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

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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 (LD₅₀>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. Pain is 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-inflammatory agents 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 (11-13). 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 °C and further in a hot air oven set at 35 °C for 48 hours to ensure complete drying. The extract was then transferred into a well closed, light resistant glass bottle and kept in a refrigerator at 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 °C 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 CO2 mixed with acetone. The flasks were then fitted into a freeze-dryer and lyophilized for 48 hrs. Afterwards, the lyophilized extract was transferred into a pre-weighed universal glass bottle and stored in a refrigerator at 4 °C awaiting biological assays [22].

2.3 Experimental animals

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

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

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).

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*

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Aqueous extract</th>
<th>Methanolic extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saccharides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*: 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 manifested 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 [29]. 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 [33, 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 activates 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 [49–50].

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-dichlorophenyl] 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  

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. 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. Furthermore, these extracts could be modifying pain messages and averting the damage caused by the pathogens thereby ameliorating pain. The results reported in this study therefore imply the analgesic efficacy of the studied plant extracts through the inhibition of the acetic acid inducing writhing in mice could be replicable to the effects conferred by this plant’s extracts in the gastrointestinal tract. ae2, 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. This has been worsened by lack of government regulation of traditional medicine leading to resurgences of unscrupulous practitioners. Furthermore, there are no clearly defined dosages, modes of preparation, modes of action and administration of herbal preparations. 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. Consequently, medicinal plants have been suspected to be toxic despite their longstanding use by many communities. 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. 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. 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. 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. In practice, preliminary toxicological studies involve acute, sub-acute, chronic, carcinogenic, and anti-reproductive effects of the study materials in model animals. 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. 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. 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. 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. This observation compares well with those of earlier investigations by other authors who established that not all phytochemicals may be present in plant parts. 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
Mbaria and 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.

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