



Research article

In vivo anti-inflammatory, anti-nociceptive, and *in vitro* antioxidant efficacy, and acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *Lonchocarpus eriocalyx* (Harms.)

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ABSTRACT

Oxidative stress causes and drives many agonising inflammatory conditions, which cause disability, financial burden, and emotional stress. The current anti-inflammatory, analgesic, and antioxidant agents are associated with adverse effects, inaccessibility, high costs, and low efficacies, thereby warranting the need for alternatives, especially from natural sources. *Lonchocarpus eriocalyx* plant is traditionally used in Kenyan communities to treat various inflammatory and oxidative stress-associated diseases; however, its pharmacologic efficacy and safety have not been empirically validated, hence this study. The *in vivo* antiinflammatory and antinociceptive efficacy of the aqueous and methanolic stem bark extracts of *L. eriocalyx* were determined using the xylene-induced ear oedema, and the acetic acid-induced writhing techniques, respectively, in experimental mice. Also, *in vitro* antioxidant activities of the studied plant extracts were investigated using the Thiobarbituric acid test for lipid peroxidation, 1, 1-diphenyl -2-picrylhydrazyl (DPPH), and Ferric reducing antioxidant power standard assay methods. Moreover, the studied extracts' acute oral toxicity effects were investigated according to the Organisation for Economic Corporation and Development (OECD) guidelines. The studied plant extracts showed significant dose-dependent inhibitions of oedema and writhing, depicting their anti-inflammatory and antinociceptive efficacy. Besides, the extracts revealed significant inhibitions of *in vitro* lipid peroxidation in varying degrees. Notably, the extracts demonstrated very strong DPPH radical scavenging and ferric-reducing antioxidant efficacies. Furthermore, the two studied plant extracts did not elicit acute oral toxicity, with LD₅₀ values of >2000 mg/kg BW, hence were considered safe. The anti-inflammatory, antinociceptive, and *in vitro* antioxidant efficacies of these extracts were attributed to antioxidant phytochemicals with diverse pharmacologic effects, especially through the amelioration of oxidative stress. Further studies on the anti-inflammatory, antinociceptive and antioxidant mechanism(s) and isolation and characterisation of responsible compounds are encouraged to spur the development of affordable, accessible, safe, and efficacious drugs.

1. Introduction

Traditional and complementary medicine plays an integral role in healthcare systems, with over 80 % of the world population, especially in Africa and Asian continents, relying on it for their day-to-day healthcare needs [1]. Herbals contain bioactive principles with broad spectra of pharmacological activities against many diseases, and have been utilised as leads for drug discovery and development [2, 3].

Inflammation is a tissue's response to noxious stimuli, such as irritants and pathogens [4]. It causes increased vascular permeability, changes in blood flow, and migration of leukocytes to the affected site, manifesting in pain, swelling, redness, heat, and loss of function of the inflamed tissue [4]. Pain and inflammation constitute the major manifestation of many pathological processes in the body, including cancer, diabetes, arthritis, among other debilitating inflammatory disorders [5]. Even though various anti-inflammatory, antinociceptive, and antioxidant drugs are

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available [6], these medications are arguably inaccessible, unaffordable, have low efficacies, and cause adverse effects [7, 8].

Oxidative stress, a phenomenon characterised by excessive production of reactive oxygen and nitrogen species and ineffective quenching/stabilisation in the body, damages essential biomolecules (proteins, lipids, and nucleic acids) [9, 10]. Oxidative damage to biomolecules triggers and exacerbates many human diseases and disorders, like cancer, diabetes, cardiac disorders, neurodegenerative disorders, and inflammation [9, 10]. Plant-derived polyphenolic, and associated antioxidant phytochemicals exhibit the broadest spectrum of bioactivity, including anti-inflammation, primarily by quenching and ameliorating oxidative stress in biological systems, thereby restoring health [9]. Consequently, research focus has shifted to investigating natural products, especially medicinal plants, as one of the most promising source of therapeutic agents for pain and inflammation [11].

Even though medicinal plants have extensive and longstanding utilisation in alternative and complementary therapy, various concerns regarding their safety have been raised [12]. For instance, no clear guidelines govern traditional medicine practice, thus allowing unscrupulous practitioners to thrive [13]. Additionally, scientific data on herb-herb and herb-drug interactions and associated effects are scanty, thus hampering effective prescriptions [14]. Moreover, there are no clearly outlined dosage forms of herbal preparations for specific diseases and expected side effects [15]. Furthermore, the lack of safety and toxicity profiles of many medicinal plants further cripples the confidence accorded to herbal medicine. As a result, it is imperative to evaluate the toxicity and safety of herbal preparations used to manage various diseases to avert the development of undesirable effects and fatalities [12, 15].

Therefore, the present study investigated the acute oral toxicity, *in vivo* anti-inflammatory, antinociceptive, and *in vitro* antioxidant effects of aqueous and methanolic stem bark extracts of *L. eriocalyx* as potential sources of efficacious, safe, accessible, and affordable anti-inflammatory, antinociceptive, and antioxidant drug agents. This plant was selected based on the ethnomedical background of its utilisation in the management of oxidative stress-related conditions like diabetes mellitus, high blood pressure, ulcers, wounds, and eye infections, by the Kenyan traditional medicine practitioners [16]. Despite its medicinal use, scientific data on its safety, anti-inflammatory, antinociceptive, and antioxidant efficacy is scanty, hence the present study.

2. Materials and methods

2.1. Collection and preparation of plant materials

Fresh stem barks of *L. eriocalyx* were collected from Cianyri village situated in Mbeere North Sub-County in Embu County, Kenya, where the plant grew naturally. The plant was selected for this study based on its traditional medicinal use by the local people. Preliminary identification was done based on its local name (*Muthigiriri*) by a renowned herbalist, after which; a voucher specimen was prepared for taxonomic characterisation and authentication at the Department of Plant Sciences, Kenyatta University. Voucher specimen number GM/002/2017 was assigned, and a duplicate specimen was prepared and deposited at the University herbarium for future reference. The collected stem barks of *L. eriocalyx* were then chopped into small pieces and broadcasted to dry under a shade at room temperature for two weeks with daily grabbing for proper drying. After that, they were ground using an electric mill (Christy and Norris Ltd., England) into a powder which was kept in a labelled khaki envelope before extraction.

2.2. Methanolic and aqueous extraction

To obtain the methanolic extract, about 0.2 kg of the powdered stem bark of *L. eriocalyx* were macerated in a 2-litre conical flask (Borosil®) containing 0.75 L of analytical grade methanol (CAS number 67-56-1;

Sigma Aldrich; Germany) for two days. Thereafter, the menstruum was separated by decantation and filtration through Whatman filter paper No.1. (Lot No# 221175). The process was repeated thrice, and the menstruum portions were combined and then concentrated *in vacuo* using a Heidolph Laborota 4001 rotary evaporator (Serial No# 0711-1187) at 50 °C [17], transferred into pre-weighed, clean, dry, labelled universal glass bottles. Further drying was done in a hot-air oven set at 35 °C for five days [18, 19].

For the aqueous extract, 50 g of powdered *L. eriocalyx* bark was soaked in 0.5 L of distilled water and heated at 60 °C for five minutes before being cooled to room temperature. The menstruum was filtered and transferred into clean freeze-drying flasks, which were then fitted into a Modulyo Freeze Dryer (Cat No# SuperModulyo230; Edwards-England) for lyophilisation for two days. The dry and lyophilised extracts were transferred into clean, dry, pre-weighed, and labelled universal glass bottles. The percentage yields of respective extracts were calculated using Eq. (1) [19], covered and stored in a refrigerator at 4 °C, and retrieved only during use [18, 19].

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of macerated power}} \times 100 \quad (1)$$

2.3. Investigation of *in vivo* anti-inflammatory, antinociceptive, and acute oral toxicity effects of the studied plant extracts

2.3.1. Experimental animals

Female Swiss albino mice aged 4–5 weeks and weighing 25 ± 2 g were sourced from the small animal breeding unit of the Kenya Agricultural and Livestock Research Organisation (KALRO) and kept in polypropylene cages with dimensions of 30 cm × 20 cm × 13 cm under standard conditions of 12-hour day and 12-hour night cycle and 360 lux lighting. The cages were furnished with softwood shavings as bedding material for the animals. They were provided with standard rodent pellets and clean drinking water *ad libitum* and were acclimatised to the laboratory settings for 72 h before experimentation. Appropriate procedures for experimental animal manipulation, handling, care, and disposal outlined by the National Research Council [20] were followed. The Kenya National Commission for Science, Technology, and Innovation (NACOSTI) reviewed, approved, and authorised this study (NACOSTI/P/19/2080 and NACOSTI/P/19/2442).

2.3.2. Preparation of administration doses

We selected four dose levels for anti-inflammatory and antinociceptive assays (4 mg/kg BW, 20 mg/kg BW, 100 mg/kg BW, and 500 mg/kg BW) of the studied plant extracts based on a prior pilot study. A 500 mg/kg BW stock dose of the aqueous and methanolic stem bark extracts of *L. eriocalyx* was prepared in normal saline according to the Organization for Economic Co-operation and Development (OECD) guidelines [21] described by Erhierhie *et al.* [22] as demonstrated in the study of Olela *et al.* [11]. The subsequent doses were prepared by serial dilution technique from the stock dose. The reference drugs (Dexamethasone (Code: PL39699/0056) and Acetylsalicylic acid (Lot No# ARO40C) were prepared using the same procedure as that for the plant extracts. The dose levels used in acute oral toxicity assays selected according to the OECD criteria [21] and were prepared individually at each step of the assay using the same procedure. All drugs were freshly prepared and administered appropriately as the experiment. Normal saline was used as the vehicle and placebo in this study.

2.3.3. Determination of *in vivo* anti-inflammatory effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

The *in vivo* anti-inflammatory efficacy of the aqueous and methanolic stem bark extracts of *L. eriocalyx* was determined using the xylene-induced ear oedema technique described by Igbe *et al.* [23] and modified by Olela *et al.* [11]. Briefly, for each studied plant extract, experimental mice were randomly assigned into seven experimental groups

(I-VII) comprising of 5 animals each. Mice in groups I-IV were orally administered with the respective extracts at doses of 4 mg/kg BW, 20 mg/kg BW, 100 mg/kg BW, and 500 mg/kg BW, respectively. Groups V and VI received the reference drug (1 mg/kg BW of Dexamethasone) and normal saline (10 ml/kg BW), respectively. Group VII mice were orally administered with normal saline at a dose of 10 ml/kg BW. After 45 min, the mice in all the experimental groups except those of the normal control group were smeared with one drop of xylene (CAS No# 95-47-6; Sigma Aldrich, Germany) on the right ear's inner pinna to induce oedema. Besides, one drop of normal saline was smeared on the inner pinna of the left ears of all experimental mice and the right ear of the normal control mice. The mice were observed for 15 min after which, they were anaesthetised with diethyl ether (CAS No# 60-29-7; Sigma Aldrich, Germany) and 6 mm diameter sections of the right ears (oedematous) and left ears (control) carefully dissected and accurately weighed using an analytical weighing machine (Model No# Shimadzu ATY224). The obtained weights were used to calculate the percentage inhibition of xylene-induced ear oedema as an indicator of anti-inflammatory activity using Eq. (2) [23], as follows.

$$\% \text{ inhibition of xylene - induced ear oedema} = \frac{x - y}{x} \times 100 \quad (2)$$

Where; x represents the weight difference in the negative control mice, and y represents the weight difference in the extract-treated/positive control/normal control group mice.

2.3.4. Determination of antinociceptive effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

We adopted the acetic-acid induced writhing method of Koster et al. [24] modified by Gupta et al. [25] to evaluate the antinociceptive effects of the studied plant extracts. In this experiment, mice were randomly assigned allotted seven groups (I, II, III, IV, VI, and VII), each having five (5) animals. Groups I, II, III, and IV were orally administered with 4 mg/kg BW, 20 mg/kg BW, 100 mg/kg BW, and 500 mg/kg BW, respectively, of either the aqueous or the methanolic stem bark extracts of *L. eriocalyx*. On the other hand, mice in groups V (positive control) and VI (negative control) were orally treated with 75 mg/kg BW of acetylsalicylic (Aspirin) and 10 ml/kg BW of normal saline, respectively. The normal control group (VII) mice were administered with normal saline (10 ml/kg BW; p.o). After 45 min, 200 μ l of 0.6 % v/v acetic acid solution (CAS No# 64-19-7; Sigma Aldrich, Germany) was intraperitoneally administered to all experimental mice except those of the normal control group to induce writhing. Each experimental mouse was observed individually, and after five minutes after acetic acid administration, a 30-minute writhing frequency was determined and recorded. The obtained frequencies were used to compute the percentage inhibition of the acetic-induced writhing, an indicator of antinociceptive efficacy using according to Eq. (3) [23, 24, 25].

$$\% \text{ inhibition of acetic acid - induced writhing} = \frac{m - n}{n} \times 100 \quad (3)$$

Where; m represents the average writhing frequency in the control group mice, and n represents the average writhing frequency in the extract-treated/positive control/normal control group mice.

2.3.5. Acute oral toxicity evaluation of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

We adopted the up-and-down procedure for acute oral toxicity testing described by the OECD [21]. Briefly, the animals were fasted for 4 h and randomly assigned into groups, each comprising of three mice. The experiment was initiated by administering a single dose of 175 mg/kg BW of the studied plant extracts orally to the first group and normal saline (10 ml/kg BW) to the control group. After that, wellness parameters, including skin fur appearance, eye colour, mucus membrane

appearance, salivation, lethargy, sleep, coma, convulsions, tremors, and diarrhoea were observed and recorded at intervals of 30 min, 4 h, 24 h, 48 h, 7 days, and 14 days, respectively, for each animal. In the absence of observable signs of toxicity or mortality during the 14 days, the subsequent higher doses of 550 mg/kg BW and 2000 mg/kg BW, respectively, were administered into new sets of mice and monitored in the same way as the first group for 14 days [23]. Thereafter, all the experimental mice were euthanised and disposed of appropriately according to the set guidelines.

2.3.6. Investigation of in vitro antioxidant efficacy of the studied plant extracts

2.3.6.1. Determination of in vitro the effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx* on in vitro lipid peroxidation. In vitro determination of anti-lipid peroxidation assay was performed according to the method of Wills [26]. Briefly, the reaction mixtures contained 2.0 ml of the TCA-TBA-HCl reagent (15 % (w/v) Trichloroacetic Acid (TCA; Lot No# LM06461808; Loba Chemie), 0.375% (w/v) Thiobarbituric Acid (TBA; Lot No# SG53121901; Loba Chemie), and 0.25 N Hydrochloric Acid (HCl; CAS No# 7647-01-0; Fisher Chemical) and 1 ml of tests extracts of different concentrations (50, 100, 150 and 200 μ g/ml) or standard (L-Ascorbic acid; CAS No# 21675-47-8; Sigma Aldrich, Germany). The resulting mixtures were incubated in a water bath (Labtech) set at 90 °C for 10 min, cooled, and centrifuged at 10,000 rpm using a Labtech Centrifuge for 15 min. The supernatants were aspirated, and their respective absorbances measured at 532 nm using a double beam UV-Vis spectrophotometer (Shimadzu UV-Vis 1601).

2.3.6.2. Determination of in vitro DPPH radical scavenging activities of the aqueous and methanolic stem bark extracts of *L. eriocalyx*. In this study, the method described by Brand et al. [27] was adopted. In this experiment, 0.3mM solution of 1, 1-diphenyl -2-picrylhydrazyl (DPPH; CAS 1898-66-4; Sigma Aldrich, Germany) was accurately prepared in analytical grade methanol for use in in vitro DPPH radical scavenging activity assay. The reaction mixtures comprised of 1 ml of 0.3 mM DPPH and 2.5 ml of each of the studied plant extract or Ascorbic acid at concentrations of 1000 μ g/ml, 100 μ g/ml, 10 μ g/ml, 1 μ g/ml, 0.1 μ g/ml and 0.01 μ g/ml respectively. The setups were incubated for 15 min in the dark, at room temperature, after which absorbance values were read at 517 nm using a Shimadzu UV-Vis (1601) microprocessor double beam spectrophotometer against a blank (2.5 ml of methanol and 1 ml of solution). The negative control setup contained 2.5 ml of 0.3 mM DPPH and 1 ml of methanol solution. The percentage of the radical scavenging activity (% RSA) for each studied plant extract was calculated using Eq. (4) described by Brand et al. [27].

$$\% \text{ RSA} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \quad (4)$$

2.3.6.3. Determination of the potassium ferricyanide antioxidant power (pFRAP) of the aqueous and methanolic stem bark extracts of *L. eriocalyx*. The ferric-reducing antioxidant power of the aqueous and methanolic stem bark extracts of *L. eriocalyx* was determined following the methods described by Oyaizu [28] and Benzie and Strain [29]. Briefly, 1 ml of assay extracts or L-Ascorbic acid at concentrations ranging from 0.01 μ g/ml to 1000 μ g/ml were mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 30 mM potassium ferricyanide (Lot No# SL60061402; Loba Chemie). The mixtures were then incubated in a water bath set at 50 °C for 20 min, after which aliquots of 2.5 ml of Trichloroacetic Acid (TCA) (600 mM) was added, mixed and centrifugation was done at 3000 rpm for 15 min. After that, 2.5 ml of the supernatants were carefully aspirated and mixed with 2.5 ml of distilled water and 0.5 ml of 6 mM ferric chloride (FeCl₃; Lot No# A21440164, Loba Chemie). The absorbances of respective supernatants

were measured at 700 nm against blank using a double beam UV-spectrophotometer (Shimadzu UV-Vis 1601).

2.4. Data management and statistical analysis

The obtained data were first tabulated on a Microsoft Excel spreadsheet (Office 365) and exported to Minitab statistical software version 19.2 (State College, Pennsylvania). Descriptive analysis was done, and results presented as Mean \pm standard error of the mean (SEM) ($\bar{x} \pm \text{SEM}$). One-Way ANOVA to determine statistical significance followed by Tukey's test for pairwise comparison and separation of means at $\alpha = .05$ was performed. An unpaired student *t*-test was performed to compare the effects of two studied plant extracts at each studied dose level. Means with $p < 0.05$ values were considered significantly different. The graphs were generated by GraphPad Prism version 9 software. The findings were presented in bar graphs and tables.

2.5. Ethical approval

This study was approved and licensed by the National Commission for Science, Technology, and Innovation (NACOSTI) of Kenya (License Number: NACOSTI/P/19/2080 and NACOSTI/P/19/2442).

3. Results

3.1. Percentage yields

Following extraction, the percentage yields of aqueous and methanolic stem bark extracts of *L. eriocalyx* were determined. The results showed that water had a higher extractive value with a percentage yield of 16.21 % compared with methanol, whose extract yield was 9.17 %.

3.2. Acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

The obtained results revealed that the aqueous and methanolic stem bark extracts of *L. eriocalyx*, at dose levels of 175 mg/kg BW, 550 mg/kg BW, and 2000 mg/kg BW, did not cause any observable acute oral toxicity effects, and lethality in the treated experimental mice (Table 1).

Therefore, according to the OECD guidelines described in document number 425, the LD₅₀ values of the two studied plant extracts were considered to be > 2000 mg/kg BW, hence safe.

3.3. In vivo anti-inflammatory effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

A positive dose-dependent increase in the percentage inhibition of xylene-induced ear oedema in mice that were orally administered with the aqueous and methanolic stem bark extracts of *L. eriocalyx* was observed in this study ($p < 0.05$; Figures 1 and 2). Notably, the percentage inhibition of the xylene-induced ear oedema in mice that were treated with 20 mg/kg BW of the two studied plant extracts and those that received the reference drug (1 mg/kg BW of Dexamethasone) was not significantly different ($p > 0.05$; Figures 1 and 2). However, the percentage inhibitions of xylene-induced ear oedema in mice treated with 100 mg/kg BW and 500 mg/kg BW of the aqueous and methanolic stem bark extracts of *L. eriocalyx* were significantly higher than those recorded in all the other treatment groups ($p < 0.05$; Figures 1 and 2).

After comparing between the effects of the two studied plant extracts, the findings revealed significantly higher inhibitions of oedema in mice treated with the aqueous stem bark extract of *L. eriocalyx*, at all the studied dose levels, compared with those treated with the methanolic extract ($p < 0.05$; Figure 3).

3.4. Antinociceptive effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

In this study, we observed a positive dose-dependent increase in percentage inhibition of acetic acid-induced writhing in mice treated with the aqueous and methanolic stem bark extract of *L. eriocalyx* ($p < 0.05$; Figures 4 and 5). At a 500 mg/kg BW dose, the aqueous stem bark extract of *L. eriocalyx* posed a significantly higher percentage inhibition of writhing than the reference drug (Acetylsalicylic acid (75 mg/kg BW), and all the other dose levels ($p < 0.05$; Figure 4).

Besides, no significant difference in percentage inhibition of writhing was observed between mice that were treated with 500 mg/kg BW of the methanolic stem bark extract of *L. eriocalyx* and those administered with 75 mg/kg BW of Acetylsalicylic acid ($p > 0.05$; Figure 5).

Table 1. Acute Oral Toxicity effects of the aqueous and methanolic stem bark extracts *L. eriocalyx* in experimental mice.

Wellness parameter	Observation											
	30 min		4 h		24 h		48 h		7 days		14 days	
	EGM	CGM	EGM	CGM	EGM	CGM	EGR	CGM	EGM	CGM	EGM	CGM
Skin and Fur appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Faecal matter consistency	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Urination and urine appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mucous membrane appearance	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Itching	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Salivation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Convulsions and tremors	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Breathing	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Coma	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Somatomotor activity	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Aggression	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Grooming	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Eyes	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Teeth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Mortality/Death	None	None	None	None	None	None	None	None	None	None	None	None

EGM: Experimental group Mice (Administered with either the aqueous, or the methanolic stem bark extracts of *L. eriocalyx* at respective dose levels); CGM: Control group Mice (Administered with 10 ml/kg BW of Normal saline only).

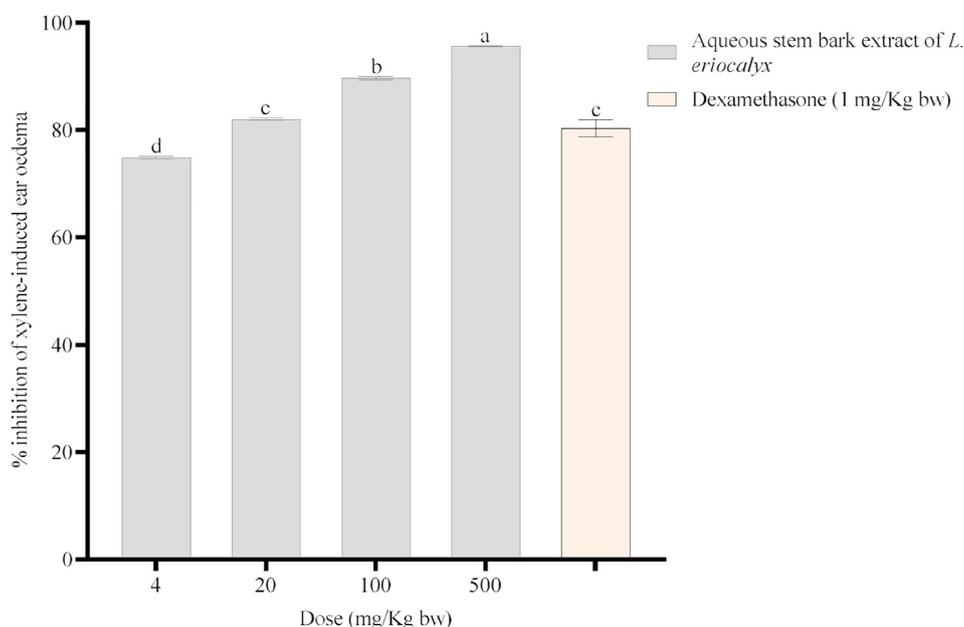


Figure 1. Percentage inhibition of the xylene-induced ear oedema in mice by the aqueous stem bark extract of *L. eriocalyx*. Bars with different alphabets are significantly different ($p < 0.05$), while those with similar alphabets are not significantly different ($p > 0.05$) (One-Way ANOVA followed by Tukey's *post hoc* test).

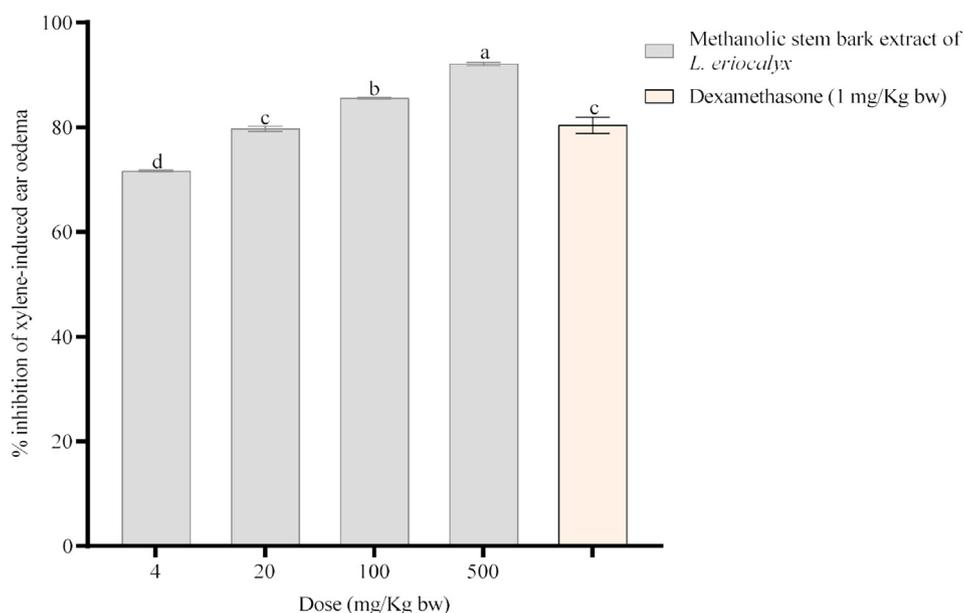


Figure 2. Percentage inhibition of the xylene-induced ear oedema in mice by the methanolic stem bark extract of *L. eriocalyx*. Bars with different alphabets are significantly different ($p < 0.05$), while those with similar alphabets are not significantly different ($p > 0.05$) (One-Way ANOVA followed by Tukey's *post hoc* test).

After comparing the effects of the two studied plant extracts on acetic acid-induced writhing in mice, the results revealed that the aqueous stem bark extract of *L. eriocalyx* had significantly higher percentage inhibitions of writhing than the methanolic extract at all the studied doses ($p > 0.05$; Figure 6).

3.5. In vitro antioxidant effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

3.5.1. Effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx* on in vitro lipid peroxidation

The results showed no significant differences in percentage inhibition of lipid peroxidation caused by the aqueous stem bark extract of *L. eriocalyx* at concentrations of 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ ($p > 0.05$;

Table 2). Similarly, the percentage inhibitions of lipid peroxidation at 150 $\mu\text{g/ml}$ compared and 200 $\mu\text{g/ml}$ were not significantly different ($p > 0.05$; Table 2); however, the percentage inhibitions of lipid peroxidation recorded at these concentrations were significantly higher than those recorded at lower concentrations ($p < 0.05$; Table 2), demonstrating a positive concentration dependency. Besides, the percentage inhibitions of lipid peroxidation produced by the methanolic stem bark extract of *L. eriocalyx* at concentrations of 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$; and at concentrations of 100 $\mu\text{g/ml}$ and 150 $\mu\text{g/ml}$ were not significantly different ($p > 0.05$; Table 2). However, the percentage inhibition of lipid peroxidation at 200 $\mu\text{g/ml}$ was significantly higher than that recorded in all the other concentrations ($p < 0.05$; Table 2). Overall, concentration-dependent increases in percentage inhibitions of lipid peroxidation were observed in the studied plant extracts (Table 2). Additionally, no

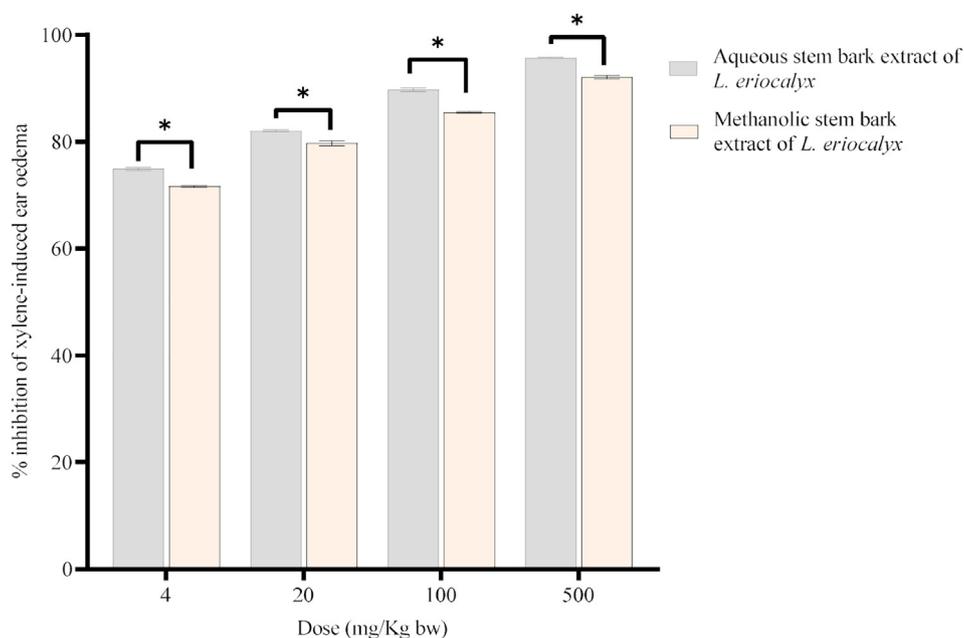


Figure 3. Comparison between the percentage inhibitions of the xylene-induced ear oedema in mice by the aqueous and methanolic stem bark extract of *L. eriocalyx*. The asterisk (*) on two bars in the same dose level indicates a significant difference ($p < 0.05$; Unpaired student *t*-test).

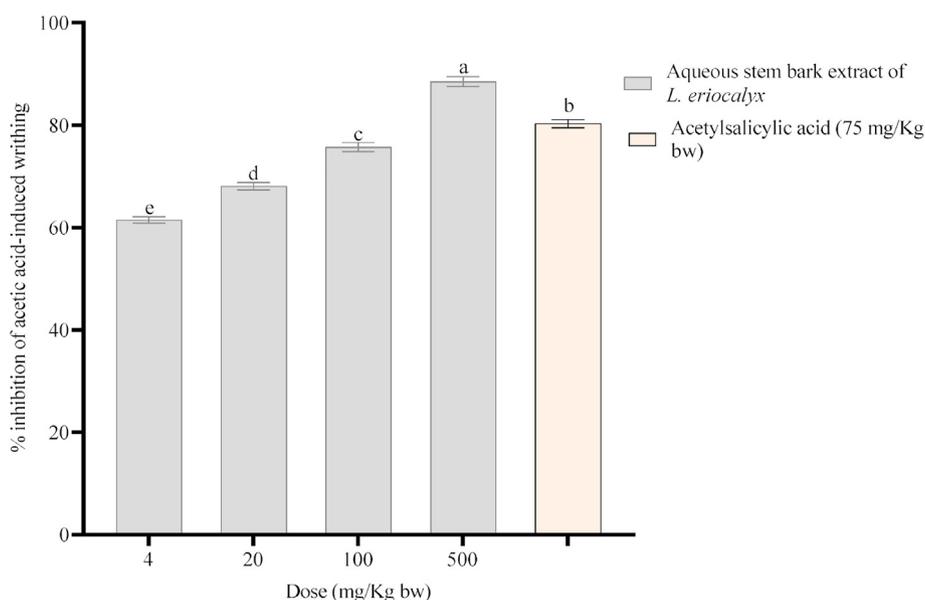


Figure 4. Percentage inhibition of the acetic acid-induced writhing in mice by the aqueous stem bark extract of *L. eriocalyx*. Bars with different alphabets are significantly different ($p < 0.05$), while those with similar alphabets are not significantly different ($p > 0.05$) (One-Way ANOVA followed by Tukey's *post hoc* test).

significant differences in the percentage inhibitions of lipid peroxidation were observed between concentrations of 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$, and between 100 $\mu\text{g/ml}$ and 150 $\mu\text{g/ml}$, of L-ascorbic acid ($p > 0.05$; Table 2). However, at a concentration of 200 $\mu\text{g/ml}$, L-ascorbic acid exhibited a significantly higher percentage inhibition of lipid peroxidation than at other lower concentrations ($p < 0.05$; Table 2).

Moreover, comparisons among the percentage inhibitions of lipid peroxidation caused by the studied plant extracts and the standard, at each of the studied concentrations, were performed in this study. The results indicated that ascorbic acid produced significantly higher inhibitions of lipid peroxidation ($p < 0.05$) at all concentrations except at 200 $\mu\text{g/ml}$, where the percentage inhibition was not significantly different from that caused by the methanolic extract of *L. eriocalyx* ($p > 0.05$; Table 2). Furthermore, the methanolic stem bark extract of

L. eriocalyx exhibited significantly higher percentage inhibitions of lipid peroxidation and the aqueous extract, at all the studied extract concentrations ($p < 0.05$; Table 2).

The concentrations of the studied plant extracts and the standard required to cause 50% inhibition of lipid peroxidation *in vitro* (IC_{50}) were also determined in this study. The aqueous stem bark extract of *L. eriocalyx* recorded the highest IC_{50} value of $>200 \mu\text{g/ml}$ (Table 2). The IC_{50} value of the methanolic stem bark extract of *L. eriocalyx* was 88.375 $\mu\text{g/ml}$, while that of Ascorbic was 34.500 $\mu\text{g/ml}$ (Table 2).

3.5.2. *In vitro* DPPH radical scavenging activities of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

In this study, dose-dependent increases in percentage DPPH radical scavenging activities of the studied plant extracts were observed

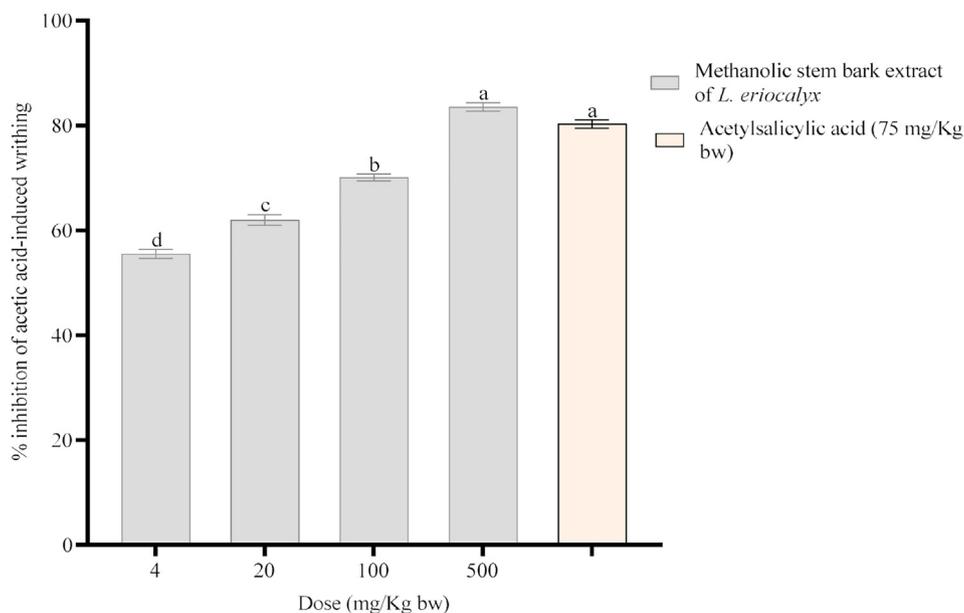


Figure 5. Percentage inhibition of the acetic acid-induced writhing in mice by the methanolic stem bark extract of *L. eriocalyx*. Bars with different alphabets are significantly different ($p < 0.05$), while those with similar alphabets are not significantly different ($p > 0.05$) (One-Way ANOVA followed by Tukey's *post hoc* test).

(Table 3). The results showed no significant differences between the percentage radical scavenging activities caused by the aqueous stem bark extract of *L. eriocalyx* at concentrations of 0.1 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$, and 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$, respectively ($p > 0.05$, Table 3). Likewise, no significant differences among the percentage radical scavenging activities produced by this extract at concentrations of 10 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ were observed ($p > 0.05$, Table 3). Overall, a significantly higher percentage radical scavenging activity of the aqueous stem bark extract of *L. eriocalyx* was observed at a concentration of 1000 $\mu\text{g/ml}$, compared to the other concentrations, depicting a positive concentration dependency ($p < 0.05$; Table 3).

On the other hand, the percentage radical scavenging activities produced by the methanolic stem bark extract of *L. eriocalyx* at

concentrations of 0.01 $\mu\text{g/ml}$, 0.1 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$, and at 0.1 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$, respectively, were not significantly different ($p > 0.05$; Table 3). However, the percentage radical scavenging activity produced by this extract at a concentration of 1000 $\mu\text{g/ml}$ was significantly higher than those produced at other concentrations, depicting concentration dependency ($p < 0.05$; Table 3).

Besides, the percentage radical scavenging activities caused by the standard (L-ascorbic acid) at concentrations of 0.01 $\mu\text{g/ml}$ and 0.1 $\mu\text{g/ml}$, and at concentrations of 10 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, and 1000 $\mu\text{g/ml}$ were not significantly different ($p > 0.05$; Table 3). However, the percentage radical scavenging activities recorded at concentrations of 10 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, and 1000 $\mu\text{g/ml}$ were significantly higher than those recorded at concentrations of 0.01 $\mu\text{g/ml}$, 0.1 $\mu\text{g/ml}$, and 1 $\mu\text{g/ml}$ ($p < 0.05$; Table 3).

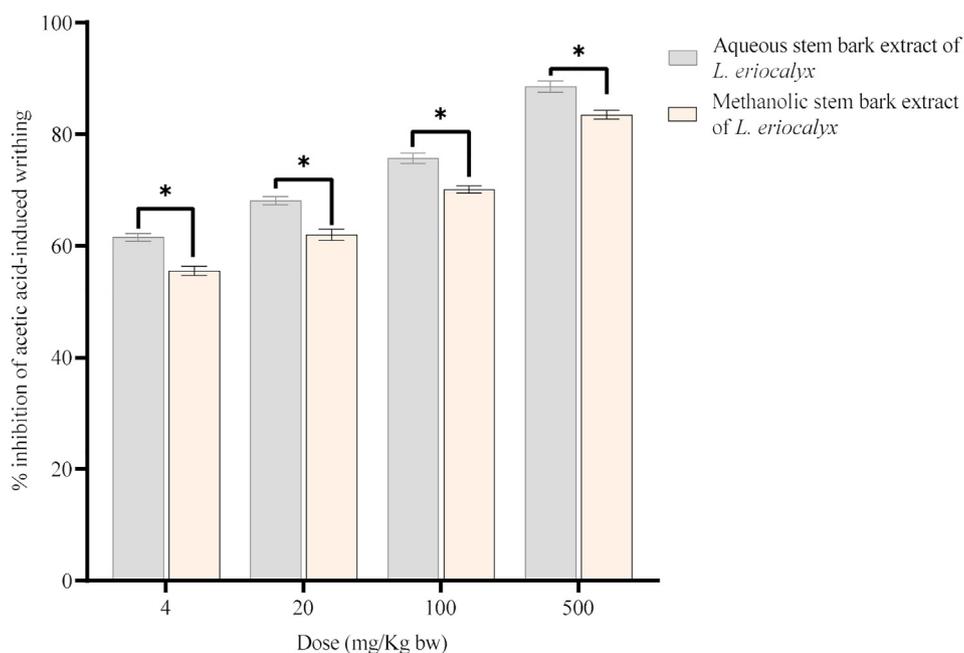


Figure 6. Comparison between the percentage inhibitions of acetic acid-induced writhing in mice by the aqueous and methanolic stem bark extract of *L. eriocalyx*. The asterisk (*) on two bars in the same dose level indicates a significant difference ($p < 0.05$; Unpaired student *t*-test).

Table 2. Effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx* on *in vitro* lipid peroxidation.

Concentration ($\mu\text{g/ml}$)	Percentage inhibition of lipid peroxidation		
	<i>L. eriocalyx</i>		L-Ascorbic acid
	Aqueous extract	Methanolic extract	
0	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
50	1.97 \pm 0.97 ^{Bc}	37.75 \pm 4.41 ^{Cb}	72.76 \pm 0.77 ^{Ca}
100	2.94 \pm 0.00 ^{Bc}	53.92 \pm 3.92 ^{BCb}	75.74 \pm 0.74 ^{BCa}
150	8.33 \pm 0.49 ^{Ac}	56.41 \pm 2.41 ^{Bb}	78.22 \pm 0.22 ^{Ba}
200	9.31 \pm 0.49 ^{Ab}	88.61 \pm 0.61 ^{Aa}	87.13 \pm 0.13 ^{Aa}
IC ₅₀ ($\mu\text{g/ml}$)	>200	88.375	34.500

Values are expressed as $\bar{x} \pm \text{SEM}$; Means with the same lowercase superscript letter across the rows and uppercase superscript letter along the columns are not significantly different ($p > 0.05$; One-way ANOVA followed by Tukey's test).

Additionally, we compared the percentage radical scavenging activities of the studied plant extracts and L-ascorbic acid at each of the studied concentrations. The results revealed that the percentage radical scavenging activities of L-ascorbic acid at concentrations of 1 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$, and 100 $\mu\text{g/ml}$ were significantly higher than those recorded by the methanolic and aqueous stem bark extracts of *L. eriocalyx* ($p < 0.05$; Table 3); notably, at these concentrations, the aqueous stem bark extract of *L. eriocalyx* exhibited significantly higher percentage radical scavenging activities than the methanolic extract ($p < 0.05$; Table 3). Generally, L-ascorbic acid showed significantly higher percentage radical scavenging activity, followed by the aqueous stem bark extract of *L. eriocalyx*, in this study (Table 3).

Furthermore, the extract concentrations required to scavenge 50 % of the DPPH radicals (IC₅₀) were determined in this study. The aqueous and methanolic stem bark extracts of *L. eriocalyx* had IC₅₀ values of 0.010 $\mu\text{g/ml}$ and 0.0095 $\mu\text{g/ml}$ while the IC₅₀ value of the standard (L-Ascorbic acid) was 0.0098 $\mu\text{g/ml}$ (Table 3).

3.5.3. Ferric-reducing antioxidant power (FRAP) activities of the aqueous and methanolic stem bark extracts of *L. eriocalyx*

The ferric-reducing antioxidant power activities of the aqueous and methanolic stem bark extracts of *L. eriocalyx* were determined in this study. Generally, the results demonstrated a concentration-dependent increase in absorbance of the reaction mixtures measured 900 nm (Table 4). The absorbance values recorded at concentrations of 50 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, and 150 $\mu\text{g/ml}$ of the methanolic stem bark extract of *L. eriocalyx* were not significantly different ($p > 0.05$; Table 4). However, at a 200 $\mu\text{g/ml}$ concentration, the recorded absorbance was significantly higher than the absorbances recorded at all the other concentrations of this extract ($p < 0.05$; Table 4).

Besides, the absorbances recorded at concentrations of 150 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ of the aqueous stem bark extract of *L. eriocalyx* were not

significantly different ($p > 0.05$; Table 4); however, these absorbances were significantly higher than those recorded at concentrations of 50 $\mu\text{g/ml}$ ($p < 0.05$; Table 4). Additionally, the absorbances recorded at concentrations of 50 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, and 150 $\mu\text{g/ml}$ of the standard (L-ascorbic acid) were not significantly different ($p > 0.05$; Table 4); however, these absorbances were significantly lower than those recorded at concentrations of 200 $\mu\text{g/ml}$ of L-ascorbic acid ($p < 0.05$; Table 4).

Moreover, a comparison among absorbances recorded at each concentration of the studied plant extracts and the standard was done. The results revealed no significant difference in absorbances obtained recorded for the aqueous and methanolic stem bark extracts of *L. eriocalyx* and L-ascorbic acid, at a concentration of 50 $\mu\text{g/ml}$ ($p > 0.05$; Table 4). At concentrations of 100 $\mu\text{g/ml}$ and 150 $\mu\text{g/ml}$, the average absorbance values obtained for the methanolic stem bark extract of *L. eriocalyx* and L-ascorbic acid were not significantly different ($p > 0.05$); however, the aqueous stem bark extract of *L. eriocalyx* recorded a significantly higher absorbance at these concentrations, than the methanolic extract and L-ascorbic acid ($p < 0.05$; Table 4). At concentrations of 200 $\mu\text{g/ml}$, the aqueous stem bark extract of *L. eriocalyx* recorded significantly higher absorbance values than the absorbances recorded for its methanolic extract counterpart and the standard (L-ascorbic acid) ($p < 0.05$; Table 4).

Furthermore, the half-effective concentrations (EC₅₀) of the studied plant extracts required to produce an absorbance value of 0.5 were determined in this study. It was observed that all the studied plant extracts had lower EC₅₀ values than that of the standard (Table 4).

4. Discussion

Fever, inflammation, and pain are critical signs that manifest in many diseases affecting humans and other animals, leading to poor quality of life, disability, depression, mortality, and financial loss [11, 30, 31].

Table 3. *In vitro* DPPH radical scavenging activities of the aqueous and methanolic stem bark extracts of *L. eriocalyx*.

Concentration ($\mu\text{g/ml}$)	Percentage DPPH radical scavenging activity		
	<i>L. eriocalyx</i>		L-Ascorbic acid
	Aqueous extract	Methanolic extract	
0	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
0.01	51.57 \pm 5.58 ^{bC}	52.09 \pm 0.34 ^{bD}	67.11 \pm 1.62 ^{cC}
0.1	68.27 \pm 0.98 ^{aB}	54.27 \pm 0.46 ^{bCD}	71.49 \pm 0.88 ^{cC}
1	69.25 \pm 2.04 ^{bB}	58.18 \pm 1.69 ^{cCD}	79.55 \pm 2.36 ^{bB}
10	77.48 \pm 2.41 ^{bAB}	61.12 \pm 3.03 ^{cC}	99.01 \pm 0.01 ^{aA}
100	82.10 \pm 3.00 ^{bAB}	68.97 \pm 0.74 ^{cB}	99.08 \pm 0.02 ^{aA}
1000	88.02 \pm 0.36 ^{cA}	97.02 \pm 0.09 ^{bA}	99.12 \pm 0.01 ^{aA}
IC ₅₀ ($\mu\text{g/ml}$)	0.010	0.0095	0.0098

Values are expressed as $\bar{x} \pm \text{SEM}$; Values with the same lowercase superscript letter across the rows and uppercase superscript letter along the columns are not significantly different ($p > 0.05$; One-way ANOVA followed by Tukey's test).

Table 4. Ferric reducing antioxidant power (FRAP) activities of the aqueous and methanolic stem bark extracts of and *L. eriocalyx*.

Concentration ($\mu\text{g/ml}$)	Absorbance ($\lambda_{900\text{ nm}}$)		
	<i>L. eriocalyx</i>		L-Ascorbic acid
	Methanolic extract	Aqueous extract	
0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
50	2.50 \pm 0.04 ^{aB}	2.51 \pm 0.01 ^{aC}	2.40 \pm 0.03 ^{aB}
100	2.56 \pm 0.04 ^{bAB}	2.77 \pm 0.05 ^{aB}	2.46 \pm 0.01 ^{bb}
150	2.62 \pm 0.05 ^{bAB}	2.95 \pm 0.06 ^{aAB}	2.49 \pm 0.03 ^{bAB}
200	2.70 \pm 0.04 ^{bA}	3.03 \pm 0.02 ^{aA}	2.57 \pm 0.02 ^{cA}
EC ₅₀ ($\mu\text{g/ml}$)	9.924	9.861	10.650

Values are expressed as $\bar{x} \pm \text{SEM}$; Values with the same lowercase superscript letter across the rows and uppercase superscript letter along the columns are not significantly different ($p > 0.05$; One-way ANOVA followed by Tukey's test).

Unfortunately, the management of pain and inflammation is expensive, and it typically entails the administration of different classes of analgesic and anti-inflammatory drugs, which are affected by various drawbacks [7]. Most of these drugs are associated with severe side effects, such as gastric ulcers, hepatotoxicity, dependency, tolerance, nephropathy, among others [32, 33, 34]. It has been established that herbal remedies are cheap, readily available, effective, and with few side effects [11, 35, 36]. However, many of the plant-based remedies, including the aqueous and methanolic stem bark extracts of *L. eriocalyx*, have not been scrutinised with scientific precision to determine their efficacy, composition, mode of action, and toxicity, hence this study.

In this study, the *in vivo* anti-inflammatory efficacy of the aqueous and methanolic stem bark extracts of *L. eriocalyx* were investigated using the xylene-induced ear technique described by Igbe *et al.* [23] and modified by Olela *et al.* [11]. Extensive research shows that xylene evokes an inflammatory response when applied topically by activating the secretion of inflammatory mediators, such as bradykinin, serotonin, and histamine. These mediators promote the vasculature's permeability and increase vasodilation leading to the accumulation of fluids at the site of the inflamed area, manifesting as oedema [11, 37]. Therefore, an extract or drug agent capable of averting or reducing the xylene-induced ear oedema is considered to possess anti-inflammatory activity. Additionally, higher percentage inhibition of the xylene-induced ear oedema is correlated with higher anti-inflammatory efficacy [11, 23]. In our study, the aqueous and methanolic stem bark extracts of *L. eriocalyx* exhibited a positive dose-dependent increase in the percentage inhibition of xylene-induced ear oedema in mice. These results demonstrate a promising antioxidant potential of these extracts. Based on the mechanism by xylene-induced inflammation, we anticipate that the studied plant extracts possess anti-inflammatory-associated phytochemicals, which modify the inflammatory cascade associated with oedema.

Most current anti-inflammatory drugs, especially topical corticosteroids, and non-steroidal anti-inflammatories, function by inhibiting phospholipase A₂, which inhibits prostaglandin synthesis [40, 41]. Consequently, it is suggestive that the studied plant extracts contain bioactive agents which confer anti-inflammatory effects by inhibiting the action of phospholipase A₂. Moreover, research has shown that synthetic anti-inflammatory drugs act peripherally, centrally, or both, to ameliorate inflammation [40, 41]. However, medicinal plant extracts have been shown to contain diverse bioactive principles with peripheral and central anti-inflammatory efficacy [42, 43]. Recently, Moriasi *et al.* [44] reported the presence of phenols, flavonoids, among other anti-inflammatory-associated antioxidant phytochemicals in the aqueous and methanolic stem bark extracts of *L. eriocalyx*. Recent reports indicate that flavonoids, phenolic acids, and terpenes inhibit the activity of various pro-inflammatory cytokines, such as TNF- α , NF- κ B, IL-6, iNOS, COX-2, NADPH oxidase, among others, thereby imparting anti-inflammatory effects [38, 45]. Therefore, the pharmacologic efficacy of the studied plant extracts is attributable to these phytochemicals, which may be exhibiting both peripheral and central anti-inflammatory

and antinociceptive properties, hence the remarkable anti-inflammatory efficacy observed in this study [39]. Nevertheless, extensive and focused investigations aimed at elucidating the specific anti-inflammatory compounds and their action mechanisms should be undertaken.

The most common presentation of many inflammatory diseases and tissue injuries is pain, and, as a result, the most commonly diagnosed symptom of many diseases [46, 47, 48, 49]. The financial and emotional burden of these conditions in affected subjects and caregivers is unquantifiable, can lead to physical incapacitation, which negatively impacts the quality of life and even death, thus warranting the need for a panacea [48]. Despite the remarkable potencies of conventionally available analgesic and anti-inflammatories, various drawbacks limiting their clinical utilisation have been reported [50, 51]. Various NSAIDs like diclofenac, acetylsalicylic acid, and ibuprofen have been shown to cause gastrointestinal problems, including peptic ulcers, gastric perforations, intestinal bleeding, gastric obstructions, among others [51, 52]. Besides, corticosteroids like cortisone, prednisone, and methylprednisolone are associated with fluid retention (oedema), delayed wound healing, osteoporosis, weight gain (obesity), and hypertension, among other side effects [51, 53, 54]. Moreover, opioid analgesics like pethidine, morphine, and codeine cause undesirable behaviour, addiction, and respiratory depression in subjects. Moreover, DMARDs, including sulfasalazine, penicillamine, methotrexate, and gold compounds, have been found to cause liver disorders, skin reactions, gastrointestinal disorders, and renal failure [51, 53, 54, 55]. Due to the many associated side effects, relative unaffordability, and inaccessibility of conventional medicines, the search for alternative and complementary agents, especially from medicinal plants, has intensified recently in the scientific arena [56, 57]. Thus, we investigated the antinociceptive efficacy of the aqueous and methanolic stem bark extracts of *L. eriocalyx* and potential sources of potent analgesic compounds.

The acetic acid-induced writhing technique of Koster *et al.* [24] modified by Gupta *et al.* [25] was used in this study to evaluate the antinociceptive activity of the studied plant extracts in mice models. In this technique, overt pain is stimulated by an intraperitoneal injection of acetic acid as a stimulus. Afterward, the chemical triggers a fast production of endogenous inflammatory mediators, including prostaglandins, which activate the primary nociceptors [58, 59, 60]. This results in abdominal contortions/writhes observed in induced animals. Research has shown that any drug agent capable of either reducing or suppressing the occurrence of writhing behaviour has a greater propensity of being a potent analgesic drug [60, 61, 62].

In this study, both the aqueous and methanolic stem bark extracts of *L. eriocalyx* exhibited analgesic/antinociceptive potency by significantly inhibiting acetic acid-induced writhing in mice. These findings are consistent with previous reports on plant extracts' antinociceptive efficacy in animal models [63, 64, 65, 66]. The reference drug, Aspirin (acetylsalicylic acid), used as a positive control in the present study, is an NSAID indicated for a variety of conditions, including pain, myocardial

infarction, angina, rheumatoid arthritis, ankylosing spondylitis, spondyloarthritis, among others [41]. It confers pharmacologic efficacy by acetylating serine moieties in the cyclooxygenase enzyme, thereby inhibiting prostaglandin and thromboxane synthesis. It non specifically inhibits both isozymes of the cyclooxygenase enzyme, which affect thrombosis and inflammation [41]. The studied plant extracts could be mimicking the Aspirin's pharmacologic mechanism; however, due to the various phytoactive constituents of the studied plant extracts, which may act individually or synergistically, further empirical validations are warranted.

Various studies have provided valuable information and knowledge on the role of oxidative stress in human diseases, health, and ageing [67, 68, 69, 70, 71, 72]. The current drugs used against many diseases linked to oxidative stress, such as inflammation are only palliative, arguably unaffordable to low-income earners, and cause adverse side effects [73, 74, 75, 76]. As a result, there is a heightened search for novel mechanisms aimed at curing, reversing, or preventing these conditions with minimal side effects and at an affordable cost [57, 74, 75]. Antioxidant compounds of plant origin are potent therapies against conditions associated with oxidative stress like diabetes, neurodegenerative disorders like Parkinson's and Alzheimer's diseases, inflammation, cardiovascular disorders, among other complex maladies [57, 67, 74, 75].

Various biochemical analytical methods have been developed to investigate *in vitro*, *ex-vivo*, and *in vivo* antioxidant potential of medicinal plants and their extracts. Studies have shown that substances with high *in vitro* antioxidant capacities can exhibit high antioxidant effects *in vivo* [17]. Our study employed three *in vitro* analytical techniques to appraise the antioxidant potential of the aqueous and methanolic stem bark extracts of *L. eriocalyx*.

The Thiobarbituric Acid Reactive Substances (TBARS) technique employed in this study is usually used to determine lipid peroxidation by-product, malondialdehyde (MDA) [10, 77]. MDA is the most prominent substance among the reactive oxygen metabolites (ROM) whose levels increase during oxidative stress [10, 69, 78]. Therefore, MDA, a marker of lipid peroxidation due to biological lipid rancidity, is used to measure oxidative stress in anti-lipid peroxidation assays [78]. The obtained results demonstrated the inhibition of lipid peroxidation by the studied plant extracts. The half inhibitory concentration (IC₅₀) and the half effective concentration (EC₅₀) values are widely used to determine the antioxidant efficacy of drugs and extracts *in vitro*. They measure a drug or extract's potential in scavenging or inhibiting free radicals' production in experimental setups. Therefore, in this context, substances with low EC₅₀/IC₅₀ values are deemed to be potent antioxidants [79].

The efficacy of the aqueous and methanolic stem bark extracts of *L. eriocalyx* in inhibiting *in vitro* lipid peroxidation was appraised according to the criterion of Blois [80] and Fidrianny *et al.* [79], which posit that extract with IC₅₀/EC₅₀ < 50 µg/ml are very strong antioxidants, those with IC₅₀/EC₅₀ of 50–100 µg/ml are strong antioxidants, those with EC₅₀/IC₅₀ values of 101–150 µg/ml are moderate antioxidants while those with EC₅₀/IC₅₀ values of >150 µg/ml are weak antioxidants. Based on this, the methanolic stem bark extract of *L. eriocalyx* was determined to be a strong inhibitor of *in vitro* lipid peroxidation, while the aqueous extract a weak inhibitor of *in vitro* lipid peroxidation. Perhaps, the aqueous stem bark extract of *L. eriocalyx*, like other extracts, could be exerting its effect through other mechanisms like those suggested earlier by other scholars [81, 82].

The DPPH stable free radical yields a deep pink colour when dissolved in methanol/ethanol, producing a maximum absorbance at 515–520 nm. As an antioxidant substance scavenges the DPPH radicals in solution, the absorbance reduces as the colour turns from pink to yellow [27, 79, 80]. As a result, as the absorbance decreases, the antioxidant capacity increases. In this study, *in vitro* DPPH radical scavenging activity of the aqueous and methanolic stem bark extracts of *L. eriocalyx* revealed remarkable antioxidant efficacy. Therefore, an extract with a high percentage of radical scavenging activity ought to be a potent antioxidant *in vitro* and *in vivo* and should give low EC₅₀/IC₅₀ values [79, 80]. As per the

criterion of Blois [80] and Fidrianny *et al.* [79], all the studied plant extracts had very low IC₅₀ values suggesting strong scavenging potency of the DPPH radical *in vitro*.

The Ferric reducing antioxidant power (FRAP) technique described by Oyaizu [28] and Benzie and Strain [28] was adopted to appraise the capacity of aqueous and methanolic stem bark extracts of *L. eriocalyx* to reduce ferric ion at low PH to ferrous ion yielding a blue coloured complex. Increasing absorbance values at 700 nm indicates high ferric reducing antioxidant power of the analyte [28]. This approach has been shown to mimic endogenous antioxidant systems, including bilirubin, among other ferredoxins [83, 84]. Using the appraisal criterion of Blois [80] and Fidrianny *et al.* [79], the aqueous and methanolic stem bark extracts of *L. eriocalyx* were considered strong antioxidants due to their low EC₅₀ values.

Research has shown agroecological, agroclimatic, and geographic factors influence the synthesis and quantity of secondary metabolites in plants. Consequently, the type and concentration of various phyto-compounds depend on the season and site from where the plant was collected [85]. Antioxidant phytochemicals are endowed with a wide range of pharmacologic bioactivities, including analgesic, cardioprotective, antidiabetic, anti-inflammatory, anti-ageing, anticancer, among others [9, 44, 76, 86, 87, 88, 89]. Therefore, the studied plant extracts' reported antioxidant efficacy was attributed to antioxidant-associated phytochemicals, which it synthesises. Indeed, this plant's medicinal use to manage oxidative stress-associated maladies, including diabetes mellitus, inflammatory conditions, among others, may be attributed to the reported antioxidant potency.

Dhanani *et al.* [90] demonstrate that it is imperative to adopt an appropriate extraction method using suitable solvents to obtain extracts having phytochemicals of the desired bioactivity. Moreover, research has indicated that antioxidant phytochemicals, which also exhibit anti-inflammatory and antinociceptive pharmacologic activities, are polar, hence extractable by methanol and water, as solvents [44, 91, 92]. Therefore, the bioactivities of the aqueous and methanolic stem bark extracts of *L. eriocalyx*, we report herein, are attributable to the bioactive principles [44] that were adequately extracted by water and methanol, based on their polarity, and the differences between the efficacies of the two extracts are due to differences in the concentration and type of these phytochemicals in respective extracts.

Despite the profound ethnomedical applications of medicinal plants in healthcare, various safety concerns have been raised, hence negatively affecting their incorporation into modern medicine [12, 35]. Insufficient empirical validation, lack of clear dosage regimens, and toxicological data, including herb-herb and herb-drug interaction data, have greatly hampered traditional medicine [35]. Therefore, toxicological evaluation of medicinal plants increases the potential of medicinal plants to act as sources of safer, easily accessible, and well tolerable drugs to make up for the deficiencies, insufficiencies, and inefficiencies of the synthetics [57, 93, 94, 95, 96].

In this study, the acute oral toxicity effects of the aqueous and methanolic stem bark extracts of *L. eriocalyx* were investigated following the OECD guideline document 425 [21]. This method has been utilised extensively to appraise the toxicity profile and safety of chemicals and plant extracts that can provide lead molecules for drug development. Research has indicated that the oral route (*p.o*) is the most suitable and inexpensive mode of drug delivery into model animals of toxicity studies [21, 97]. Moreover, studies have shown that acute oral toxicity studies in mice offer a better prediction of the human acute lethal doses in clinical setups.

In this study, orally administered aqueous and methanolic stem bark extracts of the studied plant did not elicit any observable signs of toxicity throughout the 14-day experimentation period in all three dose levels up to the limit dose of 2000 mg/kg BW. Since the LD₅₀ was projected to be > 2000 mg/kg BW, these indicated that these extracts could offer anti-inflammatory, antinociceptive, and antioxidant compounds that are orally tolerable and with fewer or no adverse side effects [21, 98].

Therefore, it is expected that if these extracts are taken orally by humans, no toxicity signs will occur. Perhaps these findings confirm the low toxicity of the studied plant extracts as they have been used traditionally for ages to manage various ailments [16]. These extracts' non-toxicity can be attributed to lack of or low abundance of toxic phytochemical compounds [44]. However, further studies are required to exhaustively establish the studied plant extracts' safety and toxicity profile to guide further development.

5. Conclusions and recommendations

Based on the obtained results, the aqueous and methanolic stem bark extracts of *L. eriocalyx* have remarkable anti-inflammatory and antinociceptive effects in experimental mice. These extracts possess remarkable *in vitro* antioxidant activities, especially by scavenging the DPPH radical and reducing the Ferric ion. The studied plant extracts can be used as alternative sources of safe anti-inflammatory, antinociceptive, and antioxidant lead compounds based on the obtained results. Further studies aimed at elucidating the specific mechanism of anti-inflammatory, antinociceptive, and antioxidant activities of the studied plant extracts are recommended. Moreover, the *in vivo* antioxidant potential of the studied plant extracts should be investigated. Also, specific phytochemicals responsible for the anti-inflammation, antinociception, and antioxidant activities should be isolated and characterised from the studied plants' extracts. Furthermore, specific mode(s) of bioactivity on claimed disease conditions like inflammation, diabetes mellitus, among others in traditional medicine, should be established and the studies advanced to clinical setups. Extensive toxicity, cytotoxicity, mutagenicity, and other related investigations should be conducted to fully establish the two studied plant extracts' safety profiles.

Declarations

Author contribution statement

Gervason Apiri Moriasi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anthony Muriithi Ireri: Conceived and designed the experiments.

Elias Mandela Nelson: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Mathew Piero Ngugi: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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