International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2021; 9(2): 48-56
Received: 04-01-2021
Accepted: 27-02-2021

John K Muchonjo
Department of Public Health, Pharmacology and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, Nairobi, Kenya

James M Mbaria
Department of Public Health, Pharmacology and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, Nairobi, Kenya

Joseph M Nguta
Department of Public Health, Pharmacology and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, Nairobi, Kenya

Gervason A Moriasi
a. Department of Biochemistry, Microbiology and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, Nairobi, Kenya
b. Department of Medical Biochemistry, School of Medicine, College of Health Sciences, Mount Kenya University, Thika, Kenya

Corresponding Author:
John K Muchonjo
Department of Public Health, Pharmacology and Toxicology, College of Veterinary and Agricultural Sciences, University of Nairobi, Nairobi, Kenya

Analgesic efficacy and phytochemical composition of the aqueous and methanolic stem bark extracts of *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae)

John K Muchonjo, James M Mbaria, Joseph M Nguta and Gervason A Moriasi

Abstract
Pain is the most widely diagnosed and managed symptoms of human diseases, with various debilitating effects. Current analgesics agents have shown low efficacy, are inaccessible, unaffordable, and elicit deleterious side effects which limit their use, thereby warranting the need for alternative and complementary strategies. *Mystroxylon aethiopicum* is widely utilized in the Agikuyu community of Kenya to treat stomachache, chronic pain, coughs, among other conditions; however, its analgesic efficacy and safety data are scanty, hence the present study. The analgesic activity of the aqueous and methanolic stem bark extracts of *M. aethiopicum* were determined using the standard acetic acid-induced writhing technique. Further, qualitative phytochemical screening for various phytocompounds in the studied plant extracts was done following standard phytochemical screening methods. The aqueous and methanolic extracts of *M. aethiopicum* possess noteworthy analgesic activity as demonstrated by the higher percentage inhibitions of writhing in the treated mice; however, the aqueous extract exhibited significantly lower analgesic efficacy than the methanolic extract (P<0.05). Qualitative phytochemical screening revealed presence of antioxidant-associated compounds including phenols, flavonoids, terpenoids, among others, which exhibit analgesic activity. All the studied plant extracts did not cause acute oral toxicity effects in experimental mice, hence safe (LD_{50}>2000 mg/Kg bw). The specific mechanisms of analgesic action, the responsible compounds should be elucidated. Moreover, extensive toxicological studies involving the studied plant extracts should be conducted to fully profile and assure their safety.

Keywords: Analgesic activity, acetic acid-induced writhing, acute oral toxicity, phytochemical screening, *Mystroxylon aethiopicum*

1. Introduction
Pain is a major symptom of many different diseases [1, 2]. It is the main prognostic indicator of many diseases and a key goal for healthcare.Generally, the burden of all types of pain affecting both children and adults, with or without defined cause are on the rise globally [3, 4]. For instance, the prevalence of neuropathic pain in cancer patients ranges from 19% to over 39.1%. The prevalence of musculoskeletal pain, one of the most causes of disability, is approximately 18.6% - 31%. One-year incidences of low back pain range from 1.5% to 38.9% with a recurrence rate of up to 80% [3, 4].
Pain arises from either the misfiring of a nerve or the damage to the tissues which is sensed by nociceptors [5]. Nociception is the process by which the nervous system (central and peripheral) responds to noxious stimuli, including injury to tissues or elevated temperatures. Such noxious conditions activate receptors responsible for relaying nociceptive information hence pain sensation. Painis caused by several agents which comprises either physical, chemical and immunological or infectious agents, which triggers nociceptive pathways and input from higher-order brain centers [6].
Conventionally, pain management involves the use of analgesic and anti-inflammatory drugs, which target and modify both peripheral and central nociceptive pathways to supress pain [7]. Even though the current analgesics and anti-inflammatories exhibit marked pain relief capabilities, they are not universally accessible, affordable, and are associated with severe side effects, including constipation, drowsiness, dizziness, stomach upset, skin itching or rash and dry mouth [8–10]. For example, aspirin (acetylsalicylic acid) and naproxen causes indigestion, stomach ulcers, kidney damage, hepatotoxicity, stroke, cerebral haemorrhage, Reye's syndrome, among others. Besides, ibuprofen, and acetaminophen cause stomach and kidney problems. Moreover, opioid analgesics cause constipation, weakened immune system, nausea, drowsiness, sweating, depression, itching, euphoria, and addiction [9].

Therefore, owing to the high prevalence rates of pain across the world and the bottlenecks of conventional drugs, there is a dire need for safe, accessible, affordable, and efficacious alternative for pain management.

Medicinal plants have a rich history of folklore usage, are relatively safer, inexpensive, and readily available [11–15]. In fact, over 80% of the global human population in the developing countries, and over 40% of individuals from developed countries use plant-derived traditional medicines for their primary healthcare needs, including pain management [16, 17]. Moreover, herbal medicines have secondary metabolites which are pharmacologically active against various diseases in addition to dietary and health promoting benefits [18, 19]. In spite of the widespread usage of medicinal plants, only a handful have been scientifically evaluated to ascertain their pharmacologic efficacy, toxicity, and safety profiles. One such plant popularly used for pain management among the Agikuyu community of Kenya is *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae).

Mystroxylon aethiopicum is a small to medium sized shrub or tree of the Celastraceae family found in bushvelds, forests and forest edges, rocky ridges, open woodlands, among other habitats [20]. Its stem bark is ethnomedically used to treat stomach-aches, chronic joint and back pains, coughs, anaemia and worm infestations in livestock [21]. Despite these ethnomedical claims, there is no scientific prove of its pain management efficacy and safety. Therefore, this study was designed to evaluate the analgesic activity, acute oral toxicity and qualitative phytochemical composition of the methanolic and aqueous stem bark extracts of *M. aethiopicum*as a potential source of safe, accessible, affordable, and potent analgesic compounds for pain treatment.

2. Materials and Methods

2.1 Plant material

Fresh barks of *M. aethiopicum* were collected from Murang'a County by a team comprising the principal investigator, supervisors, a local herbalist, and a taxonomist from the East Africa Herbarium, the National Museums of Kenya. The plant samples were collected, prepared, identified, and authenticated at the East Africa Herbarium, the National Museums of Kenya and assigned a reference number(REF:NMK/BOT/CTX/1/5) where a voucher specimen was deposited for future reference. The collected stem barks were evenly spread on a wooden bench top and air-dried for two weeks with regular grabbling for proper aeration and uniform drying in the Pharmacognosy laboratory, at the school of pharmacy, Mount Kenya University. Afterward, the dried stem barks were ground into a coarse powder using an electric plant mill, packaged in a clean labelled plastic container, and stored in a cool dry place waiting extraction.

2.2 Preparation of the aqueous and methanolic stem bark extracts

In this study, the aqueous and methanolic stem bark extracts of the *M. aethiopicum* were prepared according to the methods described by Harborne [22]. Briefly, the methanolic extract was prepared by macerating about 300g of powdered plant material in 750ml of analytical grade methanol in a 2-litre conical flask which was covered with foil paper and shaken once a day for three days. After that, the mixture was filtered thrice through a Whatman's filter paper No. 1 using a Buchner funnel apparatus. The collected filtrates were combined and concentrated *in vacuo* using a rotary evaporator at 55 ºC and further in a hot air oven set at 35 ºC for 48 hours to ensure complete drying. The extract was thentransferred into a well closed, light resistant glass bottle and kept in a refrigeratorat 4 °C awaiting biological studies.

On the other hand, about 100 g of the powdered plant material was soaked in 500 ml of distilled water and heated at 58 ºC for five minutes. Thereafter, the mixture was cooled to room temperature and filtered through Whatman's filter paper No. 1. The filtrate was transferred into freeze-drying flasks whose surfaces were coated with dry CO_2 mixed with acetone. The flasks were then fitted into a freeze-dryer andlyophilized for 48 hrs. Afterwards, the lyophilized extract was transferred into a pre-weighed universal glass bottle and stored in a refrigeratorat 4 °C awaiting biological assays [22].

2.3 Experimental animals

Healthy, 4-5weeks old female Swiss albino mice, weighing 20-25grams were obtained the animal breeding unit of the department of Public Health, Pharmacology, and Toxicology of the University of Nairobiforuse in the present study. These animals were nulliparous and non-pregnant and were held in cages measuring 35 cm (L) × 25 cm (W) × 18cm (H) with soft wood shavings as bedding material. They were housed in standard laboratory conditions (23± 2 ºC room temperature; 55-65% Relative humidity; 12-hour day/night cycle). They were offered standard mice pellets and clean drinking tap water was *adlibitum*. The mice were adequately acclimatized to lab conditions prior to experimentation.

2.4 Determination of analgesic activity of the studied plant extracts

The acetic acid-induced writhing technique described by Koster *et al.* [23] was used to determine the analgesic activity of the aqueous and methanolic stem bark extracts of *M. aethiopicum*. Briefly, experimental mice were randomly assigned into seven treatment groups comprising of six (6) mice each and treated as shown in Table 1.

Table 1: Experimental design for the determination of the analgesic activity of the aqueous and methanolic stem bark extracts of *M. aethiopicum*

Group	Treatment Administered
I: Normal Control	Normal Saline (10 ml/Kg bw; *p.o.*) only
II: Negative Control	Normal Salne (10 mg/Kg bw; *p.o.*) + 0.6% w/v acetic acid (*i.p.*)
III: Positive Control	Diclofenac (50 mg/Kg bw; *p.o.*) + 0.6% w/v acetic acid (*i.p.*)
IV: Experimental [A]	Extract (50 mg/Kg bw; *p.o.*) + 0.6% w/v acetic acid (*i.p.*)
V: Experimental [B]	Extract (100 mg/Kg bw; *p.o.*) + 0.6% w/v acetic acid (*i.p.*)
VI: Experimental [C]	Extract (200 mg/Kg bw; *p.o.*) + 0.6% w/v acetic acid (*i.p.*)

Each group consisted of 5 mice; p.o. = per os (oral route); i.p. = Intraperitoneal route; The volume of administration was 200µl; Extract: Aqueous or methanolic leaf extract of M. aethiopicum prepared in normal saline; Acetic acid was injected 30 minutes post administration of respective treatments.

Thirty (30) minutes post treatment, all the mice were intraperitoneally injected with 200µl of acetic acid (0.6% v/v) and placed in individual observation cages. After 5 minutes post acetic acid administration, the abdominal writhing frequency was recorded for each mouse continuously for 30 minutes. The results were then expressed as percentages of writhing inhibition.

2.5 Qualitative phytochemical screening of the aqueous and methanolic stem bark extracts of *M. aethiopicum*

Qualitative phytochemical tests for the presence or absence of various phytochemical groups including alkaloids, flavonoids, terpenes/terpenoids, saponins, tannins, saccharides, proteins, and phenols were performed according to the methods of Harborne [22] modified by Moriasi *et al.* [19].

2.6 Data management and statistic analysis

Quantitative data obtained from analgesic activity study were tabulated on Microsoft Excel spreadsheet (Microsoft 365) and exported to Graph Pad Prism statistical software version 8.4.3 for analysis. Data were descriptively analyzed, and results were presented as $\bar{x} \pm SEM.$ Thereafter, One-Way ANOVA was performed to determine significant differences among treatment groups followed by Tukey's *post hoc* test for pair wise comparison and separation of means. Unpaired student *t*-test statistic was done to compare between the effects of the two studied plant extracts. In both instances, $P<0.05$ was considered significant. Qualitative phytochemical screening results were just tabulated. The study findings were presented in tables and bar graphs.

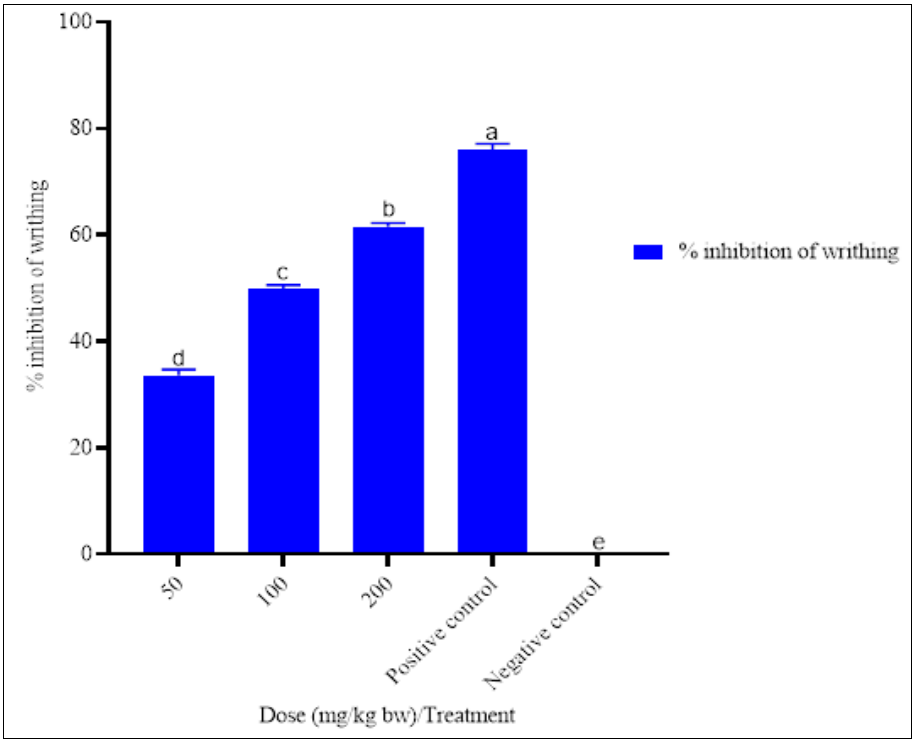
2.7 Ethical considerations

Ethical approval was granted by the Faculty of Veterinary Medicine's Biosafety, Animal Care and Use committee (FVM-BAUEC) of the University of Nairobi and referenced as REF: FVM BAUEC/2020/257. Furthermore, the National Commission for Science, Technology, and Innovation (NACOSTI) under the Ministry of Education approved this study (No: 688114).

3. Results

3.1 Effects of the aqueous and methanolic stem bark extracts of *Mystroxylon aethiopicum* on acetic acid-induced writhing in mice

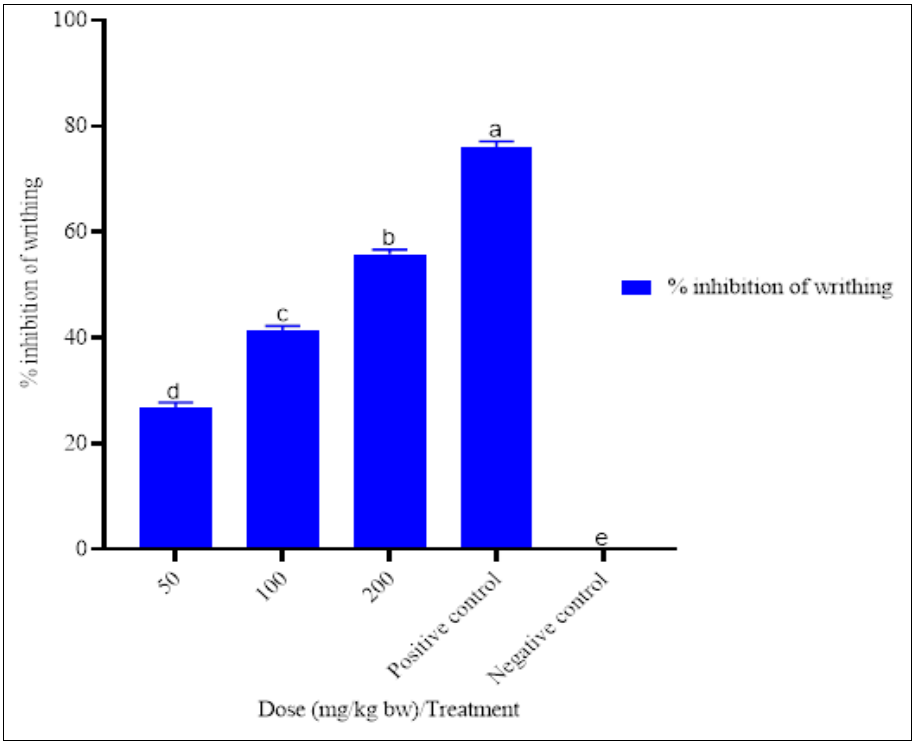
In this study, the results revealed that the aqueous stem bark extract of *M. aethiopicum* significantly inhibited writhing frequency in experimental mice, in a dose-dependent manner (Figure 1). The percentage inhibitions of writhing ranged between 33.62±1.10% at a dose of 50 mg/Kg bw and 61.58±0.67% at 200 mg/Kg bw dose level (Figure 1). Notably, the positive control (diclofenac sodium 50mg/Kg bw) exhibited the highest inhibition of acetic acid-induced writhing in mice compared with the percentage inhibitions caused by the studied extract at all dose levels ($P<0.05$; Figure 1).



Bars with dissimilar lowercase alphabet are significantly different by one-Way ANOVA followed by Tukey's test ($P< 0.05$)

Fig 1: The percentage inhibitions of acetic acid-induced writhing by the aqueous stem bark extract of *M. aethiopicum* in mice

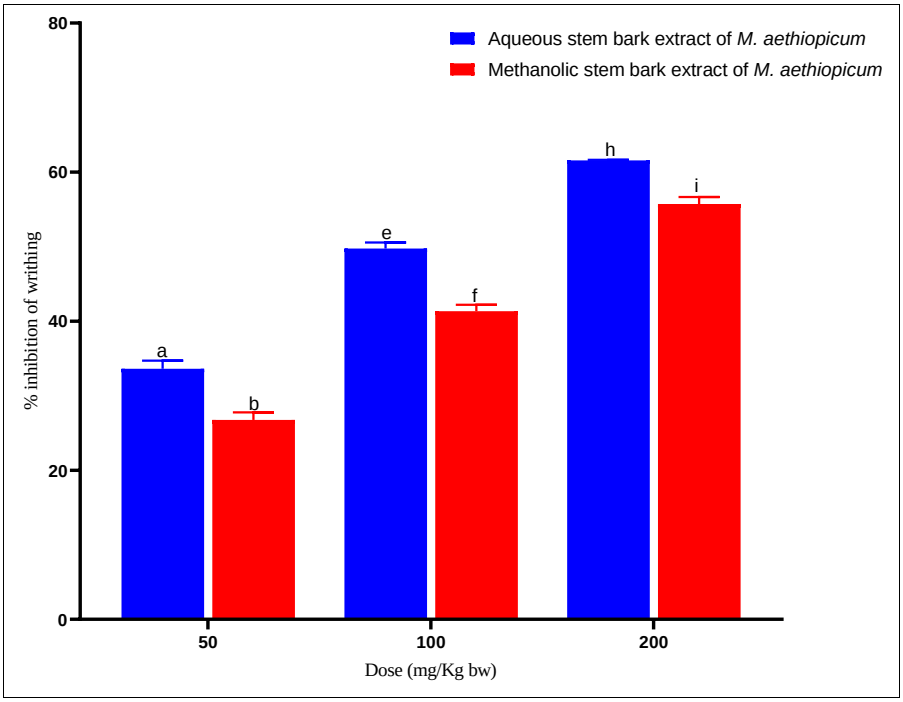
The percentage inhibition of acetic acid-induced writhing in mice by the studied methanolic stem bark extract was determined (Figure 2). It was observed that the percentage inhibitions of writhing ranged from 26.75±1.00% in the group of mice that received 50 mg/Kg bw to 55.75±0.93% in the mice treated with 200 mg/Kg bw of the studied methanolic extract (Figure 2). Overall, the reference drug caused the highest inhibition of writhing (75.99±1.12%) in experimental mice.



Bars with dissimilar lowercase alphabet are significantly different by one-Way ANOVA followed by Tukey's test ($P< 0.05$)

Fig 2: The percentage inhibitions of acetic acid-induced writhing by the methanolic stem bark extract of *M. aethiopicum* in mice

A comparison between the percentage inhibitions of writhing by the aqueous and methanolic stem bark extracts of *M. aethiopicum* was also done in this study (Figure 3). The results revealed that, at all the studied dose levels, the aqueous extract produced significantly higher percentage inhibitions of acetic acid-induced writhing in mice than those caused by the methanolic extract at the same dose levels ($P< 0.05$; Figure 3).



Bars with different alphabet letter within the same dose level are significantly different by t-test ($P< 0.05$)

Fig 3: The comparison of percentage inhibitions of writhing by the studied plant extracts in mice

3.2 Qualitative phytochemical screening of the aqueous and methanolic stem bark extracts of *Mystroxylon aethiopicum*

Following phytochemical profiling, various phytochemical groups of biological significance were detected. In both extracts, alkaloids, saponins, tannins, glycosides, flavonoids, phenols, terpenoids and saccharides were present (Table 2). However, proteins were absent in both the aqueous and methanolic stem bark extracts of the studied plant (Table 2).

Table 2: Qualitative phytochemical profile of the aqueous and methanolic stem bark extracts of *M. aethiopicum*

Phytochemical	*M. aethiopicum* stem bark extracts	
	Aqueous extract	Methanolic extract
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+
Flavonoids	+	+
Phenols	+	+
Terpenoids	+	+
Saccharides	+	+
Proteins	+	+

+: Present; -: Absent

4. Discussion

Inflammation is a multifaceted protective response to tissue injury, pathogens or irritation aimed at defending the body and maintaining health [24]. It is manifests with typical symptoms of fever, swelling, pain and redness on the site of injury [25]. While inflammation's primary goal is to defend the body against injurious stimuli, various pro-inflammatory mediators which are generated and released are key aetiologic agents that trigger and or complicate various diseases in the body [26, 27].

The most common presentation of many diseases and tissue injuries is pain, and, as a result, most diagnosed symptom of many diseases [28]. For instance, painful disease conditions including osteoarthritis, colitis, inflammatory bowel disease, rheumatoid arthritis along with other chronic conditions like neurodegenerative disorders like dementia and cardiovascular diseases among others are associated with inflammation [29–31]. The burden of these conditions to affected subjects is unquantifiable-the huge financial burden borne by the patients or caregivers and wider society as well as physical incapacitation which negatively impact the quality of life and even cause death [30]. Currently, a wide range of analgesic and anti-inflammatory drugs are being utilized to manage inflammation, alleviate pain and to reduce or stop tissue injury [32].

Analgesic and anti-inflammatory drugs generally fall under four classes namely, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease modifying anti-rheumatic drugs (DMARDs) and opioids [33, 34]. Furthermore, research has revealed that some anticonvulsant and antidepressant drugs raise the threshold in patients, thereby preventing hyperalgesia and general pain [33, 34].

Despite the remarkable potencies of the conventional drugs in managing pain and inflammation in modern medicine, various drawbacks limiting their clinical utilization have been reported [35, 36]. 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 [33, 35].

On the other hand, corticosteroids like cortisone, prednisone and methylprednisolone are associated with fluid retention (oedema), delayed wound healing, osteoporosis, weight gain (obesity) and hypertension [35, 37, 38]. Besides, opioid analgesics like pethidine, morphine, and codeine, have led to undesirable behavior, addiction as well as 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 [34, 35, 37, 38].

Due to the many associated side effects, arguably unaffordable costs and inaccessibility of the conventional medicines, the search for alternative and complementary agents and approaches have been intensified in the scientific arena [39, 40]. Of the many alternatives, medicinal plants stand a better chance of providing inexpensive, accessible, and potent analgesic products due to their rich history of use, claimed efficacy a safety [40]. Indeed, the WHO has demonstrated that over 80% of the world population especially in the developing countries use medicinal plants to cater for their primary healthcare requirements [41].

Medicinal plant contains bioactive phytochemicals which have been shown to confer either singly or in synergy with other phytoactive compounds pharmacologic value in the body [42–44]. Research has shown that bio-screening of medicinal plants claimed to treat various human maladies has a higher chance of obtaining potent pharmacologic compound leads for drug development [45]. Therefore, the present study was designed to investigate the analgesic activity and qualitative phytochemical screening of the aqueous and methanolic stem bark extracts of *M. aethiopicum* in the quest for safer, affordable, and efficacious analgesic compounds' search.

In this study, the acetic acid-induced writhing technique was used to appraise the antinociceptive activity of the studied plant extracts in mice models [46]. In this technique, overt pain is stimulated by an intraperitoneal injection of acetic acid as a stimulus. Afterwards, the chemical triggers a fast production of endogenous inflammatory mediators including prostaglandins which in turn activate the primary nociceptors [46–48]. 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 [48–50].

In this study, both the aqueous and methanolic stem bark extracts of *M. aethiopicum* exhibited analgesic potency by significantly inhibiting acetic-acid induced writhing in mice. These findings are consistent with previous reports on anti-nociceptive efficacy of plant extracts in animal models [51–54].

The reference drug, diclofenac, used in this study is a 2-[2,6-dichloranilino] phenylacetic acid\derivative clinically indicated for osteoarthritis (OA) and rheumatoid arthritis (RA) among other inflammatory diseases and mild to moderate pain [55]. Its mode of action is through the inhibition of prostaglandin synthesis through the cyclooxygenase (COX) pathway which terminate pain signal transduction [55].

It is suggestive, partly, that the studied plant extracts could be inhibiting prostaglandin synthesis in the peripheral system thus ameliorating pain. Furthermore, the results implicated the aqueous stem bark extract of *M. aethiopicum* as a more potent inhibitor of acetic acid inducing in mice compared with the methanolic stem bark extract. This could be attributable to specific phytochemical compounds that are in high concentration in the aqueous extract than in the methanolic extract [56]. Perhaps, this could be the reason behind the usage and claimed healing properties of the aqueous extract of this plant in traditional medicine. Indeed, the aqueous bark and root extracts of *M. aethiopicum* are traditionally used to manage stomach pain among other pain-associated diseases.

Pain and inflammation are induced by both endogenous and exogenous triggers [31, 57]. One of the potent triggers are microbial pathogens which infect the gastrointestinal tract and other body regions which have structures or toxins [58]. By virtue of these unique microbial elements, cells and molecule involved in the body's immunity evoke an immunologic

response with an intent of eliminating or neutralizing them and restore body homeostasis [57, 58].

The symptoms observed in inflammatory bowel syndrome, abdominal pain, gastric obstruction among others are therefore a result of inflammatory response of microbial damage to intestinal mucosa and tissues [30]. Since the aqueous stem bark and root bark extracts have been used by herbalists to manage gastrointestinal disorders, it is anticipated that these extracts modulate the body's immunity thereby clearing the insulting elements. Previous studies have demonstrated biologic activities of extracts against gastrointestinal inflammatory microbes [59].

Furthermore, these extracts could be modifying pain messages and averting the damage caused by the pathogens thereby ameliorating pain [60]. The results reported in this study therefore imply the analgesic efficacy of the studied plant extracts through the inhibition of the acetic acid induced writhing in mice could be replicable to the effects conferred by this plant's extracts in the gastrointestinal tract [33, 58, 59]. Nevertheless, the studied plant extracts appear to have active principles which have analgesic activity as evidenced by the obtained results.

In spite of the usage of medicinal plants to treat diseases in traditional medicine practice by herbalists, there is scanty research data pertaining their toxicity profiles and safety [61]. This has been worsened by lack of government regulation of traditional medicine leading to resurgences of unscrupulous practitioners [62, 63]. Furthermore, there are no clearly defined dosages, modes of preparation, modes of action and administration of herbal preparations [64, 65].

Moreover, no enough data and knowledge of herbal drug interactions with other herbal drugs, and or with conventional medicines when consumed simultaneously, or within a certain time frame are available [62, 64]. Consequently, medicinal plants have been suspected to be toxic despite their longstanding use by many communities [65]. It is, therefore, important to investigate the toxicity and safety medicinal plants, claimed to manage various diseases as framework to determining safe dose regimens and side effects if they exist.

The term toxicity is a notation of a substance being fatal, showing the degree of undesirable events because of a toxic substance interacting with cellular components [66]. This interaction varies based on the chemical and biologic activities of various toxicants and the extracellular matrix (ECM) and the cell membrane of body cells [66–68]. The interaction causing toxicity can be within individual cells, tissues, or entire organs. Further, toxicity to specialized/vital body organs can cause detrimental effects ranging from organ failure to death [66]. Toxicological evaluation of medicinal plants thus increases the potential of medicinal plants as sources of safer, easily accessible, and well tolerable drugs to make up for the deficiencies, insufficiencies, and inefficiencies of synthetics [40, 69–72].

In practice, preliminary toxicological studies involve acute, sub-acute, chronic, carcinogenic, and anti-reproductive effects of the study materials in model animals [68, 73]. There are OECD guidelines that can and has been utilized extensively to appraise toxicity profile and safety of chemicals and plant extracts that have potential of providing lead molecules towards drug development.

The form in which the drug to be administered is available, appropriate route is selected for its delivery into the body. 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 [73, 74]. Moreover, studies have shown that acute oral toxicity studies in mice offer a better prediction of the human acute lethal doses in clinical setups.

The presence of antioxidant phytochemical compounds in medicinal have been considered to play crucial functions in mitigating pain and inflammation [75]. These compounds have been demonstrated to impart oxidative stress, a key driving factor of inflammation, shun overproduction of pro-inflammatory mediators as well as reversal of cellular and tissue injury [76]. Upon this qualitative phytochemical study of the aqueous and methanolic stem bark extracts of *M. aethiopicum*, phenols, flavonoids among other antioxidant phytochemicals, associated with analgesic activity were detected. Moreover, a previous study involving gas chromatographic screening of the chloroform root bark extracts of *M. aethiopicum* reported the presence of Caryophyllene, Cubenol and Elexine, which have anti-inflammatory properties, among other antioxidant phytochemical compounds [77]. This observation compares well with those of earlier investigations by other authors who established that not all phytochemicals may be present in plant parts [78].

Although isolation of active metabolites with anti-inflammatory and analgesic effects have been isolated from the aqueous and methanolic stem bark extracts of *M. aethiopicum*, the earlier isolated compounds from the root extract could be present in the stem bark too. Consequently, the analgesic effects of the studied plant extracts reported herein, could be partly due to these compounds solely or in a combination with others yet to be determined.

5. Conclusions and Recommendations

Based on the study findings, it was concluded that the aqueous and methanolic stem bark extracts of *M. aethiopicum* have peripheral anti-nociceptive activity in experimental mice. Furthermore, the studied plant extracts possess antioxidant-analgesic associated phytochemicals.

From the results of study, the aqueous and methanolic stem bark extracts of *M. aethiopicum* can be used in the management of peripheral pain and as sources of antioxidant-analgesic compounds. This study recommends further studies aimed at isolating and characterizing specific analgesic compounds from the studied plant extracts. The specific mode(s) through which the aqueous and methanolic stem bark extracts of the studied plant exert their anti-nociceptive potency should be established. Further, toxicological investigations including acute oral toxicity studies should be conducted to fully assure the safety of the studied plant extracts. Additionally, analgesic activities of the studied plant extracts using other experimental models should be conducted. Further studies on the studied plant extracts geared towards determining their optimal doses for optimal analgesic effects are encouraged.

6. Data availability

All data in this study are included within the manuscript; however, any additional information is available from authors upon request.

7. Conflict of Interest

The authors declare that there is no conflict of interest whatsoever regarding this study.

8. Author Contributions

John Muchonjo conceived the research idea and performed the experiments under the close supervision of James

Mbariaand Joseph Nguta. Gervason Moriasi designed, guided John Muchonjo in conducting experiments, data analysis, and interpretation. All authors reviewed and approved the final manuscript for publication.

9. Funding

We did not receive any funding from any granting/funding agency/corporation in the public or private sector. This study was solely achieved through our finances.

10. Acknowledgments

We acknowledge the Department of Public Health, Pharmacology, and Toxicology of the University of Nairobi for availing the lab facility, experimental animals, reagents, microbial strains, and equipment for this study. Also, we appreciate Mr. Maloba of the Department of Public Health, Pharmacology and Toxicology (UoN), John Nzivo and Mr. Nelson of the Department of Biological Sciences (MKU) for their technical assistance. Dr. Jared Onyancha is appreciated for his pharmacognostic guidance.

11. References

1. Schaible H. Pathophysiology of pain 2004, 237–43. https://doi.org/10.1007/s00423-004-0468-9.
2. Sahlmann CO, Ströbel P. Pathophysiology of inflammation. Nuklear Medizin 2016. https://doi.org/10.1055/s-0037-1616468.
3. Henschke N, Kamper SJ, Maher CG. The epidemiology and economic consequences of pain. Mayo Clin Proc 2015;90:139–47. https://doi.org/10.1016/j.mayocp.2014.09.010.
4. Mills SEE, Nicolson KP, Smith BH. Chronic pain: a review of its epidemiology and associated factors in population-based studies. Br J Anaesth 2019;123:e273–83. https://doi.org/10.1016/j.bja.2019.03.023.
5. Kendroud S, Hanna A. Physiology, Nociceptive Pathways. Stat Pearls Publishing 2019.
6. Olela B, Mbaria J, Wachira T, Moriasi G. Acute Oral Toxicity and Anti-inflammatory and Analgesic Effects of Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* (Schumach) 2020. https://doi.org/https://doi.org/10.1155/2020/5651390.
7. Dinakar P, Stillman AM. Pathogenesis of Pain. Semin Pediatr Neurol 2016;23:201–8. https://doi.org/10.1016/j.spen.2016.10.003.
8. Maund E, McDaid C, Rice S, Wright K, Jenkins B, Woolacott N. Paracetamol and selective and non-selective non-steroidal anti-inflammatory drugs for the reduction in morphine-related side-effects after major surgery: A systematic review. Br J Anaesth 2011;106:292–7. https://doi.org/10.1093/bja/aeq406.
9. Cazacu I, Mogosan C, Loghin F. Safety issues of current analgesics: An update. Clujul Med 2015;88:128–36. https://doi.org/10.15386/cjmed-413.
10. Inflammatory Drugs, Drugs Used To Treat Gout, and Disease-Modifying Antirheumatic Drugs n.d. Abdullahi AA. Trends and challenges of traditional medicine in Africa. African J Tradit Complement Altern Med 2011;8:115–23. https://doi.org/10.4314/ajtcam.v8i5S.5.
11. Omwenga EO, Hensel A, Shitandi A, Goycoolea FM. Ethnobotanical survey of traditionally used medicinal plants for infections of skin, gastrointestinal tract, urinary tract and the oral cavity in Borabu sub-county, Nyamira county, Kenya. J Ethnopharmacol 2015. https://doi.org/10.1016/j.jep.2015.11.032.
12. Mahomoodally MF. Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. Evidence-Based Complement Altern Med 2013. https://doi.org/10.1155/2013/617459.
13. Kigen GK, Ronoh HK, Kipkore WK, Rotich JK. Current trends of Traditional Herbal Medicine Practice in Kenya: A review. J Pharmacol Ther 2013.
14. Abdullahi AA. Trends and challenges of traditional medicine in Africa. African J Tradit Complement Altern Med 2011. https://doi.org/10.4314/ajtcam.v8i5S.5.
15. World Health Organization. Traditional and complementary medicine in primary health care 2018.
16. World Health Organization (WHO). WHO Traditional Medicine Strategy 2014-2023. World Heal Organ 2013. https://doi.org/2013.
17. Moriasi G, Ireri A, Ngugi M. Cognitive-Enhancing, *Ex Vivo* Antilipid Peroxidation and Qualitative Phytochemical Evaluation of the Aqueous and Methanolic Stem Bark Extracts of *Lonchocarpus eriocalyx* (Harms.). Biochem Res Int 2020, 1–16. https://doi.org/10.1155/2020/8819045.
18. Moriasi GA, Ireri AM, Ngugi MP. *In Vivo* Cognitive-Enhancing, Ex Vivo Malondialdehyde-Lowering Activities and Phytochemical Profiles of Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* (Schum.). Int J Alzheimers Dis 2020. https://doi.org/10.1155/2020/1367075.
19. *Mystroxylon aethiopicum*. Plant Z Africa n.d. http://pza.sanbi.org/mystroxylon-aethiopicum
20. Maina JG. Herbal medicine use in murang' a county and antiflea activity and safety of *Tithonia diversifolia* and *Senna didymobotrya* extracts: A Thesis. University of Nairobi 2018.
21. Harborne JB. Phytochemical Methods A Guide To Modern Techniques of Plant Analysis, Third Edition. Chapman Hall 1998;58. https://doi.org/10.1017/CBO9781107415324.004.
22. Koster R, Anderson M, De Beer EJ. Acetic Acid for Analgesic Screening. Fed Proceedings 1959, 412–7.
23. Bukhari IA, Gilani AH, Meo SA, Saeed A. Analgesic, anti-inflammatory and anti-platelet activities of Buddleja crispa. BMC Complement Altern Med 2016;16:1–7. https://doi.org/10.1186/s12906-016-1021-4.
24. Nathan C, Ding A. Non-resolving Inflammation. Cell 2010;140:871–82. https://doi.org/10.1016/j.cell.2010.02.029.
25. Alemu A, Tamiru W, Nedi T, Shibeshi W. Analgesic and Anti-Inflammatory Effects of 80% Methanol Extract of *Leonotis ocymifolia* (Burm. f.) Iwarsson Leaves in Rodent Models 2018. https://doi.org/10.1155/2018/1614793.
26. Nasimolo J. Anti-inflammatory potential of the coral tree (*Erythrina abyssinica*): histological and immunohistochemical evidence in chronic trypanosomiasis mouse model 2013.
27. Radhika B, Nasreen Begum SK. *In-Vitro* and *In-Vivo* Anti-Inflammatory and Analgesic Activity of Bixa orellana Linn Leaf Extracts. Int J Pharm Pharmacol 2017;1:1–10. https://doi.org/10.31531/2581-3080.1000108.
28. Medzhitov R. Origin and physiological roles of inflammation. Nature 2008;454:428–35. https://doi.org/10.1038/nature07201.
29. Su HJ, Chiu YT, Chiu CT, Lin YC, Wang CY, Hsieh JY

et al. Inflammatory bowel disease and its treatment in 2018: Global and Taiwanese status updates. J Formos Med Assoc 2019;118:1083–92.' https://doi.org/10.1016/j.jfma.2018.07.005.
30. Omoigui S. The biochemical origin of pain - Proposing a new law of pain: The origin of all pain is inflammation and the inflammatory response. Part 1 of 3 - A unifying law of pain. Med Hypotheses 2007;69:70–82. https://doi.org/10.1016/j.mehy.2006.11.028.
31. Andreicut AD, Pârvu AE, Mot AC, Pârvu M, Fodor EF, Cătoi AF *et al*. Phytochemical analysis of anti-inflammatory and antioxidant effects of Mahonia aquifolium flower and fruit extracts. Oxid Med Cell Longev 2018. https://doi.org/10.1155/2018/2879793.
32. Lundberg TR, Howatson G. Analgesic and anti-inflammatory drugs in sports: Implications for exercise performance and training adaptations. Scand J Med Sci Sport 2018;28:2252–62. https://doi.org/10.1111/sms.13275.
33. True BL, Dreisbach RH, True BL, Dreisbach RH. Analgesics, antipyretics, and anti-inflammatory agents. Dreisbach's Handb Poisoning 2013, 367–78. https://doi.org/10.1201/b14640-21.
34. Carter GT, Duong V, Ho S, Ngo KC, Greer CL, Weeks DL. Side effects of commonly prescribed analgesic medications. Phys Med Rehabil Clin N Am 2014;25:457–70. https://doi.org/10.1016/j.pmr.2014.01.007.
35. Moriasi G, Nelson E, Twahirwa E. *In Vitro* Anti-Inflammatory, Antioxidant, and Qualitative Phytochemical Evaluation of the Phytexponent Preparation of Selected Plants Advanced Techniques in Biology & Medicine. Adv Tech Biol Med 2020;9:1–9. https://doi.org/10.4172/2379-1764.1000277.
36. Activation E, Britain I. Non-steroidal anti-inflammatories in the elderly 1987, 151–2.
37. Steinmeyer J. Pharmacological basis for the therapy of pain and inflammation with nonsteroidal anti-inflammatory drugs. Arthritis Res 2000;2:379–85. https://doi.org/10.1186/ar116.
38. Ghasemian M, Owlia S, Owlia MB. Review of Anti-Inflammatory Herbal Medicines. Adv Pharmacol Sci 2016. https://doi.org/10.1155/2016/9130979.
39. Shakya AK. Medicinal plants: Future source of new drugs Medicinal plants : Future source of new drugs 2016. https://doi.org/10.13140/RG.2.1.1395.6085.
40. WHO. WHO Traditional Medicine Strategy 2002–2005. World Heal Organ 2005, 1–60.
41. Phillipson JD. Phytochemistry and medicinal plants. Phytochemistry 2001. https://doi.org/10.1016/S0031-9422(00)00456-8.
42. Makkar HPS, Francis G, Becker K. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 2007. https://doi.org/10.1017/S1751731107000298.
43. Kurmukov AG. Phytochemistry of medicinal plants. Med. Plants Cent. Asia Uzb. Kyrg 2013. https://doi.org/10.1007/978-1-4614-3912-7_4.
44. Haidan Yuan, Qianqian Ma, Li Ye GP. The Traditional Medicine and Modern Medicine from Natural Products 2016. https://doi.org/10.3390/molecules21050559.
45. Gawade SP. Acetic acid induced painful endogenous infliction in writhing test on mice. J Pharmacol Pharmacother 2012;3:348. https://doi.org/10.4103/0976-500X.103699.
46. Martinez RM, Zarpelon AC, Domiciano TP, Georgetti SR, Baracat MM, Moreira IC *et al*. Antinociceptive Effect of Tephrosia sinapou Extract in the Acetic Acid, Phenyl-p-benzoquinone, Formalin, and Complete Freund's Adjuvant Models of Overt Pain-Like Behavior in Mice. Scientifica (Cairo) 2016. https://doi.org/10.1155/2016/8656397.
47. Nesa ML, Karim SMS, Api K, Sarker MMR, Islam MM, Kabir A *et al*. Screening of *Baccaurea ramiflora* (Lour.) extracts for cytotoxic, analgesic, anti-inflammatory, neuropharmacological and antidiarrheal activities. BMC Complement Altern Med 2018;18:1–9. https://doi.org/10.1186/s12906-018-2100-5.
48. Spessoto MA, Ferreira DS, Crotti AEM, Silva MLA, Cunha WR. Evaluation of the analgesic activity of extracts of *Miconia rubiginosa* (Melastomataceae). Phytomedicine 2003;10:606–9. https://doi.org/10.1078/094471103322331629.
49. Saha A, Masud MA, Bachar SC, Kundu JK, Datta BK, Nahar L *et al*. The analgesic and anti-inflammatory activities of the extracts of *Phyllanthus reticulatus* in mice model. Pharm Biol 2007;45:355–9. https://doi.org/10.1080/13880200701212973.
50. Subedi NK, Rahman SMA, Akbar MA. Analgesic and Antipyretic Activities of Methanol Extract and Its Fraction from the Root of *Schoenoplectus grossus*. Evidence-Based Complement Altern Med 2016. https://doi.org/10.1155/2016/3820704.
51. Rahman SMM, Islam N, Ahammad F. anti-diarrhoeal activities of methanol and ethyl acetate extract of Hemigraphis alternata leaves in mice 2019.
52. Jakaria M, Parvez M, Zaman R, Arifujjaman, Hasan MI, Sayeed MA *et al*. Investigations of analgesic activity of the methanol extract of *haldina cordifolia* (Roxb.) bark by using in vivo animal model studies. Res J Bot 2015;10:98–103. https://doi.org/10.3923/rjb.2015.98.103.
53. Kaushik D, Kumar A, Kaushik P, Rana AC. Analgesic and anti-inflammatory activity of pinus roxburghii sarg. Adv Pharmacol Sci 2012. https://doi.org/10.1155/2012/245431.
54. Gan TJ. Diclofenac: An update on its mechanism of action and safety profile. Curr Med Res Opin 2010;26:1715–31. https://doi.org/10.1185/03007995.2010.486301.
55. Sdayria J, Rjeibi I, Feriani A, Ncib S, Bouguerra W, Hfaiedh N *et al*. Chemical Composition and Antioxidant, Analgesic, and Anti-Inflammatory Effects of Methanolic Extract of Euphorbia retusa in Mice. Pain Res Manag 2018. https://doi.org/10.1155/2018/4838413.
56. Oschman JL, Chevalier G, Brown R. The effects of grounding (earthing) on inflammation, the immune response, wound healing, and prevention and treatment of chronic inflammatory and autoimmune diseases. J Inflamm Res 2015;8:83–96. https://doi.org/10.2147/JIR.S69656.
57. Hakansson A, Molin G. Gut microbiota and inflammation. Nutrients 2011;3:637–87. https://doi.org/10.3390/nu3060637.
58. Kilonzo M, Ndakidemi P. *In Vitro* Antibacterial Activity of Selected Tanzania Medicinal Plants. Herb Med Open Access 2016;2:1–6. https://doi.org/10.21767/2472-0151.100015.
59. Miya Gugulethu Mathews, Oyemitan, Idris Ajayi, Oyedeji Oyehan Opeoluwa, Oluwafemi Samuel

Oluwatobi, Nkeh-Chungag Benedicta Songca N, Sandile Phindile *et al*. Afr J Tradit Complement Altern Med 2016;3(6):179-185.
60. Phytochemical screening, anti-inflammatory and analgesic properties of *Pentanisia prunelloides* from the Eastern Cape Province, South Africa 2016;13:179–85.
61. Nasri H, Shirzad H. Toxicity and safety of medicinal plants. J HerbMed Pharmacol 2013;2:21–2.
62. George P. Concerns regarding the safety and toxicity of medicinal plants - An overview. J Appl Pharm Sci 2011;1:40–4.
63. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Neurol 2014;4:1–10. https://doi.org/10.3389/fphar.2013.00177.
64. Chanda S, Parekh J, Vaghasiya Y, Dave R, Baravalia Y, Nair R. Medicinal Plants-From Traditional Use To Toxicity Assessment: a Review. Int J Pharm Sci Res IJPSR 2015;6:2652–70. https://doi.org/10.13040/IJPSR.0975-8232.6(7).2652-70.
65. Subramanian K, Sankaramourthy D. Toxicity Studies Related to Medicinal Plants. Elsevier 2018. https://doi.org/10.1016/B978-0-08-102081-4.00018-6.
66. Mückter H. What is toxicology and how does toxicity occur? Best Pract Res Clin Anaesthesiol 2003;17:5–27. https://doi.org/10.1053/bean.2003.0270.
67. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Sasidharan S. Acute oral toxicity of methanolic seed extract of Cassia fistula in mice. Molecules 2011;16:5268–82. https://doi.org/10.3390/molecules16065268.
68. Saleem U, Amin S, Ahmad B, Azeem H, Anwar F, Mary S. Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb roots in albino mice as per OECD 425 TG. Toxicol Reports 2017;4:580–5. https://doi.org/10.1016/j.toxrep.2017.10.005.
69. Mishra BB, Tiwari VK. Natural products: An evolving role in future drug discovery. Eur J Med Chem 2011;46:4769–807. https://doi.org/10.1016/j.ejmech.2011.07.057.
70. Sciences DOF. Evaluation of toxicity in mice and rats and antioxydant activities of *Ruta montana* L. extracts Laboratory of Phytotherapy Applied to Chronic Diseases 2015.
71. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evidence-Based Complement Altern Med 2006;3:433–9. https://doi.org/10.1093/ecam/nel058.
72. Thukham-mee W, Wattanathorn J. Evaluation of Safety and Protective Effect of Combined Extract of *Cissampelos pareira* and *Anethum graveolens* (PM52) against Age-Related Cognitive Impairment. Evidence-Based Complement Altern Med 2012, 1–10. https://doi.org/10.1155/2012/674101.
73. Hazarika I, Geetha KM, Sundari PS, Madhu D. Acute oral toxicity evaluation of extracts of Hydrocotyle sibthorpioides in wister albino rats as per OECD 425 TG. Toxicol Reports 2019;6:321–8. https://doi.org/10.1016/j.toxrep.2019.04.001.
74. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure 2008. https://doi.org/10.1787/9789264071049-en.
75. Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME *et al*. Role of Antioxidants and Natural Products in Inflammation. Oxid Med Cell Longev 2016. https://doi.org/10.1155/2016/5276130.
76. Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T *et al*. Role of ROS and nutritional antioxidants in human diseases. Front Physiol 2018;9:1–14. https://doi.org/10.3389/fphys.2018.00477.
77. Mhuji K, Patrick AN, Musa C. Mystroxylon aethiopicum chloroform root bark extracts phytochemical analysis using gas chromatography mass spectrometry. J Pharmacogn Phyther 2017;9:44–50. https://doi.org/10.5897/jpp2017.0444.
78. Eldahshan OA, Singab ANB. Journal of Pharmacognosy and Phytochemistry. J Pharmacogn Phytochem Carotenoids 2013;2:225–34.