *Myrianthus arboreus* on anxiety index in mice.

The crude methanol extract at all the evaluated doses, dichloromethane and aqueous fraction at 250 mg/kg significantly ($p<0.05$) reduced the anxiety indices of mice in comparison to mice in control group. Of all the assessed doses, the extract at 250 mg/kg gave the lowest anxiety-reducing effect in mice. Similarly, the dichloromethane and aqueous fraction of the extract only reduced the

anxiety indices of mice at 250 mg/kg (Figure 3).

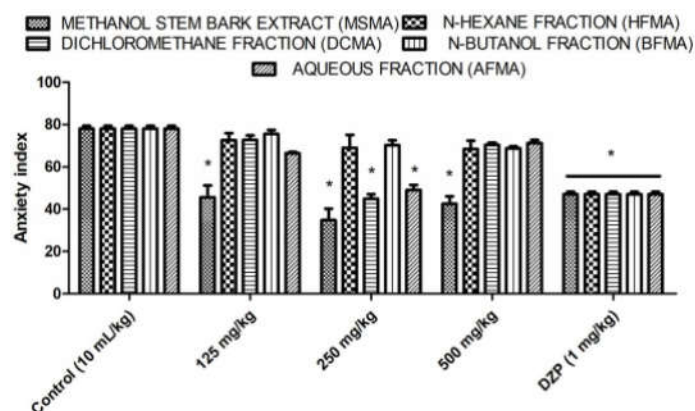


Figure 3: Effect of *Myrianthus arboreus* methanol extract and fractions on anxiety indices of mice.

Each bar represents Mean $\pm$ SEM, $n=5$, one-way ANOVA; Dunnett's post hoc test, $*p<0.05$ compared to control.

Effect of crude extract and fractions of *Myrianthus arboreus* on number of abdominal constrictions elicited by acetic acid in mice.

The extract and fractions (125 - 500 mg/kg) significantly ($p<0.05$) reduced the number of abdominal writhes induced by acetic acid in mice in comparison to mice in control group (Figure 4).

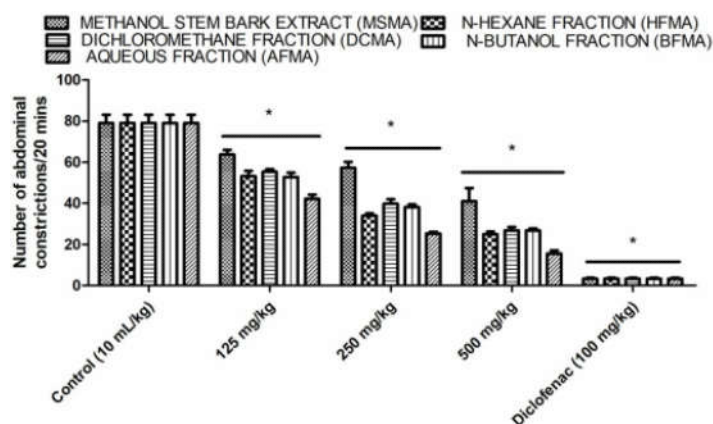


Figure 4: Effect of crude extract and fractions of *Myrianthus arboreus* on number of abdominal constrictions elicited by acetic acid in mice

Each bar represents Mean $\pm$ SEM, $n=5$, one-way ANOVA; Dunnett's post hoc test, $*p<0.05$ compared to control.

Results of FT-IR analyses

The FT-IR spectral analysis of DCMA showed several signals that correspond to various functional groups. Notably, the peak at 3332.2 cm^{-1} suggests O-H stretching, indicating the presence of alcohols or phenolic compounds. The peaks at 2922.2 cm^{-1} and 2855.1 cm^{-1} are associated with C-H stretching vibrations of methylene (CH_2) and methyl (CH_3) groups within aliphatic hydrocarbons. Additionally, the peak at

1688.5 cm^{-1} is indicative of C=O stretching, commonly observed in carbonyl compounds. The aromatic character of the fraction is supported by the peaks at 1602.8 cm^{-1} and 1513.3 cm^{-1} , which correspond to C=C stretching vibrations. Further analysis revealed additional peaks at 1461.1 cm^{-1} and 1379.1 cm^{-1} , which indicate CH_2 and CH_3 bending vibrations, respectively. The spectral range from 1319.5 cm^{-1} to 1159.2 cm^{-1} suggests C-O stretching, depicting the occurrence of ether or alcohol functionalities. Moreover, peaks at 827.5 cm^{-1} and 719.4 cm^{-1} confirmed out-of-plane bending vibrations of aromatic C-H bonds, while the low-frequency peak at 603.8 cm^{-1} may correspond to deformation modes associated with aromatic systems (Figure 5).

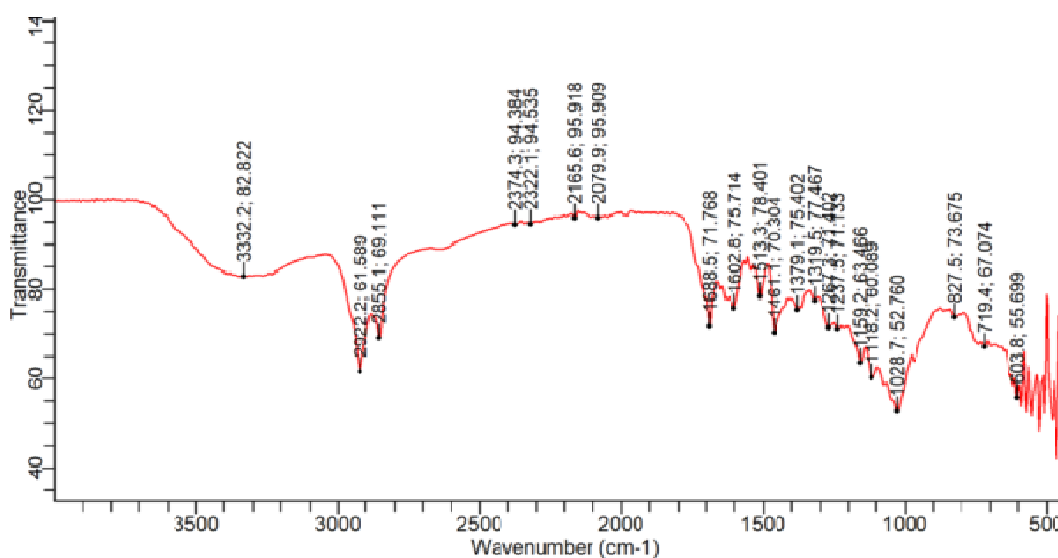


Figure 5: FT-IR analysis of the DCMA fraction of *Myrianthus arboreus* extract

Discussion

The present study assessed the anxiolytic and analgesic potentials of methanol stem bark extract and fractions *Myrianthus arboreus*

using elevated plus maze and acetic acid-induced abdominal constrictions in mice. The findings of this study showed that the extract and fractions may possess anxiolytic and peripheral analgesic potentials.

The EPM has been used as a rodent model of anxiety to evaluate anxiolytic potentials of medicinal plants (Adebayo *et al.*, 2020; Akinpelu *et al.*, 2024). The increase in number of open arm entries, and duration of stay on the open arm of EPM as well as the reduction in anxiety indices by the crude extract, dichloromethane and aqueous fractions of the extract suggest anxiolytic potentials. The extract may therefore be exerting its anxiolytic effect via GABA_A-benzodiazepine receptor neurotransmission since EPM has been reported to be sensitive to agents acting via this pathway (Murade *et al.*, 2021). Our finding is in conformity with earlier medicinal plant extracts reported to demonstrate anxiolytic potentials by increasing the parameters of open arm entry and duration of stay on the open of the elevated plus maze in mice (Adebayo *et al.*, 2020; Akinpelu *et al.*, 2024).

Acetic acid induced abdominal constriction is a very sensitive test for the detection of antinociceptive effects of agent at dose levels which may appear non detectable in other pain assessment techniques such as hot plate test (Ayanaw *et al.*, 2023). The increase in abdominal constrictions observed in control mice following the administration of acetic acid suggest the induction of algesia in mice. Acetic acid causes algesia via indirect stimulation of peritoneal nociceptors leading to the release of endogenous substances which stimulate nerve endings, thus, creating a great concentration of PGE2 and PGF2a in peritoneal fluid (Sharma *et al.*, 2019). Thus, analgesic agents working via similar mechanism as diclofenac could work to block the prostaglandin synthesis via the inhibition of cyclooxygenase enzymes (Hasan *et al.*, 2023). The reduction in abdominal constrictions by the methanol extract of *M. arboreus* and all the investigated fractions of the extract may suggest peripheral analgesic effects. It is probably therefore that the plant may contain

bioactive principle(s) which may have modulatory effect on the liberation of inflammatory mediators (histamine, prostaglandin, bradykinin, substance P, TNF- α , IL-6, and COX) triggered by acetic acid administration (Lin *et al.*, 2024; Nhung and Quoc, 2024). This medicinal plant may be interfering with these peripheral pain pathways (Jang *et al.*, 2020; Nhung and Quoc, 2024).

Collectively, this fraction indicates the presence of phytoconstituents with hydroxyl, aliphatic, carbonyl, and aromatic functionalities, which may probably be phenolic, carbohydrates, terpenoidal or steroidal compounds aligning with established analytical standards in organic compound characterization as reported in the literature (Nandiyanto *et al.*, 2023; Sanusi *et al.*, 2024). Some pharmacological activities such as anxiolytic, analgesic, antipyretic, anti-inflammatory and other biological activities have been linked to the presence of certain bioactive constituents such as flavonoids, alkaloids, terpenoids, carbohydrates and steroids in plants (Phootha *et al.*, 2022; Julie *et al.*, 2025; Sanusi *et al.*, 2025). The observed activity might be attributed to phytocompounds found in the plant extract and fractions.

Conclusion

This study concluded that the stem bark extract and fractions of *Myrianthus arboreus* may possess anxiolytic and peripheral analgesic effects. However, further studies may be carried to isolate and characterize the bioactive principle(s) responsible for its observed analgesic and anxiolytic effects.

Conflict of Interest

The authors declare no conflict of interest.

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